

Introduction

- Maternal immune activation (MIA) is associated with behavior alterations in the offspring that can impact production, costs, and management practices (1).
- The amygdala plays a central role in many neurological pathways and sexually dimorphic behaviors, and MIA's effects on this structure are not well understood.
- Porcine reproductive and respiratory syndrome virus (PRRSV) is an established model for eliciting MIA in pigs (2,3), and PRRSV is also an economic burden to the pork industry, resulting in poorer performance and productivity.

Objective

- The goal of this study was to identify molecular pathways that present differential vulnerability to MIA between sexes.
- These pathways can aid in the development of treatments to minimize the effect of MIA.

Methods

- The animal studies were approved by the Illinois Institutional Animal Care and Use Committee (IACUC) at the University of Illinois, and are in compliance with the USDA Animal Welfare Act and the NIH Public Health Service Policy on the Humane Care and Use of Animals.
- On gestation day (GD) 76, eight gilts were either intranasally inoculated with live PRRSV or with saline.
- Farrowing was induced on GD 113 10 mg of Lutalyse.
- The offspring were evenly distributed between maternal PRRSV activated (MPA) and Control gilts (CON) totaling twenty-four, with males and females in each group (Ma and Fe, respectively).
- 3 weeks after birth, pigs were anesthetized, euthanized, and amygdalae were removed and flash frozen.
- RNA was extracted from the amygdalae using EZNA isolation kit.
- RNA libraries were generated with the TruSeq Stranded mRNAseq Sample Prep kit, and 150-nt paired-end reads were generated on one lane of a NovaSeq 6000.
- Reads were aligned to the *Sus scrofa* genome (version Sscrofa 11.1 (4)) using kallisto v0.43.0 (5).
- Genes supported by > 5 transcripts per million were described using a generalized linear model and analyzed using edgeR (version 3.14.0) in the R v. 3.3.1 environment (6).
- The over-representation of functional categories was evaluated among genes that exhibited a significant MIA-by-sex interaction using the Gene Set Enrichment Analysis (GSEA) approach with the software package GSEA-P 2.0 (7).

Results

Table 1. Genes exhibiting significant (FDR-adjusted P-value < 0.1) maternal immune activation-by-sex interaction effect.

Gene Symbol	P-value	^a CON Fe- CON Ma	MPA Fe- MPA Ma	CON Fe- MPA Fe	CON Ma- MPA Ma	CON Fe- MPA Ma	CON Ma- MPA Fe
CGA	<5E-11	-5.86	0.36	0.27	6.49	0.63	6.13
VIPR2	<5E-11	-1.17	1.04	-1.03	1.18	0.00	0.14
GRM4	<5E-11	-0.97	0.91	-0.70	1.17	0.20	0.27
CRHR2	2.8E-06	-0.24	1.09	-0.36	0.97	0.74	-0.12
GRP	1.5E-05	-0.89	0.73	-0.61	1.02	0.12	0.28
PENK	1.6E-05	-0.10	0.46	-0.04	0.51	0.42	0.06
PTH1R	3.7E-05	-0.33	0.52	-0.51	0.34	0.01	-0.18
NTS	7.9E-04	-1.43	2.81	-1.78	2.47	1.03	-0.35

^aLog₂[fold change] between two maternal immune activation-sex groups: MPA = PRRSV-induced maternal immune activation; CON = control; Fe = females; Ma = males.

Table 2. Enriched informative categories (NES > |1.3|) using GSEA among the genes based on the overall maternal immune activation-by-sex interaction.

^a Category	Category Identifier and Name	^b NES	P-value	^c FDR P-value
KEGG	ssc04080:Neuroactive ligand receptor interaction	-1.84	< 1.0E-10	8.3E-02
KEGG	ssc04912:GnRH signaling pathway	-1.83	< 1.0E-10	9.9E-02
MF	GO:0005179~Hormone activity	-1.80	< 1.0E-10	2.2E-01

^aMF: molecular function; KEGG: KEGG pathway

^b normalized enrichment score; negative values indicate genes under-expression in CON females relative to males but over-expression in MPA females relative to males.

^c False Discovery Rate adjusted P-value.

Discussion

- 328 genes were found to have a significant MIA-by-sex interaction (FDR-adjusted P-value < 0.10).
- Many genes, such as vasoactive intestinal peptide receptor 2 (VIPR2), proenkephalin (PENK), and glutamate metabotropic receptor 4 (GRM4), were under-expressed in MPA relative to CON males, with the opposite profile in females (Table 1).
- The over-expression of VIPR2 in MPA compared to CON females is supported by evidence that duplications in this gene is associated with certain neurodevelopmental disorders (8). Additionally, this receptor plays a significant role in the excitatory/inhibitory circuitry of the amygdala (9).
- The under-expression of PENK and GRM4 in MPA relative to CON males are in agreement with lower expression of these genes in the brains of mice models and humans, respectively, with neurodevelopmental and behavioral disorders (10, 11).
- The KEGG pathway neuroactive ligand receptor interaction was enriched among the genes under-expressed in the amygdala of CON relative to MPA males (Table 2). This pathway encompasses neuroreceptor genes such as dopamine, serotonin, GABA, and glutamate receptors. This pattern is aligned with findings that Poly(I:C)-elicited MIA augmented the synaptic strength of glutamatergic projections from the frontal cortex into the amygdala of mice (12).
- The GnRH signaling pathway and hormone activity were enriched among the genes under-expressed in MPA relative to CON pigs (Table 2), indicating the strong impact of the sex-dependent MIA effects on neuropeptide hormones. Our results suggest that the disruption of glucocorticoid hormone balance on the hypothalamic pituitary adrenal axis initiated by MIA can have long-lasting effects because amygdala processes are regulated by glucocorticoid receptors and glucocorticoids repress GnRH secretion.

Conclusion

- The transcript and network dysregulation uncovered in this study have been associated with neurological abnormalities and behavioral disorders.
- These findings advance the understanding necessary to develop sex-dependent treatments that ameliorate the effect of MIA on the performance of pigs later in life.

Acknowledgements

- This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

References

- Canetta, S. *et al.* Maternal immune activation leads to selective functional deficits in offspring parvalbumin interneurons. *Mol Psychiatry* **21**, 956-968, doi:10.1038/mp.2015.222 (2016).
- Antonson, A. M. *et al.* Maternal viral infection during pregnancy elicits anti-social behavior in neonatal piglet offspring independent of postnatal microglial cell activation. *Brain Behav Immun* **59**, 300-312, doi:10.1016/j.bbi.2016.09.019 (2017).
- Antonson, A. M. *et al.* Altered Hippocampal Gene Expression and Morphology in Fetal Piglets following Maternal Respiratory Viral Infection. *Dev Neurosci* **40**, 104-119, doi:10.1159/000486850 (2018).
- Pruitt, K. D., Tatusova, T. & Maglott, D. R. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res* **35**, D61-D65, doi:10.1093/nar/gkl842 (2007).
- Bray, N. L. *et al.* Near-optimal probabilistic RNA-seq quantification (vol 34, pg 525, 2016). *Nat Biotechnol* **34**, 888-888, doi:10.1038/nbt0816-888d (2016).
- Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-140, doi:10.1093/bioinformatics/btp616 (2010).
- Subramanian, A. *et al.* GSEA-P: A desktop application for Gene Set Enrichment Analysis. *Bioinformatics* **23**, 3251-3253, doi:10.1093/bioinformatics/btm369 (2007).
- Morris, B. J. & Pratt, J. A. Novel treatment strategies for schizophrenia from improved understanding of genetic risk. *Clin Genet* **86**, 401-411, doi:10.1111/cge.12485 (2014).
- Rhomberg, T. *et al.* Vasoactive Intestinal Polypeptide-Immunoreactive Interneurons within Circuits of the Mouse Basolateral Amygdala. *J Neurosci* **38**, 6983-7003, doi:10.1523/JNEUROSCI.2063-17.2018 (2018).
- Gottschalk MG. *et al.* Estudos tradicionais de neuropsiquiatria e esquizofrenia: modelos animais genéticos e de neurodesenvolvimento. *Archives of Clinical Psychiatry (São Paulo)* (2013) 40:41-50.
- Meador-Woodruff, J. H. & Healy, D. J. Glutamate receptor expression in schizophrenic brain. *Brain Res Rev* **31**, 288-294, doi:10.1016/S0165-0173(99)00044-2 (2000).
- Li, Y. *et al.* Maternal and Early Postnatal Immune Activation Produce Dissociable Effects on Neurotransmission in mPFC-Amygdala Circuits. *J Neurosci* **38**, 3358-3372, doi:10.1523/JNEUROSCI.3642-17.2018 (2018).