366. Inflammation and genes in childhood asthma

P3277

Do exhaled non-invasive inflammatory markers correlate with respiratory symptoms and lung function in a longitudinal study of childhood asthma?

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Background: Worldwide the level of asthma control is far from optimal. Asthma management is currently based on symptoms and lung function indices. Non-invasive inflammatory markers like exhaled nitric oxide may have additional value for asthma management. Little longitudinal data are available.
Aim: To study the relationship between non-invasive inflammatory markers and conventional parameters, during stable periods and during exacerbations.
Methods: 40 Children with asthma (aged 5-16yrs), visited the outpatient clinic every two months during one year. In addition, 4 extra visits were planned during an exacerbation. Every visit the asthma control questionnaire (ACQ) was assessed, lung function was performed and non-invasive inflammatory markers (FeNO, and nitrate, 8-isoprostane, interleukin-5 and tumor necrosis factor-alpha in exhaled breath condensate) were measured.
Results: 16 Of the 40 children experienced an asthma exacerbation. No significant correlations were founds between non-invasive inflammatory markers and conventional parameters (Spearman, $p>0.05$).

Correlations between conventional parameters and non-invasive inflammatory markers in childhood asthma

	Stable asthma	Exacerbations
FeNO–FEV1	–0.10±0.07	–0.19±0.13
FeNO–ACQ	0.11±0.07	
8-isoprostane–FEV1	–0.002±0.08	–0.06±0.18
8-isoprostane–ACQ	–0.03±0.07	
Nitrate–FEV1	–0.05±0.07	–0.16±0.17
Nitrate–ACQ	0.04±0.06	
IL-5–FEV1	0.15±0.10	–0.08±0.11
IL-5–ACQ	–0.10±0.07	
TNFα–FEV1	0.10±0.59	–0.14±0.11
TNFα–ACQ	–0.08±0.08	

Data were expressed as mean ± standard error.

Conclusions: This study showed that exhaled non-invasive inflammatory markers provide additional information about childhood asthma compared to conventional parameters.

P3278

Cytokines and chemokines in the airway epithelial lining fluid are down-regulated in newborns of atopic mothers

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Background: We have analyzed cytokines and chemokines in the nasal airway epithelial lining fluid in asymptomatic neonates born of atopic and non-atopic parents.
Method: 18 cytokines and chemokines were assessed in the nasal airway epithelial lining fluid in 309 1 month old neonates from the novel unselected Copenhagen Prospective Study on Asthma in Childhood (COPSAC2010) birth cohort. 56% of mothers and 47% of fathers had a history of asthma, allergy and/or eczema.
Results: Children with atopic mothers had significantly lower levels of CCL26 (eotaxin-3); CXCL10 (IP-10); IFNγ, IL13, IL1β,IL2, IL4, CXCL8 (IL8), TNFα, CCL11 (Eotaxin-1), CCL2 (MCP-1), CCL13 (MCP4) and CCL22 (MDC), compared to children of non-atopic mothers: and exhibited a non-significant trend of lower levels of IL10, IL12p70, IL5, CCL4 (MIP-1β) and CCL17 (TARC); while none of the cytokines or chemokines exhibited trend of high levels in children of atopic mothers.
Father's atopic status was not significantly associated with the level of these mediators.
Conclusion: Maternal atopy (but not paternal) is associated with a general down-regulation of Th1, Th2 and regulatory cytokines and chemokines in the nasal airway epithelial lining fluid in asymptomatic neonates.

P3279

Comparison of online single-breath versus multiple-breath exhaled nitric oxide at school entry in a cohort of unselected children

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Despite requiring less cooperation there are no standards for online multiple-breath (mb) measurements of exhaled nitric oxide (eNO) with uncontrolled flow rate and no studies comparing them to more difficult online single-breath (sb) eNO measurements in young children.

Online eNOsb and eNOmb were measured by a chemoluminescence analyzer connected to an ultrasonic flowmeter in N=73 children of a birth cohort of unselected children at a mean±SD age of 6.1±0.2 years. During measurements, we aimed for 20 tidal breathing manoeuvres for eNOmb and for 3 eNOsb manoeuvres according to current standards. We compared both techniques by standard comparison methods including regression analysis and Bland-Altman plots.

After strict quality control, eNOmb and eNOsb measurements were acceptable in n=56 and n=53 children, respectively. Paired data were available for n=46 children (43.5% males). With r^2=0.85, p<0.0001, eNOmb was significantly correlated with eNOsb (mean±SD 8.2±5.8 ppb) after computing NO output or after extrapolation to an expiratory flow rate of 50 mL/s (eNOmb50, mean±SD 9.0±5.8 ppb), also on a log-log scale. The mean difference between eNOmb50 and eNOsb according to standards was -0.9 ppb with upper and lower limits of agreement of 3.9 and -5.7 ppb, respectively.

At school entry, online eNOmb with uncontrolled flow rate is highly correlated with the gold standard of eNOsb measurements controlling for expiratory flow. The wide range of limits of agreement hampers eNOmb use in population-based research. Being less dependent on cooperation at this age, it might be a promising additional tool for the clinical setting to discriminate between disease groups.

P3280

Factors associated with high levels of exhaled NO in children using asthma medication

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Background: A high fractional nitric oxide concentration in exhaled breath (FeNO) is thought to be a marker of ongoing airway inflammation and may reflect poor asthma control.

Objectives: To study factors associated with high FeNO in children using asthma medication.

Methods: Our study population consists of 602 children (age 4-12 years) who used asthma medication in the past year and participated in the PACMAN (Pharmacogenetics of Asthma medication in Children: Medication with ANti-inflammatory effects)-cohort study. FeNO was measured using an electrochemical hand-held device (Niox Mino, Aerocrine, Sweden). Data on respiratory symptoms, medication use, therapy adherence and environmental factors were collected using a questionnaire. Asthma control was defined using the Asthma Control Questionnaire (ACQ). Children with high FeNO (>25 ppb) were compared to children with normal FeNO (5-25 pbb). Logistic regression analysis was used to study factors associated with high FeNO.

Results: FeNO could be measured in 501 children (83%); FeNO was high in 134 children (27%). Children with high FeNO were older than children with normal FeNO (mean age: 9.8 vs 8.7 years, p<0.01). In addition, high FeNO was associated with a lower chance of good adherence to asthma medication (OR: 0.4; 95%CI 0.2-0.6) and a lower chance of well-controlled asthma according to the ACQ (OR 0.5; 95%CI 0.4-0.8).

Conclusions: In our cohort of children using asthma medication, high FeNO is associated with higher age, lower adherence and less well-controlled asthma at population level. Further research is necessary to assess the value of FeNO for the individual patient.

P3281

The relationship between exhaled nitric oxide measurements and subsequent asthma control in a cohort of children followed over 12-months

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Introduction: Exhaled nitric oxide (FE_{NO}) may be a useful biomarker for childhood asthma control but clinical studies have been disappointing. The aims of the present observational study was to relate FE_{NO} values with later asthma control and to derive clinically useful FE_{NO} cut off values.

Methods: Children with stable asthma were recruited. Exhaled NO was measured at two month intervals when the Childhood Asthma Control Test (CACT) was also completed.

Results: Forty nine children with stable asthma were recruited in whom 252 FE_{NO} measurements were made. Exhaled NO was inversely related to CACT after two months (rho -0.22, p=0.005, n=158) and four months (rho -0.23, p=0.009, n=127) but not six months (rho -0.12, p=0.256, n=93). There were 121 2-month periods when asthma remained well controlled (mean initial FE_{NO} 20.9ppb [95%CI 17.6, 23.5]), 12 when control was gained (35.0ppb [22.1, 55.3]), 8 when good control was lost (28.3ppb [12.4, 64.7]) and 15 when control remained poor (19.0ppb [95%CI 13.2, 27.4]); the difference in FE_{NO} between remain controlled and became controlled was significant (p=0.022). Exhaled NO of >46ppb had sensitivity of 63% and specificity of 77% for predicting future loss of asthma control (AUC 0.61). Exhaled NO of >22ppb had a sensitivity of 65% and specificity of 58% for a future reduction in CACT of 3 points (AUC 0.65).

Conclusions: Increased FE_{NO} is associated with reduced asthma control over the following four months. In this population of stable asthmatic children, increased FE_{NO} lacked precision for predicting future changes in asthma control. Exhaled NO might be useful in predicting ongoing well-controlled asthma.

P3282

A longitudinal study of exhaled nitric oxide in children – What explains intersubject variability of measurements?

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Introduction: Exhaled nitric oxide (FE_{NO}) may be a biomarker for asthma but clinical trials where FE_{NO} is used to guide asthma treatment have been disappointing. Exhaled NO measurements may not be suited to individuals with increased variability of values. We hypothesised that the variability of FE_{NO} measurements is heterogeneous within a population and sought to describe factors associated with increased variability for repeated measurements of FE_{NO}.

Methods: Children with and without asthma were recruited from the community and completed respiratory questionnaires. A subset of the cohort attended an initial phenotyping assessment which included spirometry, bronchodilator response and skin prick reactivity. Exhaled NO was measured every two months over a 12 month period. The coefficient of variation (CV) for FE_{NO} measurements was calculated for each individual.

Results: One hundred and eighty children were recruited including 49 with asthma and 893 FE_{NO} measurements were made (median 5 measurements per child). Eighty two children attended the clinical assessment. The following were not associated with altered CV: asthma, eczema or hayfever; male gender; body mass index; skin prick reactivity; spirometry; bronchodilator response; exposure to pets, smokers or damp at home. There was an inverse relationship between age and FE_{NO} CV (rho= -0.16, p=0.038). Among the asthmatic children, there was no correlation between FE_{NO} CV and treatment step or dose of inhaled steroid.

Conclusions: Measurements of FE_{NO} are variable between individuals. Younger age, but not atopy, asthma status or treatment, is associated with increased variability in FE_{NO} over 12 months.

P3283

Exhaled nitric oxide and clinical phenotype of childhood asthma

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Question. To evaluate whether exhaled NO (FENO0.05) is associated with pathophysiological characteristics such as airway tone (bronchodilator response) and inflammation (inhaled corticosteroid [ICS]-dependent inflammation), and with phenotypes of childhood asthma.

Methods: We performed multivariate and k-means cluster analyses in a population of 169 asthmatic children (age ± SD: 10.5±2.6 years) recruited in a cohort characterized in a cross-sectional design using 28 parameters of different domains: atopy, environment (tobacco), control, exacerbations, treatment (ICS and long-acting bronchodilator agonist), and lung function (airway architecture and tone).

Results: Two subject-related (height and atopy) and two disease-related characteristics (bronchodilator response and ICS dose>200 μg/d) explained 36% of exhaled NO variance. Principal component analysis isolated 9 domains and 4 clusters were identified: 1 (47%): boys, unexposed to tobacco, with well-controlled asthma; 2 (26%): girls, unexposed to tobacco, with well-controlled asthma; 3 (6%): girls or boys, unexposed to tobacco, with uncontrolled asthma and increased tone, and 4 (21%): girls or boys, exposed to parental smoking, with small airway to lung size ratio and uncontrolled asthma. Importantly, FENO0.05 was not different in these four clusters.

Conclusion: Despite its relationships with pathophysiological characteristics, FENO0.05 does not help to identify a relevant phenotype of asthmatic children.

P3284

Abnormalities in spirometry, impulse oscillometry and exhaled nitric oxide in the university freshmen who have outgrown of bronchial asthma

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Background: Incidence of asthma, particularly in childhood, is rising. Current asthma prevalence is higher in children than in adults, suggesting that children with asthma may outgrow their disease. It has been long believed that the prognosis for asthma occurring in infancy or childhood is good, and that in most patients the symptoms would resolve by the age of puberty. However, little is known about the lung function of outgrown subject. In this study, we recruited the subjects who had outgrown of asthma and performed spirometry, impulse oscillometry and exhaled nitric oxide (eNO) measurement.
Method: The freshmen in our university with a history of asthma (group A, n=54) and without a history of asthma (group B, n=63) were recruited. All group A subjects were currently free from symptoms and underwent no treatment. Lung function was estimated by CHEST-HI801 (CHEST, JAPAN). Impulse oscillometry was performed using MostGraph (CHEST, JAPAN). Exhaled NO level was measured by NO analyzer (SIEVERS NOA, USA).
Results: FEV1% was significantly lower in group A than in group B (88.6±6.1% vs 91.2±4.6%; p=0.01). The mean levels of%FEV1,%Vdot50 and%Vdot25 were lower in group A than in group B, though not significant. The levels of R5-20 and Fres were significantly higher in group A than in group B (R5-20: 0.81±0.07 cmH2O/L/s vs 0.62±0.05 cmH2O/L/s; p=0.03) (Fres: 7.9±0.4 Hz vs 6.4±0.3 Hz; p<0.01). Also, the level of eNO was significantly higher in group A than in group B (49.7±4.3ppb vs 37.4±2.6ppb; p=0.01).
Conclusions: The subjects who had outgrown of asthma still have impaired lung function, a follow-up examination of lung function may be needed.

P3285

8-isoprostane in exhaled breath condensate in healthy and asthmatic children, and its relationship with tobacco smoke exposure

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Introduction: 8-Isoprostane is an oxidative stress marker, which rises in asthma and in exposure to tobacco smoke.
Objectives: 1) Differences in the concentration of 8-isoprostane in exhaled breath condensate (EBC) between asthmatics and healthy children. And also between children with recurrent wheezes and healthy preschool children. 2) Relationship between levels of 8-isoprostane and passive smoke exposure.
Methods: 70 children (6 to 14 years) were recruited in 2 groups: 40 asthmatic and 30 healthy children. Also 46 children (8 months to 5 years) were recruited in other 2 groups: 25 with recurrent wheezes and 25 as controls. EBC was colleted through the exhaled condenser. 8-isoprostane was determined by enzyme immunoassay. Data about number of smokers living with the child, cigarettes/day and the smoking place was asked.
Results: 8-isoprostane concentrations were lower between in the group of healthy children than in children with asthma (p<0.004, Mann-Whitney U: 343, mean difference: 6.2 (4.7)). Moreover 8-isoprostane levels differ according to smoking habits of parents (ANOVA F=8.28, P<0.001) and children whose parents smoke at home have higher levels than those of nonsmoking parents (mean difference 22.4, p=0.003). Similarly happened to children from 8 months to 5 years (ANOVA F=8.23, p<0.001) and (mean difference = 141.3, p<0.001).
Conclusions: 1. There are significant differences in the concentrations of 8-isoprostane in EBC from asthmatic children 6-14 years old and healthy and also in preschool children. 2. Passive exposure to tobacco smoke increases concentrations of 8-isoprostane, in the EBC of children in both age groups.

P3286

DENND1B gene variants are associated with elevated exhaled nitric oxide in healthy neonates

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Background: Increased fractional exhaled nitric oxide (FeNO) values in neonates are associated with early transient wheeze, but risk factors driving this association are largely unknown.
Objectives: To study perinatal and genetic determinants of elevated FeNO in healthy neonates.
Methods: FeNO was measured during sedation in 253 healthy symptom-free 1-month-old neonates in relation to infant spirometry testing. The children belong to the COPSAC clinical birth cohort born to asthmatic mothers. The risk factor analyses included genetic variants in DENND1B, Filaggrin and ORMDL3; anthropometrics; demographics; socioeconomic status; paternal atopic history; maternal intake of paracetamol, antibiotics, smoking during third trimester of pregnancy; and pathogenic bacterial colonization of the neonatal airway.
Multivariate analyses were done using generalized linear models.
Results: Median neonatal FeNO level was 16.0ppb (Q1-Q3, 12.0-22.0ppb). The DENND1B risk allele, rs2786098, (major allele) was significantly associated with increased levels of FeNO (additive model, β-coefficient, 2.90ppb; 95% CI, 0.38-5.43; P=0.02). Children with atopic fathers also showed increased values of FeNO (β-coefficient, 2.90ppb; 95% CI, 0.38-5.43; P=0.02).
None of the remaining genetic or environmental risk factors were associated with neonatal FeNO levels.
Conclusion: Variants in the DENND1B locus of chromosome 1q31.3 and paternal atopy are associated with elevated FeNO levels in 1-month-old newborns prior to development of any atopic symptoms. These findings suggest that the DENND1B risk allele confer an increased risk of wheezy illnesses through modifications associated with NO metabolism very early in life.

P3287

Interleukin-10 polymorphisms influence the neonatal immune response and may be a risk factor for childhood allergic disease and wheeze

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Background: The interleukin-10 gene (*IL10*) encodes the anti-inflammatory cytokine IL-10, which is crucial for the development of immune tolerance and T cell regulation. *IL-10* polymorphisms were proposed to influence the asthma phenotype including pulmonary function. We studied the influence of *IL-10* single nucleotide polymorphisms (SNPs) early in life on Th1/Th2 lineage fate, T regulatory cells and pro-inflammatory cytokines. Furthermore, the effect of the SNPs on allergic diseases and wheeze was examined at 3 years of age.
Methods: Cord blood of 200 healthy neonates was genotyped for 8 SNPs and one deletion in *IL-10.* CBMCs were cultured unstimulated or following stimulation [Lipid A, peptidoglycan, phytohemagglutinin, house dust mite (Derp1), Derp1+Lipid A]. Treg-marker mRNA expression (FOXP3, GITR, LAG3), Th1/Th2-cytokines, TNF-α and GM-CSF were assessed. A follow-up regarding allergic diseases and wheeze was performed (3ys).
Results: The majority of *IL-10* SNP carriers showed a similar pattern of cytokine secretion and Treg-marker expression with increased IFN-γ secretion (Th1), reduced IL-5 (Th2), pro-inflammatory TNF-α and GM-CSF and reduced Treg-marker expression. The effects were primarily observed after innate and allergic stimulation. Carriers of five *IL-10* SNPs and the *IL-10* deletion showed increased risk for wheeze or atopic symptoms.
Conclusions: Polymorphisms in *IL-10* influence primarily Th1/Th2 cytokine secretion and Treg-marker expression after innate and allergic stimulation early in life. This may be relevant for immune maturation and potentially for childhood atopy and wheeze.

P3288

Phenotyping atopic dermatitis in children by filaggrin status. The COPSAC clinical birth cohort study

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Background: Filaggrin null mutations result in an impaired skin barrier function and are associated with atopic dermatitis. Clinical observations suggest a distinct phenotype for patients with the filaggrin null genotype.
Objective: To characterize and compare the clinical presentation and course of atopic dermatitis within the first 7 years of life in children with and without filaggrin null mutations.
Method: The COPSAC cohort is a prospective, longitudinal, birth cohort study of 397 Caucasian children born to mothers with a history of asthma, followed for 7 years with scheduled visits every 6 months as well as visits for acute exacerbations of dermatitis. Atopic dermatitis was defined in accordance with international guidelines and dermatitis reactions were accurately described at every visit using 35 predefined localizations and 10 different characteristics.
Results: A total of 170 (43%) of 397 children suffered from atopic dermatitis before age 7. The R501X and 2282del4 filaggrin null mutations were associated with a higher number of acute visits (3.6 vs. 2.7; p=0.04), more severe dermatitis (moderate-severe SCORAD 44% vs. 31%; p=0.14), more widespread dermatitis (10% vs. 6% of the body, p<0.001), an earlier age at onset of dermatitis (246 vs. 473 days, p<0.0001), dermatitis more often localized at exposed areas (hands, feet, extensor areas, cheeks), and with dermatitis spots characterized by an up regulation in both acute and chronic markers.

Conclusion: Filaggrin mutations represent a genetically defined endophenotype, characterized by early onset and a more severe dermatitis with particular predilection to the exposed areas of the body.

P3289

Gene expression of inflammatory markers in wheezing preschool children; the ADEM study

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Background: Wheeze affects 20-40% of preschool children. Of all wheezers 30% will have persisting symptoms after the age of six that develop into asthma which is characterized by chronic airway inflammation. Whether airway inflammation is a characteristic of preschool wheezing is poorly documented.
Aim: To assess cross-sectional differences in gene expression of several important inflammatory markers in venous blood cells in preschool children with recurrent wheeze compared to healthy controls.
Methods: In total, 202 children with recurrent wheeze (ISAAC questionnaire $\geq$2 wheezing episodes) and 50 children without respiratory symptoms, age 2-3 year were selected. Total RNA was extracted from peripheral blood mononuclear cells. Gene expression of 21 genes was assessed and corrected for geometric mean of 3 housekeeping genes. Both unadjusted and adjusted (controlling for age, gender, passive smoking and pet exposure) logistic regression analysis was subsequently conducted.
Results: Gene expression was successfully analysed in 223 children. Expression of the catalase gene (*CAT*) showed a statistically significant inverse association with recurrent wheeze ($OR_{adjusted}$ 0.65 (0.48-0.88) p=0.005). Expression of interleukin-17 (*IL17*) showed a statistically significant positive association with recurrent wheeze ($OR_{adjusted}$ 3.04 (1.33-6.92) p=0.008). None of the other genes showed a significant relationship between gene expression level and recurrent wheeze.
Discussion: A decrease of *CAT* expression and an increase of *IL17* expression was observed in preschool children with recurrent wheeze. This may point towards the importance of different inflammatory pathways in children with preschool wheezing.

P3290

The chitinase-like protein YKL-40 is elevated in children with severe asthma

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Background: Chitinase family proteins are associated with airway inflammation in adults, yet it is unknown whether levels are altered in children with asthma. Our aim was to examine chitotriosidase (CHIT1) and YKL-40 (CHI3L1) as potential biomarkers of airway inflammation in children, taking into account possible effects of genetic variation.
Methods: Blood samples were obtained from children aged 6-18 with controlled (n=39) and severe (n=57) asthma, described in Konradsen et al. (Pediatric Allergy Immunology 2011;22:9-18). Serum chitotriosidase activity and YKL-40 levels were analysed in a subset of patients and compared to 41 healthy children. DNA samples were genotyped for a null 24bp duplication in CHIT1 and the CHI3L1 promoter SNP Rs4950928.
Results: YKL-40 levels were significantly greater in severe asthmatics compared to healthy children, whereas chitotriosidase activity was no different in asthmatics compared to controls. Genetic variation affected both YKL-40 levels and chitotriosidase activity. The CHI3L1 Rs4950928 CC allele was associated with greater levels of serum YKL-40 (24.3 (16.7-29.6)) than the CG allele (19.4 (14.9-23.3), p=0.008). Children lacking the 24bp duplication in CHIT1 showed greater chitotriosidase activity (68.0 (56.6-89.7)) than those heterozygous for the duplication (35.6 (26.9-41.8), p<0.001).

	Healthy	Controlled Asthma	Severe Asthma
YKL-40, ng/ml	14.5 (9.2–20.6)	16.3 (15.0–18.3)	19.1 (13.9–23.0)*
Chitotriosidase activity, nmol/ml/hr	59.3 (40.6–93.8)	51.9 (35.7–60.1)	67.5 (38.8–85.1)

*p=0.024 vs healthy, median (IQR).

Conclusions: These results suggest that serum YKL-40 is a genetically influenced, potential new biomarker of airway inflammation in children.

P3291

Dysregulated peripheral blood miRNAs in murine experimental and human childhood asthma

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The involvement of genetic and environmental factors in asthma is evident. As miRNAs can both respond to the environment and regulate complex signalling networks at the posttranscriptional level they may be implicated in the disease.
In previous work we have identified dysregulated pulmonary miRNAs in experimental asthma (ERS Barcelona 2010, abstract 6106). In the present study we were interested to see if selected lung miRNAs are also altered in murine peripheral blood and whether these changes are similar in peripheral blood of asthmatic children. MiRNAs were quantified by rt-qPCR in blood from ovalbumin sensitized and challenged Balb/c mice and controls. Ten year old children were selected based on the following criteria: current allergic asthma, absence of acute infections and ETS exposure. Age matched controls from the same cohort were included when they never had asthma and were free from acute infections. MiRNAs were quantified in bio-banked blood samples.
Out of 10 children with diagnosed asthma 6 had a record of atopic eczema at any time and 4 had had allergic rhinitis ever.
A set of 5 differentially regulated miRNAs (miRNA 17-5p, 21, 144, 181a, 451) in murine lung were chosen to be examined in blood samples. Murine blood and lung tissue miRNAs showed inverse expression patterns. Murine und paediatric blood samples showed the same trend of regulation compared to healthy controls. In this study we found opposite expression of selected miRNAs in lung tissue compared to blood of mice with an asthmatic phenotype. This expression pattern was similar in human blood samples of asthmatic children. These results call for further studies to explore blood miRNAs as potential biomarkers in asthma.

P3292

Protection against atopic diseases through farm exposure is partially mediated by regulatory T cells

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Background: Our previous studies suggested a protection against allergic diseases through farm exposure. The underlying mechanisms may involve immune regulation through T cell subsets already present early in childhood.
Objective: To assess whether T regulatory cells (Tregs) are involved in the allergy-protective effect of farm exposure in childhood and which specific exposures are relevant.
Methods: 457 pregnant women were recruited before delivery and followed until the age of 4.5 years in Germany (n=254) and France (n=203) as part of a European birth cohort study (PASTURE/EFRAIM). Children of families living on livestock farms were compared to children of families from this region, but not living on farms. Detailed questionnaires assessed farming exposures over time. Tregs ($CD4^+CD25^{high}FOXP3^+$ expression) in peripheral blood mononuclear cells (PBMC) were assessed at 4.5 years before and following stimulation (lipopolysaccharide (LPS), PMA/Ionomycin (PI)) by flow cytometry.
Results: At 4.5 years of age, farm-exposed children showed increased Tregs following stimulation (LPS, PI), $p<0.05$). Specifically, exposure to farm milk was strongly associated with increased Tregs upon LPS and PI-stimulation ($p\leq0.001$). Exposure to stable was associated with increased Tregs after LPS and PI-stimulation (p=0.006, p=0.02; respectively).
Conclusions: Farm exposure, and particularly farm milk intake in early childhood, was associated with an increase of Tregs in early childhood upon specific stimulation. Specific contents of farm milk may modulate the immune system towards a regulatory profile potentially leading to a protective effect for the development of childhood allergic diseases.

P3293

The effects of number of siblings, day-care attendance, and farming on childhood asthma and allergies – The GABRIEL advanced surveys

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Background: Number of siblings, day-care attendance, and exposure to farming have been shown to reduce the risk of childhood asthma and allergies. It is known that families living on a farm are larger in size. We explored the interrelation and independence of these protective effects in the large GABRIELA Surveys.
Methods: The GABRIELA studies are cross-sectional multi-phase surveys on farming and asthma and allergies among 79,888 children at age 6 to 12. Detailed data on farming, number of siblings, day-care attendance, wheeze, and specific serum IgE were collected, in part from a stratified random sample (n>8,000). We used multivariate logistic regression with weighted stratified techniques where appropriate to model odds ratios with 95% confidence intervals and to test for multiplicative interaction.
Results: Farm children have more siblings and less often attend day-care facilities than their rural peers. More siblings and farming conferred independent protection from asthma and allergies. No protection by day-care attendance was found. There was no clear evidence of interaction between family size or day-care attendance and farming. The prevalence of hay fever was 12% among non-farm only children and 2% among farm children with more than 2 siblings.
Conclusion: The inverse association of farming with asthma and allergies is found in all sizes of family, with no clear tendency to total saturation or synergism. This suggests that different mechanisms may underlie these two protective factors. Combinations of family size and farm exposure markedly reduce prevalence of allergic disease and indicate the strength of environmental determinants.

P3294

Polymorphisms in 17q12-21 are associated with asthma exacerbation and lung function in asthmatic children

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Background: More then 15 studies associated genetic variants in 17q21 region with asthma. We aimed to investigate whether amongst Croatian asthmatic children, genetic variants in this region are associated with asthma severity and exacerbation.
Methods: We recruited 423 children aged 6-18 years with physician-diagnosed asthma. Information on hospital admission with asthma exacerbations was retrieved from medical records. Data on wheeze frequency and environmental tobacco smoke (ETS) exposure was collected using validated questionnaire. Lung function (FEV_1%predicted) was measured using spirometry. We analyzed 35 haplotype-tagging SNPs in 17q21.
Results: We found significant associations between 4 SNPs and hospital admissions (rs12150079, rs7212938, rs2290400, rs8067378). For example, G allele homozygotes in rs12150079 were at higher risk of being admitted to hospital than carriers of A allele (aOR 1.85, 95%CI 1.26-2.72, p=0.002); this SNP was also associated with current wheezing. Six SNPs were associated with lung function (rs9635726, rs921651, rs9900538, rs3169572, rs4795403, rs471692). In addition, we observed significant interaction between 3 SNPs (rs12603332, rs8067378, rs9303277) and *in utero* ETS exposure in relation to lung function ($p_{int}<0.04$), in that amongst children of mothers who smoked during pregnancy, major allele homozygotes had lower FEV_1%predicted than minor allele carriers, but amongst non-exposed children there was no difference in lung function between different genotype groups.
Conclusion: Variants in 17q12-21 region may be associated with asthma severity and may interact with *in utero* ETS exposure in determining lung function amongst asthmatic children.