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Characterization of the small intestinal gene expression response in a preterm pig model of necrotizing enterocolitis

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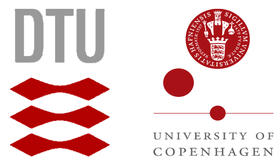

Aim
To investigate how expression of epithelial- and immune response-related genes in distal small intestinal tissue is affected by necrotizing enterocolitis (NEC) in a preterm pig model of NEC.

Conclusion
The results points to inflammation and loss of intestinal integrity as being important components of NEC. Further studies should address the relationship of inflammation related gene expression and the development of NEC in order to elucidate cause-effect relationships leading to NEC.

Introduction
NEC is a the serious gastrointestinal disease in preterm infants, and is caused by the combined effect of abnormal bacterial colonization, enteral feeding, and prematurity, including immaturity of the immune system.
In a well-established preterm pig model of NEC, the effect of diet on disease development has been studied thoroughly; however, the inflammatory response during NEC needs to be characterized to elucidate cause-effect relationships leading to NEC, and to improve and promote the use of this model as a model for human disease.

Experimental design (Figure 1)
Preterm pigs were delivered by Cesarean section and given total parenteral nutrition (TPN) for 2 days followed by enteral nutrition: bovine colostrum (n = 6) or 6 hours of milk formula followed by continued milk formula (n = 13), bovine colostrum (n = 14), reconstituted spray dried bovine colostrum (n = 7), or reconstituted pasteurized, spray dried bovine colostrum (n = 9).
Pigs were euthanized after 2 days of enteral feeding, and the gastrointestinal tract (stomach, proximal-, mid- and distal small intestine and colon) was evaluated for NEC lesions using a severity score ranging from 1–6 (6 being severe NEC). Pigs with a severity score of minimum three in any gastrointestinal region was regarded as a case of NEC. High throughput qPCR was used to investigate the gene expression of 48 genes in distal small intestinal tissue.

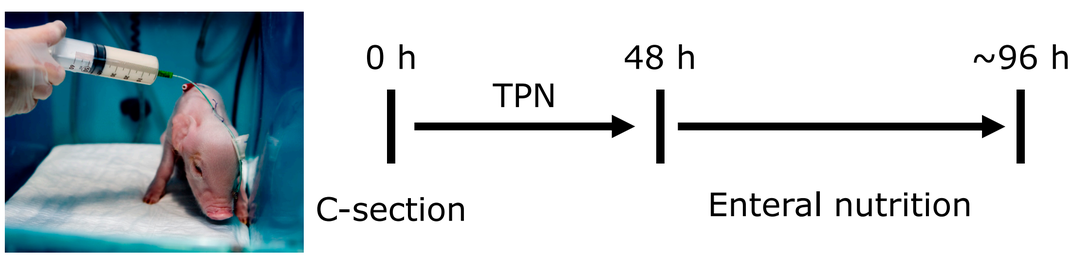

Figure 1. Experimental design.

Results
28 pigs developed NEC (mean NEC score = 3.0 ± 0.1) while 21 stayed healthy (mean NEC score = 1.0 ± 0.03).
Irrespectively of enteral diet group, the expression of four genes (*CCL3*, *IL1RN*, *IL6* and *IL8)* coding for proteins involved in inflammation was increased in pigs suffering from NEC compared to healthy pigs (**Figure 2**). With higher NEC severity in the distal small intestine, a more diverse gene expression pattern between pigs was observed in principal component analysis (PCA; **Figure 3**). Six genes involved in inflammation correlated positively, while 2 genes coding for tight junction proteins involved in maintaining intestinal permeability were negatively correlated with NEC severity (**Table 1**).

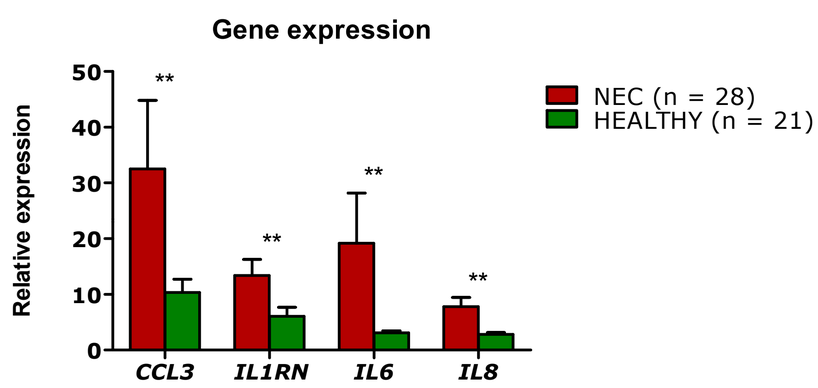

Figure 2. Relative gene expression (means ± SEM). **within each gene indicate significant differences, p < 0.01.

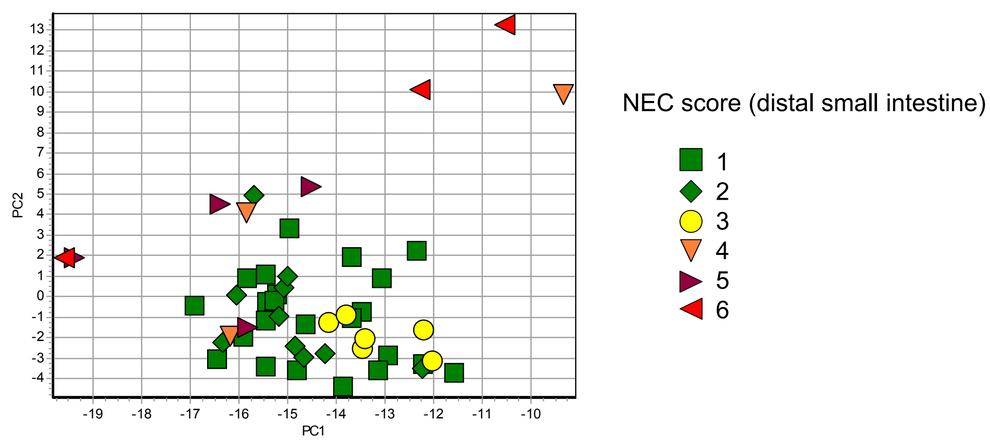

Figure 3. PCA plot of expression data.

Table 1. Spearman correlations between gene expression and distal small intestinal NEC score.

Gene	Function of protein	r	p[1]
CD14	Co-receptor for LPS detection	0.415	**
IL6	Anti- and proinflammatory	0.387	**
IL8	Chemoattractant for neutrofils	0.417	**
CCL3	Recruitment /activation of polymorphonuclear leukocytes	0.485	***
IL1RN	Inhibitor of the pro-inflammatory effect of IL1β	0.493	***
CD163	Receptor that marks monocyte/macrophage lineage	0.380	**
CLDN3	Tight junction	-0.420	**
OCLN	Tight junction	-0.364	*

[1] * p < 0.05, ** p < 0.01, *** p < 0.001