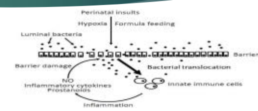
Candidate gene analysis in pathogenesis of surgically and nonsurgically treated necrotizing enterocolitis in preterm infants.

DAWID SZPECHT [1] • NATALIA NEUMANN-KLIMASIŃSKA [1] • MICHAŁ BŁASZCZYŃSKI [2] • AGNIESZKA SEREMAK-MROZIKIEWICZ [3,4] • GRAŻYNA KURZAWIŃSKA [3] • DOROTHY CYGAN [5] • MARTA SZYMANKIEWICZ [1] • KRZYSZTOF DREWS [3] • JANUSZ GADZINOWSKI [1]

1. CHAIR AND DEPARTMENT OF NEONATOLOGYPOZNAN UNIVERSITY OF MEDICAL SCIENCESPOZNANPOLAND
2.DEPARTMENT OF PEDIATRIC SURGERY, TRAUMATOLOGY AND UROLOGYPOZNAN UNIVERSITY OF MEDICAL SCIENCESPOZNANPOLAND
3.DEPARTMENT OF PERINATOLOGY AND WOMEN'S DISEASESPOZNAN UNIVERSITY OF MEDICAL SCIENCESPOZNANPOLAND
4.DEPARTMENT OF PHARMACOLOGY AND PHYTOCHEMISTRYINSTITUTE OF NATURAL FIBERS AND PLANTSPOZNANPOLAND
5.POZNAN UNIVERSITY OF MEDICAL SCIENCESPOZNANPOLAND

BACKGROUND

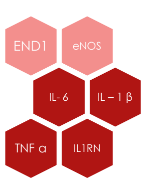
- Necrotizing enterocolitis (NEC) is one of the most severe and unpredictable complications of prematurity
- NEC is a complex, multifactorial disease; pathogenesis unclear, no specific treatment
- According to the present consensus, NEC develops in the premature intestine in the setting of improper bacterial colonization
- Altered immune response and disruption in gut blood flow are often present



Grishin A, Bowling J, Bell B et al (2016) Roles of nitric oxide and intestinal microbiota in the pathogenesis of necrotizing enterocolitis. J Pediatr Surg 51:13–17.

Chokshi NK, Guner YS, Hunter CJ, Upperman JS, Grishin A, Ford HR. The role of nitric oxide in intestinal epithelial injury and restitution in neonatal necrotizing enterocolitis. Semin Perinatol. 2008; 32(2): 92-99.

AIM of the study



- to evaluate the possible **relationship between NEC and gene polymorphisms for cytokines** (interleukin-1beta, interleukin-6; TNFalpha; interleukin-1 receptor antagonist); as well as **vasoactive agents** (endothelial NO synthase and endothelin-1)

Studied polymorphisms

- Il-1b -3953C>T
- Il-6 -174G>C
- Il-6 -596G>A
- TNFα -308G>A
- Il-1RN VNTR 86 bp
- eNOS -894G>T
- eNOS -786T>C
- END-1 -5665G>T

METHOD

- 100 neonates admitted to the Neonatal Intensive Care Unit at the Poznan University of Medical Sciences
- singletons between 24 + 0 and 32 + 0 weeks of gestation
- complete antenatal steroid therapy
- exclusion criteria: no parental consent; chromosomal abnormalities, TORCH infections; inborn errors of metabolism
- a blood sample (0.5 ml) was taken directly post-delivery and banked
- genomic DNA was extracted from blood leukocytes; polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) procedures

RESULTS

- median gestational age of enrolled infants was 28 + 4 ; median birth weight was 1124.4 ± 378.7 grams
- 22 infants developed NEC; surgery requiring NEC was present in 7 children (7% of the population); mortality rate of 22%; surgical NEC mortality rate of 28.5%

Statistical analysis showed 20-fold higher prevalence of NEC in infants with the genotype *TT* (OR 20 (3.71–208.7); p=0.0004) of eNOS *894G>T* gene polymorphism

There was a higher prevalence of allele C carriers of eNOS *786T>C* in patients with surgery-requiring NEC (OR 4.881 (1.33–21.99); p=0.013)

Our investigation did not confirm any significant prevalence for NEC development in another studied genotypes/alleles.

eNOS

- Regulation of vascular resistance is determined by a balance between the endothelial production of the vasoconstrictor peptide endothelin-1 (ET-1) and the vasodilatory free radical nitric oxide (NO)
- Nitric oxide synthase activity could be crucial for **intestinal microcirculation**
- The studied eNOS gene polymorphism in which guanine (G) is replaced with thymine (T) at nucleotide 894 (exon 7), results in a **change of the amino acid sequence** Glu298Asp.
- The -786T>C polymorphism of the eNOS gene replaces thymine with cytosine in the eNOS **gene promoter** at position 786.
- According to Veldman et al. **in the presence of *894G>T* and -*786T>C* polymorphic variants eNOS enzymatic activity is impaired**

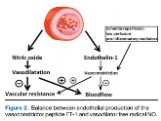


Figure 2. Balance between endothelial production of the vasoconstrictor peptide ET-1 and vasodilator free radical NO.

J Pediatr. 2011 Feb;158(2 Suppl):e25-8. doi: 10.1016/j.jpeds.2010.11.009.
Ischemia-reperfusion and neonatal intestinal injury.
Yazigi RA, Kinsella SD, Neu J.

DISCUSSION

- epidemiologic evidence shows that the most common type of necrotizing enterocolitis is not triggered by a primary hypoxic-ischemic event
- ischemia-reperfusion model of gut injury in NEC is controversial
- pro-inflammatory mediators could alter the ET-1–NO balance in favor of vasoconstriction
- decreased levels of NO in homozygous TT of eNOS 894G>T gene polymorphisms and allele C carriers of eNOS -786T>C may further promote vasoconstriction and lead to necrosis

CONCLUSION

1. Nitric oxide could play a significant role in the pathogenesis of necrotizing enterocolitis.

2. Individuals with genetic predisposition are more susceptible to NEC and surgery requiring NEC

THANK YOU FOR YOUR ATTENTION