

266. Molecular pathology and genetics in lung disease

E2994

CARD15/NOD2, CD14, and toll-like receptor 4 gene polymorphisms in Greek patients with sarcoidosis

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Background: Sarcoidosis, similarly to Crohn's disease (CD), is a complex inflammatory disease of unknown etiology. The belief that a genetic susceptibility to the development of sarcoidosis exists was derived from observations of familial clustering of sarcoidosis cases and racial differences in disease prevalence. Taking into account the remarkable similarity in the immunopathophysiology of sarcoidosis and CD, and in further exploring the genetic background of sarcoidosis, we study gene polymorphisms known for their implication in CD. These polymorphisms are in the CARD15/NOD2 gene (R702W, G908R and 3020insC), as well as mutations in the promoter of the CD14 gene (T/C at position -159) and in the TLR4 gene (Asp299Gly and Thr399Ile).

Methods: DNA was obtained from 100 sarcoidosis patients and 150 healthy individuals. Genotyping was performed by allele specific PCR or by PCR-RFLP analysis.

Results: Although CARD15/NOD2 mutations were more frequent in cases than in controls, the difference was significant only for the G908R polymorphism ($p = 0.024$). Interestingly, the same was recorded with reference to the T allele ($p = 0.002$) and TT genotype ($p = 0.017$) frequencies of the CD14 promoter. No differences were observed in the 299Gly and 399Ile allele frequencies between patients and controls. Finally, the co-existence of a mutation in the CARD15/NOD2 and the CD14 gene was associated with sarcoidosis at a higher level of significance than any of these mutations separately.

Conclusion: Our results suggest that the G908R mutation of the CARD15/NOD2 gene, as well as the T allele and TT genotype of the CD14 promoter are associated with increased susceptibility for developing sarcoidosis.

E2995

TNF-alpha and TNF-beta gene polymorphism in pulmonary sarcoidosis patients

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Sarcoidosis is a systemic granulomatous disease of unknown etiology, in which genetic factors seem to play an important role in the disease predisposition and course. -308 TNF-alpha gene (TNF-A1 and TNF-A2 alleles) and TNF-beta gene polymorphisms (TNF-B1 and TNF-B2 alleles) analysis in Polish sarcoidosis patients was performed. TNF-A2 and TNF-B1 alleles are associated with higher TNF-alpha and TNF-beta production.

The study comprised 78 sarcoidosis patients (including 18 Loeffgren syndrome subjects) in TNF-alpha polymorphism analysis, and 88 sarcoidosis patients (including 21 Loeffgren syndrome subjects) in TNF-beta polymorphism analysis. The control group consisted of 60 and 71 subjects, respectively. The genotypes were determined using PCR-RFLP assays. Pearson χ^2 test was used to analyse the differences between the groups.

In the TNF-alpha polymorphism analysis, the difference between Loeffgren and non-Loeffgren patients was significant ($P < 0.016$). TNF-A2 allele was significantly

more frequent in the RTG stage I patients than in RTG stages II/III ($P < 0.001$). There was no significant difference between sarcoidosis patients and control group. In the TNF-beta polymorphism analysis, there was no significant difference between the sarcoidosis patients, regardless RTG stage and presence/absence of the Loeffgren syndrome. The difference in allele distribution between the sarcoidosis and the control group was significant ($P < 0.03$).

The TNF-A2 allele and not TNF-B1 allele may protect from the severe course of sarcoidosis. TNF-B1 allele may increase the risk of sarcoidosis development.

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E2996

Toll-like receptor (TLR) 9 genetics in sarcoidosis patients

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Rationale: Sarcoidosis is a systemic disease characterized by a strong Th-1 response to an unknown antigen. A genome-wide search for predisposing genes in sarcoidosis (Schürmann et al 2001) revealed a susceptibility locus at chromosome 3p21.1, close to the genomic location of TLR-9. We hypothesize that genetic differences that alter TLR-9 function are involved in the aberrant immune response characterizing sarcoidosis.

Methods: We investigated the frequency of 4 known single nucleotide polymorphisms (SNPs) in the TLR-9 gene in 167 sarcoidosis patients and 196 healthy controls, all Caucasian. The sarcoidosis patients were stratified as patients with Löfgren's syndrome ($n=57$), acute/self remitting disease ($n=51$) or chronic disease, without ($n=35$) and with fibrosis ($n=24$) according to radiographic evolution over a 4-year follow-up period. Furthermore, we sequenced exon 1, exon 2 and 400bp of the promoter region of 20 patients.

Results: No differences were found in allelic or haplotype distributions between patients and controls or when comparing different clinical entities within the sarcoidosis group. The complete sequencing of exon 1 and exon 2 together with 400bp of the promoter region did, next to known SNP positions, not reveal additional genetic differences in sarcoidosis.

Conclusion: No genetic differences were found in the 4 polymorphisms, exon 1, exon 2 or part of the promoter region of the gene coding for TLR-9 in Dutch sarcoidosis patients. However, the level of TLR-9 mRNA or -protein can be controlled by other regulatory elements. Therefore, in vitro studies are currently being performed in our laboratory to investigate the functionality of TLR-9 in sarcoidosis patients in order to complete our data.

E2997

C-C chemokine receptor 2 gene polymorphism V64I and C-C chemokine receptor 5 gene polymorphism Δ32 in pulmonary sarcoidosis patients

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Background: Sarcoidosis, a multiorgan, granulomatous disease, is thought to be triggered by unknown environmental antigens in genetically predisposed subjects. It is hypothesized that genetic variation in the C-C chemokine 2 receptor (CCR2) and in the C-C chemokine 5 receptor (CCR5) genes may affect the interplay between the receptors and their ligands involved in a cascade of events characteristic of immune-mediated diseases. We investigated the distribution of the CCR2 -V64/-64I alleles, and CCR5 Δ32/ wild type alleles in Polish sarcoid patients and in the control group of healthy subjects.

Material and Methods: The genotypes were determined using PCR tests. CCR2 polymorphism was investigated in 121 sarcoid patients and in 65 unaffected subjects; CCR5 polymorphism was investigated in 82 sarcoid patients and in 70 healthy subjects. The study comprised sarcoidosis patients with radiological Stages I, II or III. Pearson χ^2 test was used to analyse the differences between the groups. **Results:** No association between the CCR2 and CCR5 variant haplotype and susceptibility to sarcoidosis was observed. We have found no statistically significant differences between the radiological stage of the disease and allele distribution.

Conclusions: Other polymorphic forms of CCR2 and CCR5 may be responsible for genetic predisposition to sarcoidosis.

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E2998

CARD15/NOD2 3020insC (1007fs) mutation is not a genetic marker for cytokine network in sarcoidosis

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The pathogenesis of sarcoidosis is defined by a highly polarized Th1 immune

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response to pathogenic tissue antigens. In chronic sarcoidosis it is likely that ongoing Th1 immunity to persistent pathogenic antigens mediates progressive tissue dysfunction. CARD15 mutations may lead to disease by causing excessive Th1 response and increased cytokine production.

Aim: To test the relationship between CARD15 mutations and non-specific serum inflammation markers combined with TGF β , IL-2, IL-12p40 serum and BALF levels.

Methods: 96 sarcoid patients were genotyped for the 3020insC (1007fs) using DNA and TaqMan SNP Genotyping Assays and had routine measurements of serum non-specific inflammation markers. In 27 patients we measured the serum and BALF TGF β , IL-2, IL-12p40 levels using commercial ELISA kits BioSource. We compared the distribution of 3020insC (1007fs) alleles according to presence (n=69) vs absence (n=27) of elevated non-specific serum inflammation markers. We compared the TGF β , IL-2, IL-12p40 serum and BALF levels among patients (n=27) with and without 3020insC (1007fs) mutation.

Results: 83 patients didn't have any 3020insC (1007fs) allele, 13 patients had at least one such allele. 3020insC (1007fs) mutation correlate with elevated non-specific serum inflammation markers (odds ratio=4.7), although the difference between groups was not significant (p=0.078). No relationship between the 3020insC (1007fs) allele occurrence and serum and BALF TGF β , IL-2, IL-12p40 levels was found.

Conclusion: While 3020insC (1007fs) allele could be genetic risk factor for non-specific inflammation markers detectable in the serum it can't be used as genetic marker of cytokine release in sarcoidosis.

E2999

Genetic studies on sarcoidosis: launch of a website

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Sarcoidosis is a systemic granulomatous disorder of unknown origin. Although the disease can manifest in almost all tissues, it is predominantly found to affect the lung. From the differences in the prevalence between ethnic groups and an abundance of cases of familial clustering it is assumed that a genetic predisposition to sarcoidosis exists. However, due to the low recurrence risk in family members and a multitude of sporadic cases, a more complex polygenic or multifactorial gene model should be postulated.

In the last decade many candidate gene have been studied, searching for associations with polymorphisms or the presence of mutations in both cases versus control samples or between clinically characteristic groups of patients. In addition, in several family-based linkage studies, scientists have tried to link inheritance of a certain genomic region to the development of sarcoidosis.

Until now over 150 papers have been published focussing on the genetic predisposition to develop sarcoidosis. We have tried to bring this information together in one comprehensive poster featuring the studied genes, the ethnicity of the group under study and the correlation to susceptibility or phenotypic development of sarcoidosis. In addition we have developed a site on the world wide web at www.geneticsofsarcoidosis.nl, where researchers can search for studies performed per gene, chromosome or ethnic group. Scientists are invited to submit their results on the genetic predisposition to sarcoidosis to the website and use the searchable database. The poster will be updated each year with the new submissions.

E3000

TGF-beta-dependent inhibitor of DNA protein (Id) expression in lung fibrosis

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Inhibitor of DNA binding (Id) proteins represent a family of transcriptional repressors essentially involved in cellular proliferation and cancerogenesis. While their role in cancer has been delineated, their role in lung disease such as lung fibrosis has been less studied. In this study, we initially characterized Id1-3 expression and localization in the lung, and analyzed their regulation in two models of lung fibrosis: In mice with orotracheal instillation of the profibrotic cytokine TGF-beta and in mice subjected to bleomycin-induced lung fibrosis. Id mRNA expression was analyzed by quantitative reverse transcription polymerase chain reaction (qRT-PCR), while Id protein expression was assessed by Western Blot and Immunohistochemistry. Id1 was expressed at low levels in the mouse lung, whereas Id2 was highly expressed in bronchial and alveolar epithelium. In contrast, Id3 was localized only in bronchial epithelial cells. Primary isolates of mouse alveolar epithelial cells, however, demonstrated expression of all three Id isoforms in vitro. Bleomycin treatment resulted in significant downregulation of Id1, whereas Id 2 and 3 were expressed at higher levels 3 weeks after bleomycin treatment. Similarly, Id3 expression was increased as rapidly as 30 min after orotracheal instillation of TGF-beta to mouse lungs. In sum, our data supports a role of Id3 in bronchial epithelial cell injury and repair in bleomycin-induced lung injury.

E3001

Development of *in vitro* assays to predict fibrogenesis and replace animal testing

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This project is part of a programme that aims to develop *in vitro* mono- and co-culture assays to predict the fibrogenic potential of inhalable cosmetic ingredients. Organic polymers S2218600, S2429901 and S2219200 (referred to as P1, P2 and P3, respectively) of varying fibrogenic potential, designed for use in hairsprays, were used as model substances.

The cytotoxicity, proliferation and differentiation induced by polymer exposure in serum-starved cultures of rat lung fibroblast-like and rat alveolar epithelial type II cell lines (RFL-6 and RLE-6TN) were studied using LDH, methylene blue and a-smooth muscle actin (a-sma) assays respectively. Fibrogenic effects *in vivo* were assayed by an instillation study in male Wistar rats (3 instillations, 12 mg/kg bodyweight in total). The results are summarised in the table.

Effects of P1, P2 and P3 *in vivo* and *in vitro*

Polymer	Interstitial fibrosis <i>in vivo</i>	Fibrosing granuloma <i>in vivo</i>	Fibroblast proliferation (% of ctrl)	AE II proliferation (% of ctrl)	α -sma expression	Cytotoxicity
P1	++	++	116.4±14.4	136.4±16.9	+	+
P2	+	o	131.9±19.6*	179.2±26.1***	++	o
P3	+	+	182.3±24.2***	154.4±25.5***	+++	+++

Cell data are mean \pm stdev, measured 24-72 h after treatment. *In vivo* data are histological scores at 22 weeks. *p<.05, **p<.01, ***p<.0001, ANOVA

Fibroblast and epithelial cell proliferation, and differentiation of fibroblasts to myofibroblasts are common features of fibrosis. Treatment with all three polymers enhanced cellular proliferation and fibroblast differentiation. No assay on its own predicted the degree of fibrogenicity *in vivo*. However ongoing work using co-culture models, including a macrophage-like cell line, may better model the fibrogenicity.

E3002

The molecular controls of resolution of inflammation: what can we learn from zebrafish?

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Although we are separated from Zebrafish by 160 million years of evolution, we share many features of the innate and adaptive immune systems. In addition, we can manipulate the genome of zebrafish, and observe the effects on inflammation *in vivo*, since they are transparent in their larval stages. This has exciting implications for the study of inflammatory diseases.

We have established a model of inflammation in the zebrafish tail, in which caspase dependent cell death is required for resolution. For example, addition of the pan-caspase inhibitor zVD added at 4 hours after tailfin injury increases the number of neutrophils present at 24 hours from 6.0±1.0 to 28.9±3.3 (mean \pm s.e.m p<0.001 n=3).

The transparency of the larvae makes these an ideal model for the study of *in vivo* inflammation, and we have generated fluorescent systems for the easy visualisation of neutrophilic inflammation and resolution *in vivo*.

We are also performing an unbiased forward genetic screen for mutants with defective resolution of inflammation, and to date have identified 38 putative mutants. These techniques allow new approaches to understanding the molecular controls of inflammation resolution.

E3003

Search for molecular mediators of alveolarization

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Background: Our knowledge about the molecular mediators of alveolarization and lung growth is very limited. A more detailed understanding of these processes may help finding new strategies in the therapy of several lung diseases which are caused by loss of gas exchange surface.

Materials & Methods: Using microarray technique (Agilent 44k whole mouse genome arrays), we compared the gene expression in lungs of newborn C57BL/6 mice 1 and 3 days after birth (induction of alveolarization) to that of adult mice. In a second step, we investigated the changes in gene expression due to compensatory lung growth in adult mice 1 and 3 days after resection of the left lobe (compared to sham-operated mice). Statistical analysis led to several genes

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with highly significant regulation; some of these were validated by real-time PCR.

Results: We identified several genes which were already found to participate in development and metabolism as well as signal transduction. In addition, there were some mediators with unknown functions or which have not been ascribed to lung growth yet. Although having found high degrees of overlap when comparing each 1 day group with the respective 3 day experiment, we found only very few genes being involved in both processes of lung growth. Those candidates sometimes even showed different directions of regulation.

Conclusions: An identification of the molecular mediators of alveolarization would be of great importance for the development of new therapies of chronic lung diseases. Our study as well as ongoing further investigations on the protein level may reveal some of these genes. Postnatal and compensatory adult lung growth mostly seem to be controlled by different genes and expression cascades.

E3004

Significance of certain predisposing genetic risk factors for venous thromboembolism

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Objective: To investigate the role of prothrombotic risk factors in patients with venous thromboembolism (VTE) and pulmonary thromboembolism (PTE).

Methods: The study group consisted of 51 patients with VTE and PTE and control group of 50 healthy volunteers. Factor V Leiden, prothrombin G20210A mutation, methylen tetrahydrofolate reductase (MTHFR) C677T genotype, total plasma homocysteine (pHcy), protein C, antithrombin III (AT III), anticardiolipin and anti-β₂-glycoprotein I antibodies were performed. Mutations were identified by polymerase chain reaction (PCR), followed by allele-specific endonuclease digestion.

Results: Twelve of the patients had PTE without deep vein thrombosis (DVT) at the time of the event. Nineteen of the patients with PTE were with DVT, 14 with PTE and systemic lupus erythematosus, 6 with PTE and chronic obstructive pulmonary disease. The antibodies to cardiolipin and β₂-glycoprotein I were elevated in respectively 41,46% and 29,41% of the patients with PTE. Heterozygous mutation of the prothrombin gene was found in 6/44 (13,64%). This frequency was significantly higher than that in a group of healthy controls subjects. The factor V Leiden prevalence was 6,52%. Genotype C677TT was found in 7 patients, all with elevated pHcy. AT III deficiency was found in 14,81%. Protein C deficiency in 22,22%.

Conclusions: The number of subjects who bore factor II G20210A or factor V Leiden, or who were homozygous for MTHFR C677T were significantly higher among patients with VTE/PTE than among controls. These data indicated that the 3 prothrombotic polymorphisms acted as risk factors for VTE/PTE. These results support the hypothesis that pathogenesis of VTE/PTE is multifactorial.

E3005

The genetic risk factors for pulmonary embolism development in various age groups of Russian patients

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At now it isn't clear what the thrombophilic mutations are the strong independent factors for high risk of pulmonary embolism (PE) development especially in the young age. The aim of this study was to investigate Leiden mutation of the factor V gene (FVL), G20210A of the prothrombin gene (F2), G-455A of the beta-fibrinogen gene (FGB), C677T of the MTHFR gene and the PIA1/A2 polymorphism in the GPIIIa gene in patients with PE. These gene polymorphisms were examined in 104 patients from 16 to 77 years (mean age 44±2) and in 321 healthy controls (mean age 41±1) with PCR-RFLP technique. Among these patients 54% individuals were before 45 years and 46% - above 45 years. We found accumulation of the FVL, A20210F2 and PIA2 GPIIIa in PE patients compared with controls - 7% and 3% for FVL (p=0.07), 7% and 2% for A20210F2 (p=0.03), 32% and 24% for PIA2 GPIIIa (p=0.09), respectively. Except this the significant increase in the A20210F2 and AA genotype of the FGB gene was detected in the PE patients before 45 years compared with subjects above 45 years - 12% and 0% for both A20210 F2 and AA FGB in these group, respectively (p=0.02). In contrast the prevalence of the TT MTHFR genotypes and PIA2 GPIIIa was higher in PE patients older 45 years compared with young patients - 14% and 0% for TT MTHFR (p=0.008), 43% and 20% for PIA2 GPIIIa (p=0.02) in studied groups respectively. Conclusion, investigating polymorphisms may increase risk of PE development in various age groups.

E3006

Gene expression profile of human airway epithelium induced by hyperoxia *in vivo*

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Human airway epithelial cells (HAEC) are susceptible to hyperoxia due to the inability to upregulate intracellular antioxidant enzymes. We hypothesized that airway epithelium must have alternative protective mechanisms against oxidative stress in the short-term oxygen exposure.

Methods: we conducted a gene expression profiling study on HAEC in response to 100% oxygen breathing for 12 hours in healthy subjects *in vivo*. To confirm some of the main pathways involved in this response, we conducted an *in vitro* study on BET-1A cells.

Results: the functional analysis based on the gene ontology classification suggests a carefully integrated cellular response to manage and degrade the damaged proteins. This includes changes associated with glutathione/thiol metabolism and cell cycle regulation, increase in the cytoprotective HSP70 molecular chaperone, and the involvement of the proteasome, ubiquitin-conjugation pathways. Because these processes require energy, upregulation of genes involved in ATP synthesis seems pivotal for cell survival. We confirmed the functional genomic analysis using BET-1A cells *in vitro* with the increase of HSP70 at the RNA level, the increase of protein ubiquitination as well as the increase of ATP release. By contrast, the use of proteasome inhibitor ALLN which magnified the level of ubiquitinated proteins after hyperoxia dramatically decrease the ATP release leading to apoptosis as shown by the increase in cleaved caspase 8. In the absence of ability to maintain energy production which probably occurs under conditions of sustained oxygen exposure, the homeostatic integrity of HAEC can no longer be guaranteed leading to cell death.

E3007

Cellular and molecular mechanisms of lung endocrine system responsibility to increasing hypoxia

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Aim: To study basal mechanism of pulmonary endocrine system responsibility to increasing hypoxia during development of papain emphysema

Material and methods: The model of papain emphysema was performed on 50 rats and 40 rabbits. The intact control included 10 rats and 10 rabbits. The euthanasia of experimental animals of studied group was carried out at the same time, that is, at 10 o'clock in the morning in 1,2 weeks after the first inhalation, and in 1,2,3 and 4 weeks after the second permissible dose of papain. Paraffin slices were stained with hematoxiline and eosine etc. For identification of endocrine cells (NEC) and bodies there was used double impregnation with argentums nitrate by Grimelius.

Results: The basal mechanism of the pulmonary endocrine system sensitivity at the onset of developing hypoxia is the wavy changes in the functional activity of NEC and NEB presenting change of the phases of increase extrusion of regulator peptides into surrounding tissue on the basis of marked intracellular synthesis and deposit of these products.

The further increase in the degree of hypoxia intensity involves additional mechanisms of adaptation and functional tension of NEC and NEB of the respiratory tract manifesting in hyperplasia and hyperfunction of NEC and NEB with increase in number and sizes of secretory granules with formation of giant hypertrophic granules with sizes to 360 nm.

Chronic hypoxia during development of emphysema is characterized by change of the phase of adaptation and functional tension to the exhaustion of neuroendocrine structures of the respiratory tract that leads to neuroendocrine insufficiency.

E3008

Association of the TNF gene promoter polymorphism with serum tumor necrosis factor-alpha levels in chronic obstructive pulmonary disease patients

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In spite of the fact, that previous studies were not able to reveal association of -308G/A polymorphism of the TNF gene in Russians patients, we supposed that TNF gene variant could be connected with important clinical features of COPD.

Aim: To study association of -308G/A polymorphism in the TNF gene with the level of tumor necrosis factor-alpha in the sputum and serum of COPD patients.

Methods: 261 Russian patients were studied: 72 COPD patients (mean age 57±12) and 189 control subjects (mean age 50±17). Quantification of TNF-alpha in the serum and sputum in COPD exacerbation and clinical remission was made by immune-assay method. DNA was extracted from white blood cells by standard

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method. Genotype by -308G/A polymorphism was determined by PCR followed by restriction digestion.

Results: Association of GG genotype of -308G/A polymorphism of TNF gene with the elevated TNF-alpha serum levels in COPD patients was detected both during exacerbation and remission periods of COPD ($p < 0.05$).

Conclusions: Elevated level of serum TNF-alpha indicates on existence of systemic inflammatory reaction. However this could not unambiguously confirm functional importance of studied polymorphism in relation to COPD as the sputum level of TNF-alpha was within the normal range.

E3009

Ultrastructural mechanisms of alveolar regeneration induction with neuropeptide EP in papain emphysema

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Purpose: To study cellular mechanisms of alveolar regeneration induction with neuropeptide EP in papain emphysema.

Material and methods: There was done papain model of emphysema in 40 not pedigree rats and 30 rabbits. EP was administered intraperitoneally into 35 rats and 25 rabbits in dose 0,2 ml (0.3 mg/kg) 4 times during a month (one injection a week) after development of emphysema. Ultra-thin lung slices were studied in comparison between groups during experiment.

Results: In experimental groups of animals treated with EP to the end of experiment there was found restoration of structural architectonic of pulmonary parenchyma. Ultrastructural analysis of the cellular composition of forming new alveoli showed presence of 2 types of cells in the content of interstice of interalveolar capillaries, the cells with lipid inclusions, distributed in the bottom of the septa and cells without lipid inclusions rich with cisterns of granular cytoplasmic net. There were noted closed intercellular contacts between alveolocytes type II (All) and lipofibroblasts (LF), as well as frequent mitoses All and endotheliocytes (EN). In new formed alveoli the intensive synthesis and secretion of surfactant All in cells was noted at the last period of experiment, as well as reduction of lipid amount in All and the forming delicate elastic framework has been revealed.

Conclusion: Neuropeptide EP induced alveolar regeneration in papain emphysema. In this case during the last period of experiment morphofunctional state of cellular structures of the novel forming alveoli appeared to be rather of full value and less different from cellular structure of lung parenchyma in intact animals.

E3010

Dependence of stage of severity of chronic obstructive pulmonary disease on metalloproteinase 1, 12, surfactant protein C, microsomal epoxid hydrolase gene polymorphisms

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Study objectives: To study dependence of stage of severity of chronic obstructive pulmonary disease (COPD) on metalloproteinase 1, 12 (MMP1,12), surfactant protein C (SFPC), microsomal epoxid hydrolase (mEH) gene polymorphisms.

Materials and methods: 60 patients with COPD were examined. Patients were divided into two groups according to severity (the first group of 27 persons - stage 1 and 2, second group of 33 persons - stage- 3 and 4). The groups were comparable in age and smoking history (pack/years). Polymorphisms of MMP1 (G-1607GG), MMP12 (A-82G and Asn357Ser), SFPC (Asn138 and Asn186), mEH (Tyr113His and His139Arg) were included in our investigation. DNA was extracted from nuclei of leucocytes of venous blood. Polymorphisms were detected by polymerase chain reaction and followed by mass-spectrometry.

Results: According to preliminary data no significant difference in distribution of allele variants among two groups of patients was detected. However, genotype CC of SFPC (Asn138) in the second group is 2 times more frequent than in the first one, and genotype GG of SFPC (Asn186) in the second group is 2.3 times more frequent than in the first one. These results need further investigation.

Conclusion: No strict dependence of the COPD severity on MMP1,12, SFPC, mEH gene polymorphisms was detected.

E3011

Typing of pseudomonas aeruginosa with random amplified polymorphic DNA; a study of molecular typing and antibiotic resistance of hospitalized patients in NRITLD

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Background: From an epidemiological point of view, it is often necessary to determine the clonality of the bacterial isolates. This is particularly important in endemic and epidemic nosocomial outbreaks to improve the management of such outbreaks.

Aim: Random amplified polymorphic DNA typing (RAPD) was used to study the genetic diversity of pseudomonas aeruginosa strains isolated from microbiology lab in NRITLD.

Material and methods: Seventy three P.aeruginosa isolates were analyzed. These strains were isolated from patient admitted to ICU (31), other wards (40), and also two specimen from ventilator and soap in ICU. All strains were identified with biochemical testing and antimicrobial susceptibility testin were carried out according to NCCLS. RAPD analysis with two sets of primers were done and electrophoretic band patterns were analysed both with Gell compare 2 and visually inspection.

Result: Phylogenetic analysis of the RAPD pattern showed rates of genetic similarity range from 40-60%. Four epidemiologically and genetically related clones each containing 2-3 isolates were identified. Most of them were from ICU. We detected high antimicrobial resistance pattern to chloramphenicol, ceftazidime, cefepim, ceftazidim (73-99%) and relatively low resistance to tazocin, imipenem and amikacin (36-44%).

Conclusion: RAPD is rapid, labour friendly method for epidemiologic study of pseudomonas aeruginosa.

A high rate of antibiotic resistance was found for pseudomonas aeruginosa mostly in ICU.

E3012

Restoration of HDAC-2 activity by curcumin & theophylline in cells exposed to oxidative stress through alteration of covalent modifications

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HDAC-2 is vital for regulating steroid function and inflammation. COPD patients, severe and smoking asthmatics all display steroid insensitivity, as a result of exposure to oxidative stress. Reactive oxygen species (ROS) have a direct inhibitory effect on HDAC-2 activity through post-translational modification. We hypothesised that HDAC activity may be restored by altering its covalent modification status. U937 cells were exposed to H₂O₂ or cigarette smoke, then incubated with 1µM curcumin or theophylline. HDAC activity was assessed by HDAC activity assay. Immunoprecipitated HDAC-2 was used to assess phosphorylation, nitration and acrolein modification status. Steroid responsiveness was assessed by IL-8 ELISA. ROS treatment reduced total HDAC activity, which was restored by theophylline or curcumin in a dose and time dependant manner. Specifically, HDAC2 and not HDAC-1 activity was decreased after the ROS stress but again HDAC-2 and activity was restored by theophylline and curcumin, correlating with a restoration in steroid responsiveness by theophylline and curcumin in both a time and dose dependent manner (EC₅₀ = 200pM and 100nM respectively). Theophylline and curcumin altered serine HDAC-2 phosphorylation, correlating to activity in ROS stressed cells. Furthermore, both theophylline and curcumin reduce tyrosine nitration (40% & 47%) and curcumin also reduced acrolein modification of HDAC-2, (55%), correlating with increased HDAC-2 activity. In conclusion, our data suggests that restoring/upregulating HDAC activity and thereby steroid function after prior reduction by oxidative stress can be achieved through pharmacological intervention.

E3013

The pharmacogenetic aspects of corticosteroid metabolism in patients with severe asthma

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Bronchial asthma (BA) belongs to the group of multifactorial diseases with different functionally related genes being involved in its pathogenesis. The aim of the study was to investigate the association of polymorphisms of multidrug resistance gene MDR1 (C3435 T), glucocorticoid receptor gene NR3C1 (Ile 559 Asn), gene IL 13 (Arg 130 Gln). Genotypes were investigated by PCR and analysis of the length of restriction fragments. 22 BA patients were included; 17 of them had severe and 5 - moderate course of the disease. The frequency of MDR1 C/T was 50% (n= 11), T/T -18,2% (n= 4), C/C- 31,8% (n= 7). The FEV₁ (74,07% ± 6,42%) were found in patients with C/T gene significantly more higher if compared to these with C/C (52,62% ± 3,54%), $p < 0.05$. The osteoporosis and Cushing syndrome were significantly revealed more frequently (rate 1,82 ± 0,12) in patients with C/T genotype, than in T/T homozygotes (1,25 ± 0,25), $p < 0.05$. The daily dose of per os glucocorticosteroids (GC) was higher in patients with C/T genotype (19,40mg ± 5,75 mg), if compared to C/C genotype (15mg ± 5 mg). Patients with heterozygote (C/T) 81,81% (n= 9) had severe course of the disease steroid-dependent asthma. There were not revealed any mutations in gene NR3C1 (Ile 559 Asn) and in gene IL 13 (Arg 130 Gln) in all the patients under the investigation. Thus, we identified significant association of MDR1 (C3435 T) gene polymorphism with severe steroid dependent asthma. That may be related with possible role of genetic control mechanism in corticosteroids transport from the cell. This mechanism can possibly determine different cell sensitivity to GC, (GC) therapeutic dose, and different rate risk of clinical side effects.

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E3014

Association between polymorphism of tumour necrosis factor α -308 gene promoter and asthma: a meta-analysis

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Background: Tumour necrosis factor alpha (TNF α) gene is one of several candidates for asthma and polymorphic. A number of studies have investigated the polymorphism of TNF α -308 in relation to asthma susceptibility in different populations. However, the results have been inconsistent.

Methods: We have addressed the inconsistency in studies of the association of the polymorphism of this gene with asthma by systematically reviewing the published data and performing a meta-analysis. We searched the MEDLINE database for case-control studies from 1966 to October 2005. Pooled odds ratios with 95% CI by random-effects model were calculated.

Results: Fifteen eligible studies, comprising 2409 patients with asthma and 3266 controls, were included. Using the random-effects model, the pooled result demonstrated that the TNF2 allele is associated with susceptibility to asthma (OR = 1.37, 95% CI = 1.02 to 1.84, P=0.04) (figure 1). The ORs for asthma susceptibility in TNF2 homozygote individuals were significantly increased at 2.01 (95% CI = 1.26 to 3.20, P=0.009) and 1.51 (95% CI = 1.02 to 2.22, P=0.041) when compared with the TNF1 homozygote and TNF2/1 heterozygote, respectively. The pooled OR for asthma risk in TNF2/1 heterozygote was also significantly higher relative to that in TNF1/1 homozygote (OR = 1.47, 95% CI = 1.01 to 2.13, P=0.045).

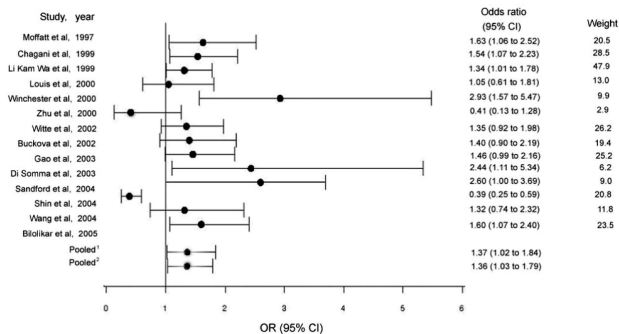


Fig. 1

Conclusion: The TNF2 allele confers a significant risk for developing asthma.

E3015

Gene polymorphisms of endothelial nitric oxide synthase in patients with mild and moderate bronchial asthma

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Bronchial asthma (BA) is a pulmonary disease characterized by chronic inflammation of the airways and bronchial hyperresponsiveness. Previous studies suggest that asthma is a multifactorial disease influenced by genetic and environmental factors. However, the nature of the genetic factors remains unknown. Nitric oxide (NO) plays a key role as a vasodilator, neurotransmitter, and inflammatory mediator in the airways, and it is produced in increased amounts in asthma. Nitric oxide, including that produced by endothelial constitutive nitric oxide synthase (eNOS), may regulate vascular and airway tone in the lungs and may influence various aspects of airway homeostasis. We collected and performed PCR genetic polymorphism of the eNOS gene in 47 patients with BA (males -11, females -36) and 69 healthy individuals (residence of St-Petersburg). We examined the relationship between 4 Δ /4b polymorphism eNOS gene and severity of asthma of 47 patients in this group. There was a significant difference in the frequencies of 4a alleles and the 4a/4a genotypes of this polymorphism between patients with moderate persistent BA and mild persistent BA. These data provide the evidence that the eNOS gene are associated with heavier current of asthma. Genetic, as well as environmental factors, may contribute not only to the manifestation of the disease, but also to its severity. Polymorphisms of the endothelial nitric oxide (eNOS) genes have been implicated in the pathogenesis of asthma.

E3016

Relationship between N-acetyl transferase-2 gene polymorphism and risk of bronchial asthma

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There are still uncertainties as to the mechanism of many pathological conditions, among them allergic diseases. It has been suggested that acetylation rate may be a factor that influences the development of allergic diseases. The aim of the present study was to investigate further whether the genetic polymorphism of the NAT2 plays a role in susceptibility to bronchial asthma disease.

97 patients with bronchial asthma and 104 healthy individuals were participated in this study. DNA was extracted from the leucocyte by high pure template preparation kit. NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A polymorphisms of NAT2 were detected by using LightCycler-NAT2 mutation detection kit by real time PCR with LightCycler instrument. We found that mutant NAT2*5A (OR: 3.84, 95% CI: 1.08-13.6) and NAT2*6A (OR=5.27, 95% CI: 1.06-26.05) genotype could be associated with a high risk for the development of bronchial asthma according to the genotype. After grouping phenotype, the risk for bronchial asthma was more than two times higher (OR=2.7, 95% CI: 1.07-6.97) in individuals with the slow NAT2*5A acetylator phenotype compared to the fast phenotype. Our study suggests that the NAT2 slow acetylators may be a determinant in susceptibility to asthma disease. This finding may have implications for the theories for the pathogenesis of the disease as well as for therapeutic aspects.

E3017

Molecular-genetic study of asthma in Volgo-Ural region of Russia

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Asthma is a common chronic respiratory disease, the development of which is determined by the interaction between genetic and environmental factors. To date, numerous DNA-loci and candidate genes have been reported to show linkage and association to asthma. Cytokines play an integral role in the coordination and persistence of the allergic inflammation of the airways in asthma. In the present study, we analyzed associations between polymorphic variants of cytokine and its receptor genes (IL4, IL4R α , IL9, IL10, TNF α) and asthma. The asthma group consisted of 156 patients, the control group included 203 unrelated nonasthmatic subjects from Volgo-Ural region of Russia. The genotyping of SNP polymorphisms was performed by PCR followed restriction analysis. We have detected significantly differences of allele and genotype frequencies of -590C>T polymorphism of the IL4 gene between asthma patients and healthy donors of Tatars. The frequencies of T allele and TT genotype were higher in asthma patients (30,3% and 9,09%) compared with control groups (17,21% and 1,64%). The distribution of genotype frequencies of I50V polymorphism of the IL4R α gene differed between patients and healthy donors of Russians. Increased frequency of II genotype was found in asthma patients (40% vs 24,14%). The analysis of Q576R polymorphism of the IL4R α gene, T113M polymorphism of the IL9 gene, -627C>A polymorphism of the IL10 gene and -308G>A polymorphism of the TNF α gene detected that there are no significant differences between patients and healthy donors. The data of this study revealed association of polymorphisms of the IL4 (-590C>T) and IL4R α (I50V) genes with asthma in Volgo-Ural region of Russia.

E3018

Intermittent hypoxia aggravates the sensitivity to the infarction in the rat heart: protection by erythropoietin

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Background: An acute intermittent hypoxia (IH) for 4 h with a minimal FiO₂ of 5% induces a delayed higher cardiorespiratory sensitivity to the infarction in the rat. Recent studies suggest that recombinant human erythropoietin (rhEPO) can be considered as a pharmacological pre- and post-conditioning agent since administration of rhEPO before and after cardiac ischemia is known to induce protection against ischemic injury improving functional recovery and reducing apoptosis and necrosis. Thus we investigate whether pre- and post-conditioning by rhEPO could protect against higher sensibility to the infarction induced by previous IH in the isolated rat heart.

Methods: Conscious adult male rats were exposed to 1-min cycles of hypoxia (FiO₂ 5%) / normoxia (FiO₂ 21%), during 4 h. The hearts were isolated, 24 h later, and subjected to a 30-min no-flow global ischemia followed by 120 min of reperfusion. For some hearts, rhEPO (5 IU/ml) was infused for either 10 min

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before ischemia or 15 min at the reperfusion. Infarct-to-ventricle (I/V) ratio was then assessed using a colorimetric technique with planimetric analysis.

Results: The delayed higher sensitivity to the infarction induced by 4 h of acute IH with a minimal FiO₂ of 5% (I/V: 38±1 vs 27±4% in normoxic controls) was prevented by rhEPO infusions either before ischemia (I/V: 27±4 vs 28±2% in normoxic controls) or at reperfusion (I/V: 23±3 vs 25±2% in normoxic controls). Infarct size data were compared using one-way analysis of variance (ANOVA) with a statistical significance set at $p < 0.05$.

Conclusion: Pre- and post-conditioning by rhEPO is able to prevent delayed higher cardioresensitivity to the infarction induced by previous IH.

E3019

Genetic variations and lung function

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Pulmonary functions like lung volumes, compliance, diffusing capacity have been found to vary among common laboratory inbred mouse strains (C3H/HeJ, BALB/cByJ, C57BL/6J, A/J, JF1/Msf, PWD/PhJ). C3H and BALB strains versus the JF1 and PWD strains represent the two phenotypic extremes in this case. Reinhard *et al.* (2005) have identified the genetic loci with linkage to these lung function parameters. The genes *Rxrb*, *Pdgfb*, *Rln1*, *Sod3* and *Tgfb3* are located within the chromosomal regions with highest linkage scores and thus considered to be the major candidates. In order to detect the genetic basis for the variation in lung functions among the above mentioned strains of mice, the candidate genes were analysed for strain specific variations. Missense polymorphisms have been detected in the genes coding for relaxin1 (*Rln1*; Leu-98-Pro, Leu-109-Phe, Val-173-Ile); superoxide dismutase 3 (*Sod3*; Asn-21-Asp, Glu-57-Gln, Pro-152-Leu, and Ala-186-Ser); platelet derived growth factor beta (*Pdgfb*; Ile-183-Val) and transforming growth factor beta receptor type III (*Tgfb3*; His-96-Tyr, Ser-391-Pro, Met-765-Pro, Pro-772-Ala) in JF1 and PWD mice. However no amino acid polymorphism was detected in case of retinoic acid receptor beta (*Rxrb*). The identified polymorphisms results in altered secondary structure and hydrophobicity of the respective proteins that could interfere with their essential functioning, presumably during lung development, thereby playing significant role in modulating the lung function. Supported by NIH (HL70542) and NGFN (IGR0430).

E3020

Progression towards the development of the universal highly discriminative panel of VNTR loci for *Mycobacterium tuberculosis* genotyping

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Background: The identification of variable number tandem repeats (VNTRs) in *M.tuberculosis* has offered the possibility of high throughput genotyping with discrimination similar to RFLP-IS6110 typing. Development of a panel of highly discriminative loci suitable for differentiation of *M.tuberculosis* strains circulating in various geographical areas is of vital importance for TB epidemiology and control.

Methods: 232 *M.tuberculosis* strains isolated in London in 1998 were fingerprinted using IS6110 RFLP, spoligotyping, and VNTR typing using following panels: MIRU (loci 2,4,10,16,20,23,24,26,27,31,39,40), ETR (A,B,C), VNTR 0424, 0531, 1955, 1982, 2074, 2163a, 2163b, 2347, 2401, 3171, 3232, 3239, 3336, 3690, 4052, 4156.

Results: Combination of IS6110 RFLP with spoligotyping yielded 11 clusters comprising of 25 strains and 207 individual genotypes. MIRU/ETR panel of 15 loci failed to achieve comparable discrimination yielding 26 clusters consisting of 80 strains. Expanding of the panel of VNTR loci to 31 loci allowed to improve the discrimination dramatically, giving the level of discrimination virtually identical to that provided by RFLP/spoligotyping (11 clusters comprising of 27 isolates). The calculation of the HGDI followed by the reduction analysis allowed to identify the minimum panel of 16 loci (MIRU10,26,27,31, ETR-A, 0424, 1955, 1982, 2074, 2163a, 2163b, 2347, 3232, 3336, 3690, 4052) providing the same discrimination as 31 loci altogether. The loci identified may be considered as prospective universal panel for *M.tuberculosis* genotyping in various geographical settings including high TB burden areas with dominance of highly conserved genotypes.

E3021

Microcirculation impairment in weaning failure from mechanical ventilation

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Objective: Disconnection from mechanical ventilation (MV) may induce an imbalance between O₂ demand and supply. We tested the hypothesis that changes in tissue O₂ saturation (StO₂) and in O₂ consumption rate in pts who fail to wean from MV, can be detected with near-infrared spectroscopy (NIRS).

Methods: We studied 35 consecutive pts (aged 65±15, SOFA 5±2) during a 2-hr T-piece weaning trial. The trial was judged successful if the pt was able to sustain spontaneous breathing without distress. Respiratory rate (RR), SaO₂ and minute ventilation (V_E) were measured before disconnection from MV and at 2 hrs in pts with successful trial, or at reconnection in those with a failed one. Thener muscle StO₂ and O₂ consumption rate were measured by NIRS (InSpectra, Hutchinson) at the same time points by the arterial occlusion method.

Results: 19 pts had successful and 16 a failed trial. SaO₂, RR and V_E on MV did not differ between 2 groups. During weaning, SaO₂ decreased in failure group from 97.8 to 88.5% ($p < 0.001$), and the frequency/tidal volume (fr/tv) index increased from 30 to 188 br/L ($p < 0.001$). In success group, SaO₂ did not change, while fr/tv increased from 32 to 80 br/L ($p = 0.001$). StO₂ on MV did not differ between failure and success groups (73% vs 79%). In failure group, StO₂ decreased to 67% ($p = 0.013$) at the end, while it did not change in success group. O₂ consumption rate on MV was not different between the 2 groups. In failure group, O₂ consumption rate was significantly lower at the end of the trial compared to the beginning (17.1±7.5 vs 15.5±4.8, $p = 0.019$).

Conclusion: NIRS can detect a decrease in StO₂ and in O₂ consumption rate in pts who fail to wean from MV, indicating microcirculation impairment.

E3022

Investigation of the main complex histocompatibility antigens in patients with pulmonary tuberculosis

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The aim of this study was to define presence of associative links between of the main complex histocompatibility antigens (HLA system) and development of acute progressive forms of pulmonary tuberculosis with multi drug resistance *M.tuberculosis*.

Material and methods: In this study was included 64 patients (30m, 34w, main age 35±5.3) with pulmonary tuberculosis and 200 healthy volunteers. In all persons have been performed investigations of antigens of HLA system with the aid of identifying HLA antigens A, B, Cw loci microlymphocytotoxic test (Terasaki P.). It was defined a degree of relative risk (RR), frequency of antigens, genes, haplotypes and phenotypes.

Results: In patients with pulmonary tuberculosis as against healthy persons occurred significantly often ($p < 0.01$ or $p < 0.05$) in HLA phenotypes Cw2 (RR-3.92), 8 (RR-30.85), 9 (RR-7.07) antigens, haplotyp A0B8 (RR-54.19). In patients with acute progressive forms of pulmonary tuberculosis with multi drug resistance *M.tuberculosis* occurred also significantly often ($p < 0.01$ or $p < 0.05$) in HLA phenotypes A24 (RR-2.83), B7 (RR-26.73), 61 (RR-10.32) antigens, haplotyp A0B13 (RR-4.14). In healthy persons as against patients with pulmonary tuberculosis occurred significantly often ($p < 0.01$ or $p < 0.05$) in HLA phenotypes B63 (RR-10.47), Cw3 (RR-30.85) antigens.

Conclusions: Revealing of antigens A24, B7, 61 in phenotype of HLA system, haplotyp A0B13 allows to form groups of risk of development of acute progressive forms of pulmonary tuberculosis with multi drug resistance *M.tuberculosis*, whereas presence of B63, Cw3 antigens may be linked to resistance to pulmonary tuberculosis.

E3023

Analysis of melting temperatures in defining resistance to isoniazid and rifampicin by PCR based techniques

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Objective: to develop a strategy for defining resistance using the deviation of melting temperatures (ΔT_m) in the detection of resistance associated mutations (RAM) by LightCycler PCR.

Methods: 41 susceptible and 29 resistant clinical isolates were used (with known genotypic resistance). The molecular evaluation was performed by adapting a protocol previously described (Torres *et al.* 2002); sets of primers and probes that targeted the rpoB gene (2 sets) for RMP resistance and katG and inhA for INH resistance were used.

Results: Since we found no matching with the melting temperatures published by Torres, we developed an alternative strategy for estimating mutations in the targeted sequence by measuring each time the ΔT_m from the T_m of H37Rv reference MT strain. ROC curves were used to determine the cut-off of ΔT_m predictable for mutations associated with resistance, which were established in

Table I. ΔT_m for INH resistance

inhA / Kat G	0.175 / 0.61	0.21 / 0.76	0.245 / 0.96	1.3 / 1.45	2.6 / 2.9
Se %	100	96	96	89	89
Sp %	76	88	95	95	97.6

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Table II. ΔT_m for RMP resistance

rpo1 / rpo2	0.50 / 0.70	0.75 / 0.80	0.91 / 0.95	1.12 / 1.12	1.35 / 1.23
Se %	85.7	85.7	85.7	67.8	57
Sp %	42.8	66.6	73.8	80.9	92.8

order to obtain best values for sensitivity (Se) and specificity (Sp) of the tests (tables I, II). The results were better for IHH (Se 96%, Sp 95%) than for RMP (Se 85.7%, Sp 73.8%).

Conclusion: The best accuracy for genotyping compared to phenotyping resistance was for INH resistance using katG. An alternative strategy for estimating mutations in the targeted sequence is by measuring each time the ΔT_m from the H37Rv reference strains' T_m .

E3024

Towards an understanding of pulmonary arterial hypertension (PAH): gene regulation patterns in pulmonary arterial resistance vessels of patients with PAH

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The pathogenesis of PAH is still largely unknown and thus a causal therapy is not yet available. To identify important PAH perpetuating genes, optimal conserved human lung tissue from PAH patients was used to analyze differential gene expression compared with healthy lung tissue.

Methods: Lung tissue was shock-frozen directly after explantation. Sections of pulmonary arterial resistance vessels were isolated by laser microdissection (6 individuals per group). The extracted RNA was reverse transcribed. cDNAs of each 3 individuals were pooled, amplified and Cy-labeled by SMART. Labeled products were hybridized on 44k 60mer oligo-spotted microarrays (Agilent).

Results: From 1251 genes with a statistically significant expression ($p < 0.001$), 41% (59%) were down (up) regulated in PAH. 27% of the genes were not annotated. The major groups of GO processes among the annotated genes were physiological (29%) and cellular (19%). The major groups of GO molecular function were binding (24%) and catalytic (11%). The largest subgroup consists of transcription factors and DNA binding proteins. Regulated genes were statistically significant over represented in the KEGG wnt and integrin-mediated cell adhesion pathways.

Conclusions: The results suggest that factors of the wnt and integrin-mediated cell adhesion pathways play a central role in the pathogenesis of chronic pulmonary hypertensive vasculopathy in PAH.