**Supplemental files**

**Impact of Insulin-like Growth Factor 1 and Preterm Birth on Kidney Development in Necrotizing Enterocolitis-sensitive Pigs**

Jingren Zhong1, Thomas Thymann1, Per Torp Sangild1,2,3, Duc Ninh Nguyen1 and Tik Muk1\*

1 Section for Comparative Pediatrics and Nutrition, Department of Veterinary and Animal Sciences, University of Copenhagen; 2 Department of Pediatrics, Odense University Hospital, Odense, Denmark; 3 Department of Neonatology, Rigshospitalet, Copenhagen, Denmark

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**Supplemental Table S1 List of gene names, primer sequences and amplicon length of primers used in the study.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene name** | **Gene symbol** | **Sequence (5' to 3')** | **Sequence (5' to 3')** | **Amplicon length** |
| HPRT1 | Hypoxanthine phosphoribosyltransferase 1 | TATGGACAGGACTGAACGGC | ATCCAGCAGGTCAGCAAAGA | 115 |
| CASPASE3 | Caspase 3 | CCGGAATGGCATGTCGATCT | CATGGCTTAGAAGCACGCAA | 189 |
| GATA3 | GATA binding protein 3 | ACCCCTTATTAAGCCCAAGC | TCCAGAGAGTCGTCGTTGTG | 92 |
| IL10 | Interleukin 10 | GTCCGACTCAACGAAGAAGG | GCCAGGAAGATCAGGCAATA | 73 |
| IL6 | Interleukin 6 | TGGGTTCAATCAGGAGACCT | CAGCCTCGACATTTCCCTTA | 116 |
| TBET | T-box transcription factor 21 | CTGAGAGTCGCGCTCAACAA | ACCCGGCCACAGTAAATGAC | 121 |
| TNFA | Tumor necrosis factor alpha | ATTCAGGGATGTGTGGCCTG | CCAGATGTCCCAGGTTGCAT | 120 |
| HIF1A | Hypoxia-inducible factor 1 alpha | TGTGTTATCTGTCGCTTTGAGTC | TTTCGCTTTCTCTGAGCATTC | 96 |
| KIM1 | Kidney injury molecule-1 | ATGTACCCTTGGGTAACCGC | AACGTAGAACATGCCCCTCG | 164 |
| NGAL | Neutrophil gelatinase-associated lipocalin | AAGACGGCAGCTACAACGTC | GACACCACACGCACGACATA | 157 |
| LRG1 | Leucine Rich Alpha-2-Glycoprotein 1 | TGACCTGCACATCCTTGACC | CAGAAAGCCCTCTTCGAGCA | 133 |
| IGFBP7 | Insulin like growth factor binding protein 7 | ATCGTGACACCCCCTAAGGAC | ATAGTGACCCCTTTGTACCTTGT | 120 |
| WNT11 | Wnt family member 11 | CTTGACCTGGAGAGAGGGACC | ATGAGGAGCCCGTAGCTGAG | 197 |
| WNT4 | Wnt family member 4 | CACAAGGCTTCCAGTGGTCA | GCATGTGTGTCAGGATAGCCTTC | 168 |
| WNT9b | Wnt family member 9B | CTGCTCGAGTGCCAGTTTCA | CCGTCTCCTTGAAACCTCTCTT | 94 |
| VEGFA | Vascular endothelial growth factor A | ATGCGGATCAAACCTCACCA | TGTCACATCTGCAAGTACGTTCG | 224 |
| TGFB1 | Transforming growth factor beta 1 | GCAAGGTCCTGGCTCTGTA | TAGTACACGATGGGCAGTGG | 97 |
| TGFB2 | Transforming growth factor beta 2 | GCGCGATTTGCAGACTTGAG | ATGTAAAGTGGACGCAGGCA | 170 |
| REN | Renin | TCTCCGTCTACTACAGCAGGA | CCTCACAGACACCCCTTTCA | 152 |
| RET | Ret proto-oncogene | GGAAGATAACCAGGACCCGC | GTTCTTGTGGTAGCGGTGGA | 124 |
| SIX2 | SIX homeobox 2 | CTACCCCTCACCCCGAGAGA | TTCTCGCTGTTCTCCTCGTACC | 141 |
| CTNNB1 | Catenin beta 1 | ATTGAAGCTGAGGGAGCCAC | GAACTGGTCAGCTCAACCGA | 152 |
| AT1 | Angiotensin II receptor type 1 (AGTR1) | GGTCTACATCCAGGTGCATT | GGGGCAGTCATCTTGGATTC | 116 |
| CDH1 | E-cadherin | GCTGGACCGGGAGAGTTTTC | TGAAGATGGGTGGGTTGTCG | 131 |
| GDNF | Glial cell derived neurotrophic factor | CCTGGGCGTGTGGATGTTTA | GCCATCTGTTTATCGGGGGA | 132 |
| IGF-1 | Insulin-like growth factor 1 | ATTTCTTGAAGGTAAAGATGCA | CAGCCCCACAGAGGGTCTCA | 117 |

**Supplemental Table S2 Circulating IGF-1 contents (ng/ml) in the plasma of pigs.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **Group** | | |  | ***p*-value** | |
|  |  |  | **Preterm** | **IGF-1** | **Term** |  | **Preterm/IGF-1** | **Preterm/Term** |
| **Day 5** |  |  | n=37 | n=24 | n=7 |  |  |  |
| **IGF-1 contents** | |  | 10.64±0.64 | 50.19±3.96 | 16.13±2.94 | | <0.001 | 0.048 |
|  |  |  |  |  |  |  |  |  |
| **Day 9** |  |  | n=15 | n=21 | n=7 |  |  |  |
| **IGF-1 contents** | |  | 18.74±2.69 | 87.63±11.71 | 46.87±7.15 | | <0.001 | 0.125 |
|  |  |  |  |  |  |  |  |  |
| **Day 19** |  |  | n=13 | n=16 | n=7 |  |  |  |
| **IGF-1 contents** | |  | 42.94±2.53 | 139.32±9.75 | 45.45±8.55 | | <0.001 | 0.999 |
|  |

**Supplemental Figure S1 The relative expression of kidney function, injury and immunity genes in pigs.** From PND5 to 19, the n values for preterm are 23-24, 13-14, 11; for IGF-1 are 12-13, 22, 14-16; for term are 7, 6, 7. Data are presented as mean ± SEM. \*,\*\*, \*\*\* means *p* < 0.05, 0.01 and 0.001, respectively.

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**Supplemental Figure S2 Pearson correlation analysis of kidney inflammation and injury related gene expression on PND5.** (A), (B), (C) and (D) are the correlation of *KIM1* to *TNFA*, *LRG1* to *TNFA*, *KIM1* to *IL10*, and *LRG1* to *IL10*, respectively.

Graphical user interface, application

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