Background and aims Bronchopulmonary dysplasia (BPD) is an importantmorbidity in premature infants with an multifactorial aetiology. In recent years, both genetic and epigenetic mechanisms were suggested to play an important role in BPD development. NO (Nitric Oxide) which is produced along with L-Citrulline by the oxidation of L-Arginine and catalysed by three different isoforms of NOS (NOSynthase), is a short-lived free radical involved indiverse physiological and pathological processes. It consists of 3 types such as neuronal NOS, endothelial NOS and inducible NOS. All the NOS genes are expressed in airway epithelial cells and they are important for physiological functions in the airways. The aim of this study was to investigate possible association between eNOS gene polymorphism and development of BPD in preterm infants.
Methods One hundred and twenty two blood samples DNA isolation was carried out using the PureLink™ Genomic DNA Mini Kit and the concentration of the DNA samples was measured by nanophotometerImplen P 300. For the SNP analysis of eNOS (*rs1799983*) optimised primers were used. Real Time Polymerase Chain Reaction (QRT-PCR) was carried out in a CFX96 thermocycler. Chi-square $\chi 2$ test, Fisher's exact test, the odds ratio and confidence intervals were calculated for the comparisons of allelic and genotype frequencies.
Results Comparison of the allele frequency distribution revealed the presence of G allele as a highly significant risk factor for development of BPD (p = 0.000*; OR 4.07, 95% CI 2.066–8.009) compared to the T allele. The distribution of the T allele in eNOS was found to be similarly distributed amongst BPD (51.9%) and healthy control groups (48.1%). This study demonstrated that the frequency of the GG genotype (25.37%) of the eNOSgene was higher in babies with BPD rather than TT (53.6%) and TG (59.4)genotype, when these genotypes were compared with the healthy control groups. No healthy infants were seen to carry the GG genotype (p = 0.000*; OR 1.89, 95% CI 1.514–2.148). The TT genotype (p = 0.019*, OR 0.39, 95% CI 0.180–0.870) also displayed a susceptibility fordeveloping BPD. Heterozygous TG genotype (p = 0.631; OR 0.63, 95% CI 0.527–2.873) was not associated with the development of BPD.
Conclusion To our best of knowledge, noinvestigation of the eNOS gene polymorphism has previously been documented in BPD. Thefindings of this study demonstrated that the GG genotype in eNOS gene was highly significant for BPD.

ESPR – Young Investigators Presentations

O-214 GROWTH TRAJECTORIES AND BONE MINERAL DENSITY IN CHILDREN WITH SUBCLINICAL CELIAC DISEASE: THE GENERATION R STUDY

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Background Positive anti-tTG levels have been associated with reduced weight and bone mineral density (BMD) in clinical celiac disease (CD). However, effects in subclinical CD are not known.
Objective To assess associations between anti-tTG levels, growth and BMD in children with subclinical CD.
Methods In a population-based prospective cohort study, serum samples were analysed for anti-tTG levels at 6 years of age. (n = 4,*442*) Children were categorised into 2 groups: negative (< 7 U/ml, n = 4,*249*), or positive anti-tTG (>7 U/ml, n = *57*). Positive tTG levels were further categorised into 2 categories based on the > 10 times upper limit of normal (ULN) levels (70 U/ml). Height, weight and BMI z-scores were obtained using Dutch reference growth charts. BMD was measured by Dual-energy X-ray absorptiometry (DEXA). Multivariable linear regression and linear mixed models were performed.
Results Delayed growth in weight SDS/year (-0.05; 95% CI -0.09,-0.01) and BMI SDS/year (-0.10; 95% CI -0.18, -0.01) from 6 months until 6 years was observed in children with positive anti-tTG levels. Height growth tended to be delayed over time (-0.02 SDS/year; 95% CI -0.06, 0.02). A lower height (-0.29; 95% CI -0.55,-0.04), weight (-0.38; 95% CI -0.64,-0.12), BMI (-0.26 95% CI -0.49,-0.03) and BMD (-0.26; 95% CI -0.45, -0.08) at 6 years of age was found in children with positive anti-tTG levels.
Conclusion Positive anti-tTG levels in children without gastrointestinal symptoms at 6 years of age were associated with reduced BMD and delayed growth trajectories until 6 years. This suggests that subclinical CD has consequences for BMD and normal growth.

O-215 NECROTIZING ENTEROCOLITIS IS ASSOCIATED WITH HIPPOCAMPAL NEURON LOSS, MICROGLIAL ACTIVATION AND INCREASED IL-8 LEVELS IN PRETERM PIGS

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Background and aims Preterm birth predisposes to neurological sequelae. Necrotizing enterocolitis (NEC) may further increase the susceptibility to neurological damage, possibly via gut-derived inflammatory signals. To investigate this, we test if NEC severity and intestinal permeability in formula-fed preterm pigs is associated with histopathology, microglial activation, and increased proinflammatory cytokine levels in the hippocampus.
Methods Forty-four preterm piglets were fed increasing doses of formula and euthanized on day five. Macroscopic NEC lesions were scored in five regions of the gut (stomach, proximal, middle, and distal small intestine, colon). Intestinal permeability was assessed by urinary lactulose-mannitol-ratio. Hippocampal IL-1β and IL-8 levels were determined by ELISA. Histopathology, neurodegeneration, and microglia were investigated by analyses of hematoxylin-eosin, Fluoro-jade B (FJB), and Iba-1 stained coronal sections, respectively.
Results Proximal, middle, and distal small intestinal NEC score, and intestinal permeability correlated positively with IL-8 levels (all $p < 0.05$) but not with IL-1β. In preterm piglets with severe NEC lesions, numerous shrunken, hyperchromatic neurons were observed. Neurodegeneration was confirmed by positive FJB staining. Iba-1 positive cells with a morphology resembling

activated microglia populated the area in which neurons had disappeared.

Conclusions Acute development of NEC is associated with neuron loss, microglial activation, and increased IL-8 levels in the hippocampus of preterm pigs. Gut inflammatory disorders and increased intestinal permeability may affect the immature brain and contribute to long term neurological disorders.

O-216 MELATONINE REDUCES BBB BREAKDOWN IN A RAT MODEL OF NEONATAL EXCITOTOXIC DAMAGE

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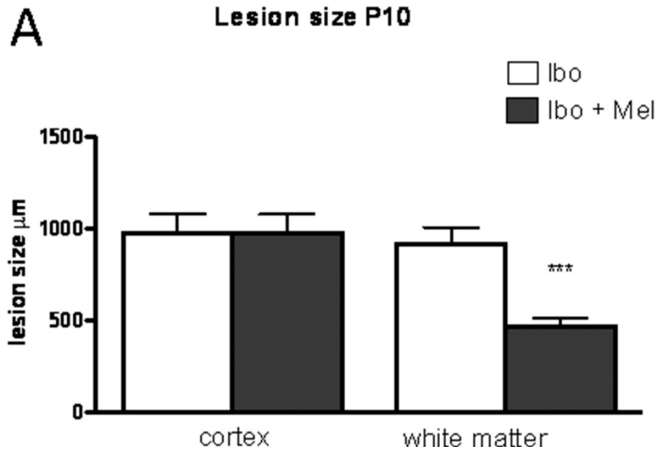
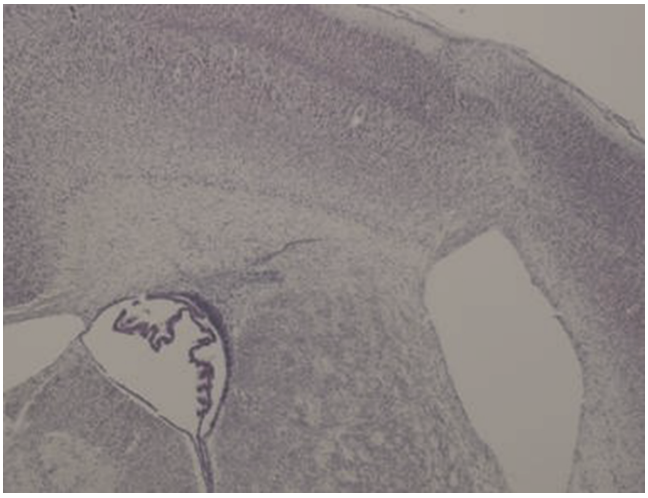
Objective The Blood-brain barrier (BBB) is a complex structure that protects the central nervous system (CNS) extracellular fluid from peripheral insults. Understanding the molecular basis and functioning of the BBB has a significant potential for future strategies to prevent and treat neurological disorders. The aim of our study was (1) to investigate BBB alterations in an excitotoxic model and (2) to test the protective properties of melatonin.

Methods The glutamate analogue ibotenate was injected intracerebrally in postnatal day 5 (P5) rat pups to mimic excitotoxic injury. Rats were sacrificed at P5+2 h, P5+4 h, P5+18 h. Lesion size and location of tight junction (TJ) proteins were determined by immunohistochemistry and BBB leakage after ibotenate injection by dextran staining. BBB proteins gene expression (TJs efflux transporters and detoxification enzymes) was determined on cortex and plexus. A group of pups was treated with melatonin (5 mg/kg, intraperitoneal).

Results Dextran extravasation was found 2 h after the insult, suggesting a rapid BBB breakdown that resolved at +4 h. A significant reduction in extravasation was observed in melatonin-treated pups. Molecular Biology, immunohistochemistry and electron microscopy showed a dynamic BBB modification during the first 4 h, partially reversed with melatonin. Lesion size evaluation confirmed melatonin white matter neuroprotection.



Abstract O-216 Figure 1 A quantitative analysis of lesion size in cortical plate and white matter induced by ic injection of ibotenate (ibo) in the presence or in the absence of ip melatonin injection 5 mg/kg



Abstract O-216 Figure 2 B, C Cresyl-violet stained sections showing brain lesions induced by ibotenate ic injected on P5 rat pups and studied at the age of P10. (B: PBS ip injection, C:5 mg/kg melatonin ip injection)

Interpretation Our study, for the first time, evaluates the BBB at a very early time point, and it demonstrates that excitotoxicity causes early BBB disruption and that at this phase melatonin neuroprotects by preventing TJ proteins modifications, before acting as an anti-inflammatory and antioxidant molecule, and promoting axonal regrowth.

O-217 ASSESSMENT OF MYOCARDIAL FUNCTION IN PRETERM INFANTS WITH CHRONIC LUNG DISEASE USING TISSUE DOPPLER IMAGING

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Background Chronic lung disease (CLD): oxygen requirement at 36 weeks corrected gestational age (CGA) is a significant neonatal morbidity which can have adverse effects on cardiac function until pre-school age.[1] Conventional echocardiographic techniques such as fractional shortening (FS) and left ventricular output (LVO) may not identify cardiac dysfunction in preterm infants. We have previously demonstrated that tissue Doppler imaging (TDI) is useful in assessment of myocardial function in these patients.[2]

Objectives To compare myocardial function in preterm infants born at.

Methods 50 preterm infants with CLD (25 receiving low flow nasal cannula oxygen and 25 receiving non-invasive positive airway pressure) and 22 without CLD (controls) had an echocardiogram at approximately 36 weeks CGA. Myocardial function was evaluated using FS, LVO and TDI. Ethical approval and written parental consent were obtained.

Results Median GA and birth weight of infants with CLD was lower than controls (27 wk (23–31) vs. 29 wk (23–31); 829 g (500–1790) vs.1030 g (570–1700)). There was no difference in persistence of PDA, tricuspid regurgitation, left ventricular FS and LVO between the groups. However, using TDI right ventricular peak systolic (S') and late diastolic velocities (A') ($p < 0.001$) were all significantly higher in CLD cases compared with controls.

Conclusion Cardiac dysfunction in this vulnerable group of patients can be better identified with TDI compared to FS and