491. Severe asthma and developing allergy in childhood

Fardous Heili,1 Marielle Pijnenburg,2 Leonie Vanlaeken,3 Wim Timmes,2 Bart Rottier,2 1Pediatric Pulmonology, Universitair Medical Center, Groningen, Netherlands; 2Pediatric Pulmonology, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands

Introduction: Bronchospasy and endobronchial biopsies in children are useful tools in the evaluation of difficult asthma and other pediatric airway diseases. The yield of these procedures in clinical practice is uncertain. Therefore our aim was to evaluate if bronchospasy and endobronchial biopsies change management.

Methods: We prospectively collected data on diagnosis and treatment before and after bronchoscopy in all children undergoing bronchoscopy with endobronchial biopsies between 0 and 18 yrs. Difficult asthma was defined as uncontrolled asthma despite high dose ICS dose and LABA. In bronchoscopy biopsies reticular basement membrane (RBM) thickness was measured.

Results: Of the 74 children (37 male, mean age 7.8 yrs), 26 had asthma and 13 had difficult asthma. The diagnosis of children undergoing bronchoscopy changed in 31.3% of the cases: 10 children got a second diagnosis, which could explain the persistent symptoms, 8 children were misdiagnosed. In these 18 children airwaymalaclia (n=10) and atopic abnormalities (n=7) were the most common. In 9.5% of the cases biopsies changed the diagnosis; in 3 of them ciliary dyskinesia was suspected. In 25% medication was changed after bronchoscopy.

Conclusions: Bronchoscopy showed an important role to reveal underlying causes of persistent symptoms such as airwaymalaclia and atopic abnormalities. In 25% of patients bronchoscopy led to a change in treatment. We found no significant difference in RBM thickness between nonasthmatic, asthmatic children and children with difficult asthma.

4709 IL-33 and airway remodelling in paediatric severe therapy resistant asthma

Nicola Ullmann1,2, Alex Adams1,2, Cara J. Bossley2, Tim Oates1,2, Claire M. Lloyd,3 Andy Bush1, Sejal Saglani1,2. 1Paediatric Respiratory, Royal Brompton and Harefield NHS Trust, London, United Kingdom; 2Department of Paediatric Respiratory, Royal Brompton and Harefield NHS Trust, London, United Kingdom; 3Department of Paediatrics, Federico II University, Naples, Italy; 4Department of Paediatrics, Queen Mary Hospital, Chinese University of Hong Kong, China

Background: Associations have been reported between epithelial interleukin (IL)-33 expression and severe asthma in adults, but its role in the pathophysiology of paediatric severe therapy resistant asthma (STRA) remains unknown.

Objective: We quantified IL-33 expression in endobronchial biopsies from children with STRA and explored its relationship with parameters of airway remodelling.

Methods: Children (n=55) (median age 11.3 yrs; range 5.4-16.4 yrs) with STRA were recruited from three UK hospitals. Wheezing pattern was multi-triggered (n=46) and episodic (n=115). There was no significant difference in IL-33 expression in STRA compared to that in age matched non-sensitised patients (n=90) had negative SpIgE and SPT. Age, atopy, symptoms, medications, lung function and airway inflammation were assessed.

Results: IL-33 expression was increased in STRA compared to STRA (Spearman r=0.35; p=0.018), but not in controls (Spearman r=0.35; p=0.01). Increase of IL-33 expression was associated with increased submucosal (r=0.35; p=0.018) and BM thickness (r=0.35; p=0.018). There was no significant association between IL-33 with medication. Some children had chronic pulmonary leukotriene activation, and therefore higher baseline IL-33 expression.

Conclusion: Children with STRA and SAFS had earlier asthma onset (0.5 years [0-12.5] vs 1.5 [0-12.5], p=0.006), higher total IgE (637 IU/mL [12-6737] vs 177 [1-10881], p=0.002) and sum of inhalant allergen specific IgE (SPT) positivity to Aspergillus fumigatus, Alternaria alternata or Cladosporium herbarum. Non-atopic patients (n=90) had negative spIgE and SPT. Age, atopy, symptoms, medications, lung function and airway inflammation were assessed.

Results: SAFS children were mainly boys (57/76(75%) vs 43/90(48%), p<0.01), had earlier asthma onset (0.5 years [0-12.5] vs 1.5 [0-12.5], p=0.006), higher total IgE (637 IU/mL [12-6737] vs 177 [1-10881], p=0.002) and sum of inhalant allergen specific IgE (SPT) positivity to Aspergillus fumigatus, Alternaria alternata or Cladosporium herbarum. Non-atopic patients (n=90) had negative spIgE and SPT. Age, atopy, symptoms, medications, lung function and airway inflammation were assessed.

Results: SAFS children had a trend for lower FEV1 (72% predicted [29-121] vs 75.5 [23-125], p=0.18) and FVC (90% predicted [36-138] vs 95 [30-123] p=0.13), more bronchodilator reversibility (59/73 (81%) vs 42/81 (52%), p<0.001; 78 IU/mL [0-400] vs 19 [0-243]; p=0.02). SAFS children had a trend for lower FEV1 (72% predicted [29-121] vs 75.5 [23-125], p=0.18) and FVC (90% predicted [36-138] vs 95 [30-123] p=0.13), more bronchodilator reversibility (59/73 (81%) vs 42/81 (52%), p<0.001; 78 IU/mL [0-400] vs 19 [0-243]; p=0.02). SAFS children had a trend for lower FEV1 (72% predicted [29-121] vs 75.5 [23-125], p=0.18) and FVC (90% predicted [36-138] vs 95 [30-123] p=0.13), more bronchodilator reversibility (59/73 (81%) vs 42/81 (52%), p<0.001; 78 IU/mL [0-400] vs 19 [0-243]; p=0.02). SAFS children had a trend for lower FEV1 (72% predicted [29-121] vs 75.5 [23-125], p=0.18) and FVC (90% predicted [36-138] vs 95 [30-123] p=0.13), more bronchodilator reversibility (59/73 (81%) vs 42/81 (52%), p<0.001; 78 IU/mL [0-400] vs 19 [0-243]; p=0.02). SAFS children had a trend for lower FEV1 (72% predicted [29-121] vs 75.5 [23-125], p=0.18) and FVC (90% predicted [36-138] vs 95 [30-123] p=0.13), more bronchodilator reversibility (59/73 (81%) vs 42/81 (52%), p<0.001; 78 IU/mL [0-400] vs 19 [0-243]; p=0.02). SAFS children had a trend for lower FEV1 (72% predicted [29-121] vs 75.5 [23-125], p=0.18) and FVC (90% predicted [36-138] vs 95 [30-123] p=0.13), more bronchodilator reversibility (59/73 (81%) vs 42/81 (52%), p<0.001; 78 IU/mL [0-400] vs 19 [0-243]; p=0.02).

Conclusions: Children with STRA and SAFS had earlier onset asthma, more atopy and bronchodilator reversibility, and were more often given prednisolone. We randomly controlled treatment of antifungal therapy in paediatric SAFS.

4712 Urinary eosinocidns and preschool wheeze phenotype

Mubin Ibrahim1, Chimeda Nwokoro1, Marek Sanak2, Steve Turner3, Jonathan Grigg3 1Centre for Paediatrics, Blizzard Institute, Barts and the London School of Medicine and Dentistry, London, Greater London, United Kingdom; 2University Department of Medicine, Jagiellonian University School of Medicine, Krakow, Poland; 3Department of Child Health, University of Aberdeen, United Kingdom; 4Department of Child Health, University of Leicester, United Kingdom

Rationale: Preschool wheeze follows two broad clinical patterns: i) multi-trigger wheeze (wheeze both with and between colds) and ii) episodic viral wheeze (wheeze exclusively with colds). Whether these patterns reflect stable, distinct phenotypes with different underlying inflammation remains unclear.

Hypothesis: We hypothesised that preschool children with multi-trigger wheeze have chronic pulmonary leukotriene activation, and therefore higher baseline urinary eosinocidns, than episodic viral wheeilers.

Methods: Children aged 10 months to 5 years with wheeze and no respiratary comorbidity were recruited from three UK hospitals. Wheezing pattern was obtained from parental history. Urine was collected between viral-triggered attacks and frozen at -70°C. Samples were analysed using high performance liquid chromatography tandem mass spectrometry for a range of eosinocidns (Table 1).

Results: See Table 1. There was no significant demographic difference between multi-triggered (n=46) and episodic (n=115) wheeilers. There was no significant difference in baseline eosinocidn excretion between the 2 groups (P=NS by unpaired t-test, Table 1).

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Conclusions: We found no evidence that the multi-trigger phenotype is associated with increased baseline eosinophil activation.

Dedicated to the memory of Prof Andrzejs Szczeklik who would have been a co-author.

**Table 1. Baseline urinary eicosanoids (pg/mg of creatinine)**

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Episodic</th>
<th>Multitrigger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukotriene B4</td>
<td>1216.47</td>
<td>928.71</td>
</tr>
<tr>
<td>13,14-dihydro-15-keto-PG2</td>
<td>455.03</td>
<td>625.38</td>
</tr>
<tr>
<td>15-deoxy-delta12,14-PGJ2</td>
<td>361.61</td>
<td>606.20</td>
</tr>
<tr>
<td>13,14-dihydro-15-keto-PGE2</td>
<td>1281.95</td>
<td>1837.72</td>
</tr>
<tr>
<td>tetranor-PGE-M</td>
<td>35655.09</td>
<td>34995.67</td>
</tr>
<tr>
<td>tetranor-PGD-M</td>
<td>45810.02</td>
<td>43065.58</td>
</tr>
</tbody>
</table>

**4713** Changes in skin prick test (SPT) from the age of 1 to 6 years (yrs) and relationship to specific IgE and atopic dermatitis: The French arm of PASTURE European study

Stéphanie Drillon1, Anne Leydier1, Caroline Roduit1, 3 Erika von Mutius4, Judith Régnier1, Jean Salmon1, 5 Dominique Angele Vuillon1, 5 Jean Charles Dalphin1, 5 Amélie Thonnard1, 5 Pierre Coin1, 5 Cyril Lefebvre1, 5 Audrey Dechamps1, 5 Christelle Thiville1, 5 Laurence Lemaire1, 5 Céline Parrot1, 5 Aurore Bouchard1, 5 Virginie Boulet1, 5 Adeline Morvan1, 5 Fabrice Prevot1, 5 Angèle Lécouterie1, 5 Pierre Gobat1, 5 Dominique Vannier1, 5 Marie Claire Vatin1, 5 Jean Claude Lefaix1, 5 Marie Laure Darmon1, 5 Vincent Kaukel1, 5

The “PASTURE” study (Protection against Allergy: STUDY of Rural Environment) examines the influence of exposure to a dairy-farm environment on the occurrence of allergy in a cohort of European children from the 3rd trimester of pregnancy to 10 yrs.

The aim of this study was to analyze the course of SPTs to aero- and food allergens from the age of 1 to 6 yrs in the children of PASTURE, whether they live or not on a farm and to evaluate their relationship to specific IgE and atopic dermatitis (AD).

Two hundred and four children, which 95 of the farmer group, were included. SPTs, AD assessment and specific IgE assays were performed at 1, 4.5 and 6 yrs.

One hundred and forty children participated in all three visits. The prevalence of positive SPTs increased with age (9.5% at 1 yr, 14.2% at 4.5 yrs, 22.6% à 6 yrs). It was lower in the farmer group, regardless of age and category of allergens considered (statistically significant only for seasonal allergens at 4.5 yrs). Positive SPTs were transient at 1 yr whereas they were persistent between 4.5 and 6 yrs. The prevalence of AD was not significantly different in the two groups and decreased with age. Positive SPTs at 1 yr were predictive of the occurrence of AD during the follow-up. The correlation between SPTs and specific IgE was poor for an IgE cut-off at 0.35 UI/mL but increased with age and with higher cut-off.

In conclusion, our study showed that skin reactivity increased with age, was lower in the farmer group, and investigated potential reverse causation in analysis that excluded children with an IgE cut-off at 0.35 UI/mL but increased with age and with higher cut-off.

**4714** DNA methylation in asthma and allergy related genes is influenced by farm exposure and time trends in early childhood

Sven Michel1, Florence Brunazot1, Jon Gennusa1, Juha Pekkanen4, Charles Dalphin1, Josef Riedler2, Nicolas Mazaleyrat1, Erika von Mutius1, Juliane Weber1, Charlotte Braun-Fahrlander3,1, Roger Lauener4,11, Michael Kabesch1, Jrg Tost1, the PASTURE Study Group. 1Department of Department of Pediatric Pneumology, Allergy and Neonatology, Hannover Medical School, Hannover, Germany; 2Laboratory for Epigenetics, Centre National de Genotypage, CEA–Institut de Génomique, Evry, France; 3Institute of Epidemiology and Medical Biometry, Um University, Ulm, Germany; 4National Institute for Health and Welfare, THL, Kuopio, Kuopio, Finland; 5Department of Respiratory Disease, Universite de Franche-Comte, Besanon, France; 6Children’s Hospital Schwarzach, Children’s Hospital Schwarzach, Schwarzach, Austria; 7University Children’s Hospital, LMU, Munich, Germany; 8Institute of Social and Preventive Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland; 9University of Basel, University of Basel, Switzerland; 10Children’s Hospital and Christine Kühne-Center for Allergy, University of Zurich, Switzerland; 11Christine Kühne-Center for Allergy Research and Education, Hochbergklinik Davos, Davos-Wolfgang, Switzerland

Genes and environmental influences are crucial for the development of asthma and atopic diseases. These factors may act upon each other through epigenetic mechanisms.

We studied the rural birth cohort PASTURE (Protection against allergy: study in rural environments) to investigate if (a) epigenetic patterns in asthma candidate genes are influenced by farm exposure in general and (b) change over the first years of life and (c) whether these changes may contribute to the development of asthma.

DNA was extracted from cord blood and whole blood at age 4.5 in 48 samples per time point. We analyzed DNA methylation of 23 regions in ten asthma and allergy candidate genes (ORMDL1, ORMDL2, ORMDL3, CHI3L1, RAD50, IL15, IL4, STAD1, FOXP3 and RUNX3) by pyrosequencing and compared differences between strata.

In cord blood, regions in ORMDL1 and STAD1 showed a hypomethylation while regions in RAD50 and IL15 were hypermethylated when DNA from farmers was compared to non-farmers (lowest p-value 0.001 for STAD1). Only small associations between asthma status and methylation were observed in our population.

Changes in methylation over time occurred in 14 out of 23 gene regions investigated (lowest p-value for IL15, p=1.57*10^{-8}). Interestingly, these differences clustered in the genes highly associated with asthma (ORMDL family) and IgE regulation (RAD50, IL15 and IL4) but not in the T-regulatory genes (FOXP3, RUNX3). Our study indicates that DNA methylation changes significantly in early childhood in specific asthma and allergy related genes in peripheral blood cells while also early exposure to farm life seems to influence methylation patterns in certain genes.

**4715** Does breastfeeding influence development of atopy? A cohort study

Cristian Dorgan1, Ania Pescatore1, Ben Spycher1, Caroline Beardmore2, Michael Silverman2, Claudia Kuehn1, 1 Institute for Social and Preventive Medicine, University of Bern, Switzerland; 2Department of Infection, Immunity & Inflammation, University of Leicester, United Kingdom

Aim: It has been postulated that breastfeeding (BF) reduces the risk of allergy in children. However, recent studies failed to show a protective effect or suggested an increased risk of atopy with BF. We examined the association between BF and atopy, measured by skin prick test (SPT), in a large population-based cohort, stratifying by maternal hayfever.

Method: We assessed BF in the first years of life in children from Leicestershire, UK. At school age (9-14 yrs), we performed SPT for 4 allergens (cat, dog, dust, grass) in 1515 children. A child was defined as atopic if any wheal size was >3mm.

We used logistic regression, adjusting for age, sex, ethnicity, birth season, family history of atopy and socio-economic status. We stratified by maternal hayfever and investigated potential reverse causation in analysis that excluded children with hayfever or wheezing in infancy.

Results: 465 (31%) children were not breastfed, 435 (29%) had BF <3 months (mo), 256 (17%) BF=4-6 mo and 348 (23%) BF >6 mo. 437 mothers (29%) had hayfever. We found no evidence for differences in risk of atopy by BF; the odds-ratios (95% confidence intervals) for BF<3 mo, BF=4-6 mo and BF>6 mo were 0.97 (0.69,1.37), 1.07 (0.72,1.59) and 1.19 (0.83,1.71), respectively, compared with no BF (p-trend=0.297). We did not find differential association by maternal hayfever (p-interaction=0.489) nor evidence for reverse causation.

Conclusion: Using objective measures and controlling for reverse causation, our study did not find evidence for an effect of breastfeeding on atopy in children of mothers either with or without hayfever. We can continue recommending breastfeeding for atopic and non-atopic mothers.