72. Genetics of airway diseases and treatment

P432

LSC 2011 Abstract: Association of IL-9 and IL-4R genes and their phenotypes among Sudanese with asthma

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Background: Asthma is a "complex" heritable disorder, candidate genes that may be involved in the pathogenesis of asthma including: interleukin 4 (IL-4), IL-5, IL-9, and IL-13 (Postma et al. 1995). Asthma prevalence *in Sudan* was found to be 12.5% in children aged 13-14 year in the Capital Khartoum (Mohamed et al. 1999).
Objectives: To detect polymorphisms of IL-9 in chromosome 5 and iIL-4R in chromosome16 contributing to asthma and to estimate the environmental components: Total immunoglobulin E levels, skin prick test, and eosinophil count in Sudanese population.
Methods: Seventy, nuclear and extended families were sampled in the initial phase of the study. Ventilatory function, skin, prick test blood sample for DNA analysis, immunoglobulin E, eosinophil count were carried out in the whole sample. Genotyping for IL-9, IL-4R and IL-13, polymorphisms using PCR were also carried out for a subset of the sample.
Results: Phenotypic analyses of the pedigrees suggest a likely genetic cause for asthma, as shown in one extended family. Level of total immunoglobulin E was found to be 71% in asthmatic, and 24% in non asthmatic. Eosinophil count was found to be 58% in asthmatic, while found to be 17.4% in non asthmatic. Hypersensitivity symptoms to six allergens showed positive skin test. (p = 0.00). Genotyping for IL-9, IL-4R suggest the presence of association for bothIL-9 and IL4R (*P*=0.008&0.007).
Conclusions: Asthma runs in families showing strong linkage to genes. There seems to be sufficient phenotypic and genotypic indicators to suggest a genetic predisposition component to asthma among Sudanese, and warrant some furthers investigation.

P433

Polymorphisms in *toll-like receptor 4* are associated with severity but not susceptibility for asthma in a Chinese Han population

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Background: Toll-like receptor 4 (TLR4) links human innate and adaptive immunity via bacterial endotoxin recognition, and has a considerable role in the pathogenesis of asthma. The effects of the genetic variants in *TLR4* on asthma are still largely unknown.
Aims and objectives: This study aimed to evaluate the effects of polymorphisms in *TLR4* on asthma risk and asthma-related phenotypes in a Chinese Han population.
Methods: We consecutively recruited 318 unrelated adult asthmatic patients and 352 healthy volunteers. Four tagging single nucleotide polymorphisms (SNPs) in *TLR4* gene were detected using GenomeLab SNPstream or TaqMans Genotyping. We conducted case-control and case-only association studies between the selected tagging SNPs in *TLR4* and asthma or asthma-related phenotypes.
Results: We found no evidence to support a significant association between *TLR4* SNPs and asthma susceptibility. However, our results revealed that the TT homozygote of rs1927914 was associated with lower FEV_1% in asthmatic patients. An evidently positive association was found between the TT genotype of rs1927914, or the GG genotype of rs10983755 and rs1927907, and asthma severity (*P*=0.024, 0.009, 0.013, respectively), which indicated that the C allele of rs1927914, and the A allele of rs10983755 and rs1927907 had a protective effect on asthma severity.
Conclusion: *TLR4* polymorphisms do not contribute to asthma susceptibility, but may influence the severity of asthma.

P434

Vitamin D binding protein variants associate asthma susceptibility in a Chinese Han population

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Background: Asthma is a genetically heterogeneous disease. Polymorphisms of genes encoding components of the vitamin D pathway have been reported to associate with the risk of asthma. Previously, we demonstrated that the vitamin D status in serum was associated with lung function in Chinese asthma patients. In this study, we tested whether polymorphisms of vitamin D receptors (*VDR*), vitamin D 25-hydroxylase (*CYP2R1*) and vitamin D binding protein (*GC*) were associated with asthma in a Han Chinese Population.
Methods: We sequenced 8 exons of VDR and all 5 exons of CYP2R1 and identified only two mutations on the coding regions in a Han Chinese case-control cohort of asthma. These two polymorphism markers were rs2228570 on exon 4 of *VDR* and rs12794714 on exon 1 of *CYP2R1*. We then genotyped the two markers in the cohort that consisted of 467 asthma patients and 288 unrelated healthy. We also genotyped two common polymorphism marker rs4588 and rs7041 in vitamin D binding protein (group-specific component, GC) gene by a PCR–restriction fragment length polymorphism (RFLP) method. We analyzed the association between these polymorphisms and asthma susceptibility and asthma relevant traits.
Results: Polymorphism markers in *VDR* and *CYP2R1* were not associated with asthma in the Han Chinese cohort. The variants of vitamin D binding proteins were associated with asthma susceptibility. Compared with Gc1, Gc2 was strongly associated with the risk of asthma (OR=1.35, 95% CI= 1.01-1.78 p=0.006).
Conclusions: The results provide supporting evidence for association between GC variants and asthma susceptibility in the Chinese Han population.

P435

Is there a relation between asthma associated gene polymorphisms and recurrent wheezing in preschool children? The ADEM study

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Background: About 20-40% of preschool children experience wheezing. One in three wheezers will have persistent symptoms after the age of six and develop asthma. It is unknown which gene variants contribute to wheezing.
Aim: To explore the relation between asthma associated single nucleotide polymorphisms (SNPs) and preschool recurrent wheezers.
Methods: We selected 202 recurrent wheezers (ISAAC questionnaire $\geq$2 wheezing episodes) and 50 controls aged 2-4 years. Saliva and buccal swaps were used for DNA extraction. Chi square tests were performed on 23 SNPs in 15 genes. Results were expressed in unadjusted odds ratio (OR) with 95% confidence interval. Multiple testing was corrected by the Benjamini and Hochberg False Discovery Rate.
Results: In total 134 boys and 116 girls participated with an average age of 3.3 years. All SNPs had a high call rate (94.0-99.2%). The frequencies of a genetic variant in *ADAM33* (rs511898) differed statistically significantly between cases and controls leading to OR=2.4 (1.2-4.9) for CT genotype and OR=1.0 (0.4-2.5) for TT genotype compared to CC genotype (p=0.03). The frequencies of a genetic variant in *ORMLD3* (rs7216389) differed statistically significant between cases and controls leading to OR=3.7 (1.7-8.2) for CT genotype and OR=2.7 (1.2-6.3) for TT genotype compared to CC genotype ($p<0.01$). Neither remained statistically significant after multiple testing correction (rs511898 p=0.36, rs7216389 p=0.07).
Conclusions: We observed some evidence for the association of the ORMDL3 SNP rs7216389 and recurrent wheeze. The T-allele was more frequent in recurrent wheezers, which is consistent with previous studies in asthma.

P436

Association of ADAM33 gene polymorphisms with asthma in Volga-Ural region of Russia

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ADAM33 is the first reported asthma-susceptible gene identified by positional cloning. ADAM33 is located on chromosome 20p13 and codes for a protein

important for cell fusion, cell adhesion, cell signaling, and proteolysis. Four polymorphic variants 11434C/A (rs44707), 6716 G/C (rs2787095), 7667 G/A (rs2485700), 400 A/G (rs2280091) were genotyped in 530 patients with physician-diagnosed asthma, aged 2-60 years (177 Russians, 110 Tatars, 68 Bashkirs and 175 mixed origins), and 366 nonasthmatic individuals (123 Russians, 91 Tatars, 51 Bashkirs and 101 mixed origins) from Volga-Ural region of Russia. A subgroup analysis of children and adults was conducted separately. Genotypes were determined by the PCR-RFLP method. Data were analyzed using the chi-square test with Haploview software. We found significant association of rs44707A allele (p=0.009, OR=1.56) with asthma susceptibility in Russian patients. Also the frequency of allele rs44707A was increased in adult asthmatics Russian ethnicity compared to controls (p=0.02, OR=1.73). Allele rs2280091G was found to be overrepresented in Bashkirs with asthma (p=0.04, OR=2.51). There were no significant differences in the allele frequencies in SNPs rs2787095, rs2485700 between asthma patients and control group. We constructed the haplotypes of cases and controls. Haplotype analysis showed that the haplotype CACA (rs44707, rs2787095, rs2485700, rs2280091) was more common in the control group than in the case group ($p=1\times10^{-4}$) and offered a protective effect against asthma in Russians. In summary, our results suggest an association between the ADAM33 gene polymorphisms and asthma in Russians and Bashkirs.

P437

Use of partition tree to identify genetic marker combination predicting bronchodilator response by inhaled short-acting beta 2 agonist

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Objective: To identify genetic marker to predict bronchodilator response by inhaled short-acting β2 agonist after methacholine-induced bronchoconstriction.
Methods: A total of 480 patients were enrolled, who showed ≥20% decrease of FEV1 in methacholine bronchial provocation test and subsequently inhaled 2 puffs of salbutamol (200 mcg), then FEV1 was re-measured 10 minutes later. Twenty SNPs in 10 genes related with airway inflammation, structural changes, or smooth muscle function was scored by high throughput technique. They were TNFα (-308G>A), IL13 (-1111C>T,-1510A>C,431G>A), IL4RA (-3223T>C, 223G>A,1727A>G), VEGFR1 (-2021G>A, -59C>T, 3204T>A), VEGFR2 (889G>A, 1416A>T), TGFβR3 (44T>C, 2753G>A), FGFR4 (28A>G, 1162G>A, 538A>G), CSF2 (536C>T), IL3 (80T>C) and ADRβ2 (79C>G). The partition tree was used to investigate SNP combinations. The first partition was done by the most significant SNP discriminating good responders from poor in dominant model of minor frequency allele. Then subsequent partition was performed by the other SNPs until statistical significance disappeared.
Results: The SNP, -2021G>A in VEGFR1, was the most significant and used in the first partition (25.7±19.8% vs. 20.0±13.8% increase, p<0.001). The best responders with AA or AG of -2021G>A and CC of -1111C>T were discovered after second partition (30.3±16.7% increase). The poorest responders with GG of -2021G>A, GG of 2753G>A, and GG of 1162G>A were discovered after third partition (17.2±15.4% increase).
Conclusions: The combination of genetic markers related to the airway inflammation or structural changes can be useful in predicting bronchodilator response by short-acting β2 agonist.

P438

Association between beta2 adrenergic receptor (*ADRβ2*) haplotype pair and severe asthma in an Australian caucasian population

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Background: Studies in mild asthmatics showed adverse outcomes with chronic use of short or long acting beta agonists in individuals with *β2* adrenergic receptor (*ADRβ2)* polymorphisms including *ADRβ2*+46G>A. The extent to which *ADRβ2* polymorphisms contribute to severe asthma is unknown, hence the association of *ADRβ2* polymorphisms with asthma severity was investigated.
Methods: Caucasians with mild (n=201) or severe asthma (n=118, defined by ATS criteria 2000) and non-asthmatics (n=200) were recruited. All subjects (mean age 53.1, 60% atopic) were genotyped for 12 *ADRβ2* polymorphisms (-1023, -709, -654, -468, -367, -47, -20, +46, +79, +252, +491, +523). Haplotype frequency and haplotype pair was determined via PHASE.
Results: Severe, mild and non-asthmatic cohorts were matched by age but distinguishable from each other in terms of symptoms, lung function, medication use and health care utilisation (p <0.001). Genotypic and allelic frequency was similar across cohorts for all polymorphisms. Nine Caucasian haplotypes were identified and not associated with asthma diagnosis or severity per se. In addition, 12 new haplotypes were identified, 2 (frequency ≥ 1%) and were found exclusively in asthmatics. Haplotype pair 2/4 was associated with severe compared with mild (OR 1.84 95%CI 1.13, 2.97, p value=0.01) or non-asthmatics (OR 1.92, 95%CI 1.19, 3.11, p value=0.008).
Conclusions: *ADRβ2* haplotype pair 2/4 is associated with severe asthma. Known haplotypic difference in receptor expression and acute bronchodilator response in mild compared to severe asthmatics suggests a role for *ADRβ2* haplotypes in the development and/or treatment response in severe asthmatics.

P439

Pharmacogenetic control of bronchial obstruction in steroid depend asthma (SDBA) patients

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Multidrug resistance gene (MDR1) encodes P-glycoprotein 170 (Pgp170), which realize efflux of glucocorticosteroids (GCS) from the cell. C3435T of gene MDR1 in SDBA patients is supposed to be associated with dose of GCS, their anti-inflammatory activity, degree of bronchial obstruction.
Methods: Blood samples were taken from 40 SDBA patients and 103 controls. Genotypes were analyzed using PCR-RLFP method.
Results: Homozygous 3435C in SDBA patients were observed more frequently, than in controls: 35% (n=14) vs 8% (n=9) ($\chi2=15.56$, p<0.001; OR=6.6; CI95% 3.2-9.6). Mean daily dose of oral GCS (oGCS) was higher in patients with CC genotype if compared with geterozygous ones: 2.86 mg/day vs 1.86 mg/day (p=0.041).

Lung function in SDBA patients with different C3435T genotypes of MDR1 gene

Characteristics	M ± m			p
	CC (1)	CT (2)	TT (3)	
Vmax50, %	23.38±3.7	37.13±5.4	16.95±4.4	(2-3) 0,048
Vmax50 increase, %	26.05±3.2	33.65±11.9	87.25±23.4	(1-3) 0,041
Vmax75, %	19.01±3.1	26.72±5.3	11.4±0.5	(2-3) 0,037
Vmax75 increase, %	16.87±6.3	16.81±11.3	87.5±13.4	(1-3) 0,007; (2-3) 0,005
Sgaw	0.05±0.01	0.07±0.01	0.03±0.01	(2-3) 0,031
Sgaw increase, %	55.00±17.3	19.16±5.0	106.3±38.3	(2-3) 0,025

In patients with allele C bronchial obstruction was less severe. However, if compared with TT genotype, post-broncholytic increase of velocity characteristics and Sgaw was not so marked.
Conclusion: Patients with allele C require higher oGCS doses, probably, due to enhanced activity of Pgp170 and accelerated GCS efflux. In patients with TT genotype significant obstruction may be due to inadequate oGCS dose, while reversibility of distal obstruction may be due to intact function of bronchial smooth muscles and their sensitivity to GCS.

P440

Effect of *ADRB2* polymorphism on the airway response to cold air in asthmatics

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Background: Previously we've found out that the decline in β_2-adrenoreceptors (β_2-AR) function affects airway response to cold air.
Objective: The aim of our study was to reveal the contribution of Arg16Gly SNP (rs1042713) in the development of cold airway hyperresponsiveness (CAHR) in asthmatics.
Methods: The study included examination of 60 mild to moderate asthmatics of Caucasian race, mostly non-smokers (mean age 36±1.39). All the patients underwent spirometry before and after the challenge with 3-minute isocapnic (5% CO_2) cold air (-20°C) hyperventilation (ICAH). More than 10% drop in FEV_1 was interpreted as a positive result. Intracellular cyclic adenosine monophosphate (cAMP) concentration in lymphocytes was measured before and 30 min after ICAH under in vitro stimulation with 10^{-6}M epinephrine. PCR-RFLP analysis was used for genotyping.
Results: Arg16Arg genotype dominated in the group with CAHR ($\chi^2=7.47$; p=0.02). Mean FEV_1 drop differed between homozygous patients (16.03±2.07 in Arg16 vs. 8.66±1.12 in Gly16, p=0.0045). cAMP concentration didn't depend on genotype before the challenge, but 30 min after there was a significant fall in cells ability to produce cAMP in subjects with Arg16Arg genotype (p=0.03). Moreover, Arg16 homozygotes had lower cAMP level as compared to Gly16 (48 (32.8; 61.6) and 78 (59.9; 81.3) pM/10^6cells, respectively; p=0.0048).

Conclusions: These data suggest a definite proof for contribution of primary β_2-AR dysfunction into the development of CAHR in our population sample. CAHR was strongly associated with Arg16Arg genotype. Blunted cAMP response in Arg16Arg subjects indicates inherited predisposition of their β_2-AR to acute desensitization during the ICAH.

P441

Role of -511C>T and +3953C>T SNPs of *IL1B* gene as risk factors of COPD and bronchial asthma in Bulgarian population

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A common characteristic of COPD and bronchial asthma is the chronic inflammation in the airways. In the current case-control study we investigated -511C>T promoter polymorphism and +3953C>T silent polymorphism of *IL1B* and their haplotypes as candidate risk factors of COPD and Bronchial asthma. We genotyped 163 patients with COPD, 47 with Bronchial asthma and 174 control individuals using Taqman genotyping assay for *IL1B* -511C>T and PCR-RFLP-based method for +3953C>T.

We did no observed significant differences in genotype frequencies of studied SNPs between controls and patients both with COPD and asthma, however the minor T allele of *IL1B* -511C>T was less frequently found in controls (0.305) than the patients with COPD (0.377, p=0.046) and especially with asthma (0.415, p=0.0002). The carriers of *IL1B* -511T allele (TT and TC genotypes) had 1.56-fold higher risk for development of COPD (p=0.045) and 2.25-fold higher risk of Bronchial asthma (p=0.019). The performed estimations of *IL1B* haplotypes showed that T_C haplotype (alleles found to determine enhanced expression of IL-1b), appeared to be associated with higher risk of COPD (OR=1.25, p=0.231) and asthma (OR=1.78, p=0.035) compared to the most common C_C haplotype and with 1.70-fold higher risk of COPD (p=0.018) and 1.37-fold higher risk of asthma (p=0.313) compared to the C_T haplotype, associated with lower *IL1B* expression.

We suggest that the -511C>T promoter SNP and +3953C>T silent polymorphisms in exon 5 of *IL1B* may influence the genetic predisposition of COPD and Bronchial asthma: the carriers of alleles and haplotypes supposed to define higher IL-1b levels are more susceptible for these diseases.

P442

Association of IL-1β (-511 C>T) and IL1RN 86-bp VNTR polymorphism suspectibility with chronic obstrutive pulomary disease between male and female patients in northern Indian population

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Background: Chronic obstructive pulmonary disease (COPD) is characterized by chronic obstruction of expiratory flow affecting peripheral airways, associated with chronic bronchitis (mucus hypersecretion) and emphysema (destruction of airway parenchyma), together with fibrosis, tissue damage and inflammation of the small airways. Interleukin 1 (IL-1) and other cytokines play a central role in chronic inflammatory diseases. our hypothesis is IL-1β (-511C>T) and IL-1RN 86-bp VNTR polymorphism may be associated with COPD susceptibility.
Methodology: A case–control study was performed in 412 subjects (204 COPD patients and 208 healthy controls) to analyze the association of polymorphisms IL-1β (-511C>T) and IL-1RN 86-bp VNTR of genes in susceptibility to COPD in northern Indian population. All subjects were genotyped through polymerase chain reaction and Restriction fragment length polymorphism.
Results: The frequencies of IL-1β (-511C>T) and IL-1RN (variable number tandem repeat) genotypes, alleles, and haplotypes did not differ significantly between patients and controls. However, In IL1 RN allele 1/2 and 2/2 were significant associated with the female COPD patients, with odds ratio [OR] = 2.60; 95% confidence interval [CI] = 1.24-5.44; p=0.011; OR=5.60; 95% CI = 0.95-32.96; p=0.057).
Conclusion: Both the polymorphism IL-1β (-511C>T) and IL1RN showed sex-specific association with COPD in north India.

P443

Matrix metalloproteinase (*MMP*) -1 and -3 promoter polymorphisms in Bulgarian patients with COPD

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COPD is a chronic disease of the lung that is associated with abnormal chronic inflammation in the airways and development of extensive tissue remodeling. MMPs are proteolytic enzymes that play an essential role in tissue remodeling. Polymorphisms in *MMP* gene promoters have been found to alter transcriptional activity. Particularly, 2G allele of -1607insG polymorphism of *MMP1* has been associated with augment transcription of MMP-1, whereas 6A allele of -1171insA polymorphism of *MMP3* – with reduced gene expression.

The aim of our work was to investigate whether the insertion-deletion polymorphisms of *MMP1* (-1607insG) and *MMP3* (-1171insA) have a role as risk factors for developing and progression of COPD. We genotyped 163 Bulgarian patients with COPD and 172 control individuals using PCR-RFLP-based methods.

The analyses showed no significant difference in both *MMP1* and *MMP3* genotype and allele distribution between controls and patients with COPD. We did not find correlation of the genotypes with the age of disease onset, however the COPD patients homozygous for 2G allele (2G/2G) of *MMP1* had, although not significantly, lower values of the spirometric index FEV1% pr. (49.83±14%) compared to the patients with other genotypes (52.37±16, p=0.375). In particular, this difference was higher, reaching a statistical significance, in the subset of patients with smoking history (44.18±13 vs. 50.91±14, p=0.034).

Our data suggest that -1607 2G allele of *MMP1* gene and -1171 6A allele of *MMP3* gene do not represent risk factors for development of COPD, however the homozygous 2G/2G genotype of *MMP1* seems to affect the lung function, especially of smokers, possibly by enhancing *MMP1* gene expression.

P444

VEGF gene polymorphisms are associated with post-natal lung function development

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Background: Vascular endothelial growth factor (VEGF) is involved in angiogenesis and suggested to be involved in airway remodeling and asthma susceptibility.
Objective: To study single nucleotide polymorphisms (SNP) in the VEGF gene in relation to lung function and hyperresponsiveness at birth and at six years of age, and development of asthma at six years of age.
Methods: We analyzed 13 SNPs in the VEGF gene from 411 children born to asthmatic mothers from the Copenhagen Prospective Study on Asthma in Childhood birth cohort. Lung function measurements at birth were obtained using the raised volume rapid thoracoabdominal compression technique during repeated methacholine challenges (FEV0.5, PD15). Lung function and methacholine challenges were repeated at six years of age (FEV1, PD20).
We analyzed the SNPs and outcomes using an additive genetic model in a linear regression analysis adjusting for appropriate confounders and corrected for multiple testing using the Bonferroni threshold as level of significance.
Results: Three SNPs, from the same linkage disequilibrium block, were associated to FEV1 at age 6: rs699947 (P = 2.03E-05), rs833069 (P = 0.00149) and rs3025010 (P = 0.00177). Stratifying for asthma did not alter the individual effects.
We found no association between SNPs and lung function or hyperresponsiveness at birth, or hyperresponsiveness or asthma at age 6.
Conclusion: VEGF gene variants were associated with lung function at school age but not at birth, and independent of asthma. This suggests a role of VEGF and VEGF gene variants for postnatal lung function development. Possible interaction between VEGF gene variants and post-natal environmental risk factors should be targeted in future studies.

P445

Predictive role of *MMP-2* genetic variants on severe hematologic toxicity of NSCLC patients treated with first-line, platinum-based chemotherapy

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Purpose: *MMP-2* plays an important role in hematopoietic recovery after chemotherapy-induced myelosuppression. In this study, we investigated the association between *MMP-2* SNPs and the incidence of adverse hemtologic events in advanced NSCLC patients treated with platinum-based chemotherapy.
Methods: A retrospective pharmacogenetic association study was performed in 1004 Chinese patients with advanced NSCLC receiving platinum-based regimens (including NP, GP, TC, TP). Information about grade 3 or 4 hematologic toxicity (neutropenia, anemia, thrombocytopenia) was available. 16 tag SNPs of *MMP-2* were assessed.

Results: There were 6 SNPs significantly associated with risk of grade 3 or 4 hematologic toxicity in platinum-based treatment. The variant homozygotes of rs12934241 exhibited the most significant influence on the occurrence of severe neutropenia (P=9.3×10^{-5}). In stratification analysis by chemotherapy regiments, we found the greates correlations in patients receiving cisplatin-gemcitabine: 7 polymorphisms were found be associated with severe neutropenia, especially rs12934241 (2.9% for C/C *v* 66.7% for T/T; P=4.9×10^{-5}). Consistent results were found in *MMP-2* haplotype analyses. In patients receiving cisplatin-navelbine, we also observed 6 SNPs significantly associated with grade 3 or 4 hemtologic toxicity. However, in cisplatin/carboplatin-paclitaxel treatment groups, no correlation with such toxicity was found.
Conclusions: Our study, for the first time, provides evidence for the predictive role of *MMP-2* polymorphisms on severe chemotherapy-related hematologic toxicity variability among platinum-treated advanced NSCLC Chinese patients.

P446
EGFR, _HER2_ and _KRAS_ mutational status according to adenocarcinoma patterns/sub-types
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Lung adenocarcinomas represents about 42% and 28% of NSCLC diagnosed in women and men. Several subtypes are recognized by WHO. *EGFR*, *HER2* and *KRAS* gene mutations have been described.
Authors intends to identify differences between adenocarcinomas subtypes/patterns concerning *EGFR*, *HER2* and *KRAS* mutations, gene copy number and protein expression.
45 lung adenocarcinomas were evaluated for *EGFR*, *HER2* and *KRAS* mutational status by PCR, fragment analysis and direct sequencing, *EGFR* and *HER2* gene copy number by fluorescence in situ hybridization (FISH). EGFR and c-erbB-2 protein expression was evaluated by immunohistochemistry (IHC).
8 cases showed *EGFR* exon 21 mutation (38,75%). In two cases the mutation was present in only one pattern of the tumour. 10 cases showed *EGFR* exon 19 deletions (32%), one case with the mutation present in only one of the patterns. 4 cases showed synchronous exon 19 deletions and exon 21 point mutations. Of the 14 cases with *EGFR* mutations 10 cases were FISH positive (71%). *KRAS* mutations were identified in 5 cases (16%), one coexisting with *EGFR* exon 21 point mutation. All cases were *HER2* wild-type. 8 cases with *EGFR* mutations demonstrated EGFR protein expression and 6 cases were negative.
EGFR mutational status and FISH results showed a moderate agreement/concordance. Concordance between *EGFR* FISH results and mutational status with EGFR IHC expression was fair.
Frequently, when a mutation is identified it is present in all the patterns of 1 adenocarcinoma. Mutations of *HER2* do not seem to be important in lung adenocarcinomas pathology. *KRAS* and *EGFR* mutations are in general mutually exclusive, but in rare cases they may coexist.

P447
Genetic analysis of two novel mucin-like genes in the disease-susceptibility locus for diffuse panbronchiolitis
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Background: Diffuse panbronchiolitis (DPB), which is characterized by chronic inflammation in respiratory bronchioles and sinobronchial infection, is a complex genetic disease affecting East Asians. DPB is strongly associated with HLA-B54 in Japanese and HLA-A11 in Koreans. We hypothesized that a major susceptibility gene for DPB might be located between the HLA-A and HLA-B loci and recently cloned two novel mucin-like genes designated panbronchiolitis related mucin-like 1 and 2 (PBMUCL1 and PBMUCL2) in the candidate region. We found disease-associated genetic polymorphisms in the new genes.
Objectives: The aim of the study is to compare the genetic polymorphisms of the genes between Japanese and European descent, because genetic predisposition to DPB has been assumed only in Asians.
Methods: We genotyped polymorphisms in 50 DNA samples of European descent and compared their frequency and haplotype structure with 108 Japanese DPB patients and 98 Japanese controls. Expression of PBMUCL1 transcript was investigated in primary-cultured human bronchial epithelial (HBE) cells.
Results: The haplotypes were different between European descent and Japanese. The mRNA expression pattern of the HBE cells with Asian-specific haplotype was analyzed.
Conclusions: Further analysis of newly identified mucin-like genes may provide insights into the pathogenesis of the disease.

P448
Genetic epidemiology of hereditary hemorrhagic telangiectasia complicated with pulmonary arteriovenous malformation
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Background: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by aberrant vascular development such as arteriovenous malformation (AVM). We report here a genetic epidemiologic study in a county, A, in the Akita prefecture (population 1.1 million) located in northern Japan.
Method and subjects: A total of 137 pedigree members were traced of which 81 were alive and 37 were affected by HHT. Complications associated with pulmonary arteriovenous malformations (PAVM) were proven in six out of seven families.
Results: Linkage analysis in two large families complicated with PAVM revealed a linkage to the HHT1 locus (encoding endoglin; ENG). Three novel mutations were found in four families, all of which led to a frame shift: a G to C transversion at the splicing donor site of intron 3 (Inv3+1 G>C) in one family, one base pair insertion (A) at nucleotide 828 (exon 7) of the erdoglin cDNA in two large families (a828-29 ins A), and a four base pair deletion (AAAG) beginning with nucleotide 1120 (exon 8) of the endoglin cDNA (c.1120-1123 delAAAG) in one family. The insertion of A in exon11 (c.147O-1471 insA) mutation was found in one family.
Summary and conclusion: The population prevalence of HHT in the county was estimated to be 1:8,000-1:5,000, roughly comparable with those reported in European and U.S. populations, which is contradictory to the traditional view that HHT is rare among Asians. We recommend that families with HHT be screened for gene mutations in order that high-risk individuals complicated with PAVM receive early diagnosis and treatment initiation that will substantially alter their clinical course and prognosis.

P449
Genetic polymorphisms in _TNF_ genes and tuberculosis in cystic fibrosis patients
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The genes for tumor necrosis factor-α (TNF-α; *TNF*) and lymphotoxin-α (LT-α; *LTA*) are arranged in tandem within MHC III region of chromosome 6 in the same transcriptional orientation. This formation is conserved, even in marsupials, suggesting that there may be some functional advantage to this arrangement. The proteins encoded by *TNF* and *LTA* are known to interact as complementary factors in various cell signaling networks. Single nucleotide polymorphism sites for *TNF* (-308 G>A) and *LTA* (252 A>G) were investigated with TaqMan allelic discrimination assay. 190 cystic fibrosis (CF) subjects were enrolled into the study. The distribution of allelic variants of *TNF* and *LTA* genes in CF patients did not differ from those in health subjects of Moscow and European populations. We did not find any association between *TNF* genotypes and CF lung disease progression. In the same time the carriers of -308A allele more frequently had asthma and other atopic disorders as compared to patients homozygous for -308G allele (14.9 *vs* 5.3%, $p<0.05$). There was no case of tuberculosis among patients with 252G alleles. In the same time in 7.9% of 252A/A carriers tuberculosis infection was documented ($p<0.03$). Besides, the subjects with genotype 252A/A demonstrated a significant elevation of plasma TGF-β1 in comparison with carriers of 252G allele (72.6 *vs* 32.4 pg/ml; $p<0.05$). Our data confirm the critical importance of -308 A *TNF* allele for asthma development and provide robust evidence that *LTA* gene variants are involved in tuberculosis etiology.