

Final Report—April, 2009

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Direct Sponsor: NC Pork Council

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Proposal Title: Identification of pig genes that play a role in infection caused by porcine reproductive and respiratory syndrome (PRRS) virus

Project Summary: Porcine Reproductive and Respiratory Syndrome (PRRS) is a contagious disease of pigs and a worldwide problem for the swine industry. PRRS virus (PRRSv) infections can cause respiratory problems in pigs at all stages of development, disrupt growth, increase pre-weaning mortality, and induce abortions in sows. Strategies for controlling PRRS have mostly been based on management practices that reduce exposure to the virus and on development of vaccines that reduce losses. Despite these control measures, PRRS continues to be a great threat because the virus can easily mutate and make current vaccines less effective. A more permanent and economic solution to PRRS would be to develop strains of swine that are genetically resistant or tolerant to viral infection. To achieve this, we first need to know how certain genes of both the virus and pig function in the disease process.

The objective of this proposal is to identify and map the specific pig genes that are actually involved in PRRSv infection. To achieve this goal, we have used DNA microarray analysis to identify these genes. This technique determines if a gene is turned on or off, and can do so for thousands of genes at once. We can tell which genes are turned on and off in response to viral infection by comparing the on/off profile of infected cells with the on/off profile of uninfected cells. This information will let us decide which genes are likely to be important for the disease process. Finally, we will use a genome mapping approach to locate the identified genes in the pig genetic map.

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Porcine macrophage cells before and after PRRS viruses infection were collected, and their mRNAs were purified and labeled with Cy3 (green color; 24h PRRSV-infection) or Cy5 (red color; 24h mock-infected) by RT (reverse transcription) reactions. Both labeled mRNA sets were competitively hybridized onto 13K Porcine Oligoarray distributed by Michigan State University. A total of 587 genes were differentially expressed with fold changes greater than 2.5 (examples shown in Table1 and 2). Of the 587 differential genes, 222 genes were up-regulated and 547 genes were down-regulated upon PRRSV infection. In order to understand the biological significance of these results, bioinformatics analysis was carried out using the Database for Annotation, Visualization and Integrated Discovery (DAVID) accessible at <http://david.abcc.ncifcrf.gov/>. DAVID is a web-based program developed at the National Institute of Allergy and Infectious Diseases (NIAID), NIH. DAVID web-tools facilitate microarray data interpretation by grouping functionally related gene groups and identifying enriched biological themes. A homology search of pig genes was performed to obtain their human orthologs. These human orthologs were used for functional and pathway mapping analyses because in several human genes and/or proteins are better annotated compared to other species. The 587 differential genes were significantly enriched for gene ontology (GO) terms that

related to several different biological processes including program cell death or apoptosis (44 genes), transcription response to biotic stimuli (17 genes), co-activator activity (16 genes) and stress response (8 genes). In addition, pathway analysis was performed using BioRag (Bioresource for array genes) at www.biorag.org, provided by the Arizona Cancer Center and Southwest Environmental Health Sciences Bioinformatics Core. Differentially expressed genes were assigned to various metabolic, cellular and regulatory pathways. Interestingly, several immune related pathways were also identified in this dataset and are summarized in Table 3. As shown in Figure 1, the BioRag program assigned differential genes from the microarray analysis to several immune related pathways including Jak-STAT signaling, antigen processing and presentation, cytokine and inflammatory response and complement and coagulation cascades. These results suggest that PRRSV infection activates various immune pathways, the identification of which will provide a better understanding of PRRSV pathogenesis and candidate genes for further study.

This set of preliminary results that gathered in this project has enabled us to put together a more comprehensive proposal designed to address the questions generated above. We were successful in obtaining funding for this new proposal from the USDA (grant title: “Host cell-virus interactions required for PRRSV,” awarded from USDA PRRS-CAP Integrated Program. PIs: Liu, Rutherford, and Yoo). In addition, the technique of gene expression analysis developed from this work allowed us to apply to a different study which involved iron (Fe) dietary treatment. Two manuscripts are under review and this grant has been acknowledged in the manuscripts.

Table 1. Up-regulated gene list

Gene Annotation	Log2 ratio
homologue to GP 16041824 gb AAH15802.1 Unknown	10.16
homologue to SP P18583 SON_HUMAN SON protein	9.39
similar to GP 11876970 emb CAC19003. Human epithelial-restricted with serine box	9.37
homologue to SP P28480 TCPA_RAT T-complex protein 1 alpha subunit	9.31
similar to PIR G02371 G02371 U1-snRNP binding protein homolog - human	8.91
GP 12847957 dbj BAB27774. putative	8.40
homologue to GP 12006035 gb AAG44723.1 NPD016	8.28
homologue to GP 15011995 gb AAH10892.1 Unknown	7.90
homologue to SP Q00973 CAG2_HUMAN Beta-1 4 N-acetylgalactosaminyltransferase	7.65
similar to GP 10438785 dbj BAB15342. unnamed protein product	7.51
homologue to GP 14328050 gb AAH09239.1 Unknown	7.51
homologue to GP 1247124 emb CAA01866.1 TGR-CL10C	7.50
homologue to SP P09012 RU1A_HUMAN U1 small nuclear ribonucleoprotein A	7.28
similar to EGAD 31728 32798 PH-20 homolog	6.96
similar to GP 5689507 dbj BAA83037.1 KIAA1085 protein	6.51
weakly similar to GP 12838275 dbj BAB24148. putative	6.46
similar to SP O96018	6.24
alpha-lactalbumin	6.16
mahogany [Sus scrofa]	6.07
similar to SP P09581 KFMS_MOUSE Macrophage colony stimulating factor I receptor	6.07

homologue to PIR S61532 S61532 RET oncogene fusion partner RFG - human	5.93
similar to SP Q9UNZ5 L10K_HUMAN Leydig cell tumor 10 kDa protein homolog	5.86
olfactory receptor [Sus scrofa]	5.84
heat shock protein 70.2 [Sus scrofa]	5.78
homologue to SP Q00610 CLH1_HUMAN Clathrin heavy chain 1	5.65
Arabidopsis thaliana papain-type cysteine endopeptidase XCP2	5.55
homologue to GP 5762305 gb AAD51094.1 COP1 protein	5.51
homologue to SP Q29486 PPBT_FELCA Alkaline phosphatase	5.50
homologue to SP Q02734 CAG6_RAT	5.50
similar to GP 10438776 dbj BAB15338. unnamed protein product	5.40
similar to SP Q9NTQ9 CXB4_HUMAN Gap junction beta-4 protein	5.31
similar to GP 12653555 gb AAH00551.1 lysophospholipase-like	5.30
similar to GP 13477117 gb AAH05013.1 hypothetical protein FLJ22347	5.23
similar to GP 2654606 gb AAC39546.1 putative mitotic checkpoint protein kinase	5.21
homologue to PIR S27226 S27226 NADH dehydrogenase	5.16
homologue to GP 14042445 dbj BAB55249. unnamed protein product	5.12
weakly similar to PIR T21659	5.11
homologue to GP 1495483 emb CAA66379.1 RAD54	5.04
homologue to GP 13489043 gb AAK27794.1 growth/differentiation factor 7	5.00
NADPH-cytochrome P-450 oxidoreductase	4.99
similar to GP 15559497 gb AAH14114.1 Similar to RIKEN cDNA 1200014D22 gene	4.97
branched chain aminotransferase [Sus scrofa]	4.90
homologue to GP 9247092 gb AAF86279.1 Ras related small G protein RAL-A	4.87
similar to GP 12082168 dbj BAB20793. protein phosphatase 2A inhibitor-2 I-2PP2A	4.87

similar to GP 14250444 gb AAH08658.1 Unknown	4.83
similar to GP 17389633 gb AAH17842.1 pronapsin A	4.78
ribosomal protein S4 [Sus scrofa]	4.62
similar to GP 12804117 gb AAH02911.1 Unknown	4.56
homologue to GP 12653747 gb AAH00659.1 Unknown	4.49
homologue to GP 10241708 emb CAC09439. hypothetical protein	4.45
GP 14790021 gb AAH10856.1 Unknown	4.44
GP 12653445 gb AAH00494.1 eukaryotic translation initiation factor 2B subunit 2	4.41
homologue to PIR JC4945 JC4945 ADP-ribosylation factor 1 - mouse	4.41
similar to GP 15209726 emb CAC51162. unnamed protein product	4.40
folate binding protein [Sus scrofa]	4.38
homologue to GP 15559382 gb AAH14058.1 presenilins associated rhomboid-like protein	4.34
homologue to SP O95985 TP3B_HUMAN DNA topoisomerase III beta-1	4.24
Arabidopsis thaliana mRNA for rubisco activase	4.23
heat shock protein 70 [Sus scrofa]heat shock protein 70	4.21
homologue to GP 15080543 gb AAH12010.1 Similar to RIKEN cDNA 5031425D22 gene	4.21
similar to GP 220434 dbj BAA00500.1 heparin binding protein 44	4.21
homologue to GP 17900927 emb CAD19357. unnamed protein product	4.19
homologue to GP 6634025 dbj BAA20833.2 KIAA0379 protein	4.16
apical organic cation transporter [Sus scrofa]	4.15
complement component 3 [Sus scrofa]complement component C3 [Sus scrofa]	4.11
similar to GP 12484136 gb AAG53983.1 SMHS2	4.11
homologue to GP 4929575 gb AAD34048.1 CGI-53 protein	4.04
homologue to GP 14602914 gb AAH09952.1 Similar to hypothetical protein FLJ12838	4.03
homologue to GP 204270 gb AAA41202.1 guanylate cyclase	4.02

Ig heavy chain variable VDJ region 3.98

Ig heavy chain variable VDJ region	3.98
similar to GP 14279576 gb AAK58638.1 interferon-induced protein 1-8U	3.94
Arabidopsis thaliana papain-type cysteine endopeptidase XCP2	3.92
SP P01252 THYA_BOVIN Prothymosin alpha. [Bovine]	3.91
similar to GP 12698027 dbj BAB21832. KIAA1741 protein	3.90
homologue to GP 15559417 gb AAH14077.1 Unknown	3.90
homologue to PIR S29170 S29170 annexin VII - mouse	3.89
prointerleukin-1 beta	3.88
homologue to SP P08760 KAD3_BOVIN GTP:AMP phosphotransferase mitochondrial	3.87
homologue to GP 4325215 gb AAD17301.1	3.84
similar to SP Q92844 TANK_HUMAN TRAF family member-associated NF-kappa-b	3.80
homologue to SP P57780 AAC4_MOUSE Alpha-actinin 4	3.78
homologue to GP 3327166 dbj BAA31651.1 KIAA0676 protein	3.77
SP P33552 CKS2_HUMAN Cyclin-dependent kinases regulatory subunit 2	3.72
GP 12845815 dbj BAB26910. putative	3.72
galanin-like peptide precursor [Sus scrofa]	3.71
similar to GP 5106572 gb AAD39760.1 transcriptional activator SRCAP	3.70
similar to GP 4063841 gb AAC98480.1 lysosomal pepstatin insensitive protease	3.67
homologue to GP 12847425 dbj BAB27564. putative	3.63
homologue to PIR JE0272 JE0272 low density lipoprotein receptor-related protein 6	3.59

Table 2. Down-regulated gene list

Gene Annotation	Log2 ratio
similar to GP 10433831 dbj BAB14034. unnamed protein product	-4.55
similar to SP Q14690 RRP5_HUMAN RRP5 protein homolog	-4.28
ribosomal protein [Sus scrofa]	-4.13
SP P15154 RAC1_HUMAN Ras-related C3 botulinum toxin substrate 1	-4.02
homologue to SP P26430 SL52_RABIT Sodium/nucleoside cotransporter	-3.94
homologue to GP 5689525 dbj BAA83046.1 KIAA1094 protein	-3.94
butyrophilin [Sus scrofa]	-3.80
similar to GP 10437626 dbj BAB15080. unnamed protein product	-3.74
ribosomal protein [Sus scrofa]	-3.72
SP P05216 TBA6_MOUSE Tubulin alpha-6 chain. [Chinese hamster]	-3.67
transcription factor A	-3.63
homologue to GP 14035880 emb CAC38536. unnamed protein product	-3.62
weakly similar to GP 15126581 gb AAH12225.1 Unknown	-3.61
similar to GP 10439777 dbj BAB15565. unnamed protein product	-3.60
similar to GP 8896138 gb AAF81254.1 pregnancy-associated glycoprotein 4	-3.59
SP P05216 TBA6_MOUSE Tubulin alpha-6 chain. [Chinese hamster]	-3.58
similar to GP 18027830 gb AAL55876.1 unknown	-3.56
homologue to GP 14042513 dbj BAB55278. unnamed protein product	-3.48
GP 12652885 gb AAH00198.1 Similar to CG11985 gene product	-3.47
homologue to SP O95741 CNE6_HUMAN Copine VI	-3.46
similar to GP 12841075 dbj BAB25067. putative	-3.41
similar to GP 9507250 gb AAF37320.2 glutaredoxin 2	-3.40
similar to GP 15214627 gb AAH12440.1 Similar to mitochondrial ribosomal protein L24	-3.39
homologue to GP 3600028 gb AAC63519.1 dysferlin	-3.37
homologue to PIR JC5922 JC5922 syntaxin 10 - human	-3.32
V-2 lysine vasopressin receptor [Sus scrofa]	-3.27
GP 6841570 gb AAF29138.1 HSPC175	-3.26

GP 6841570 gb AAF29138.1 HSPC175	-3.26
SP P05216 TBA6_MOUSE Tubulin alpha-6 chain. [Chinese hamster]	-3.25
TATA box binding protein	-3.20
non-muscle myosin light chain [Sus scrofa]	-3.16
homologue to GP 12653059 gb AAH00294.1 Unknown	-3.16
homologue to GP 16741618 gb AAH16614.1 Unknown	-3.15
inward rectifying K channel [Sus scrofa]	-3.15
ribosomal protein [Sus scrofa]	-3.13
retinoic acid receptor gamma [Sus scrofa]	-3.11
homologue to GP 5107509 pdb 1BLX B Chain B P19ink4dCDK6 COMPLEX	-3.10
similar to GP 16740625 gb AAH16196.1 Unknown	-3.07
pro-hormone convertase [Sus scrofa]	-3.07
SP P05216 TBA6_MOUSE Tubulin alpha-6 chain. [Chinese hamster]	-3.07
glutathione peroxidase [Sus scrofa]	-3.07
preproendothelin	-3.06
similar to GP 13274194 emb CAC33877. dJ686N3.2.1	-3.06
similar to GP 12803877 gb AAH02782.1 Unknown	-3.05
homologue to GP 14286298 gb AAH08950.1 Unknown	-3.05

homologue to GP 13938185 gb AAH07211.1 Similar to RIKEN cDNA 5031439A09 gene	-3.04
homologue to GP 13992542 emb CAC38115. single-stranded DNA binding	-3.04
similar to GP 6606076 gb AAF19050.1 aquaporin 8	-3.03
homologue to GP 11320879 gb AAG33935.1 aldehyde dehydrogenase-6	-3.03
similar to GP 16226067 gb AAL16066.1 putative methyltransferase WBMT	-3.01
Randomly generated negative control	-3.00
SP P05216 TBA6_MOUSE Tubulin alpha-6 chain. [Chinese hamster]	-2.98
homologue to SP P70452 STX4_MOUSE Syntaxin 4. [Mouse]	-2.97
mannose-binding lectin [Sus scrofa]	-2.97
homologue to GP 13279068 gb AAH04266.1 Unknown	-2.97
homologue to SP Q9BXS5 A1M1_HUMAN	-2.95
weakly similar to GP 7301881 gb AAF56989.1 CG7912 gene product	-2.91
similar to GP 1469205 dbj BAA09490.1 The KIAA0141 gene product is novel.	-2.90
homologue to GP 10435511 dbj BAB14605. unnamed protein product	-2.89
homologue to PIR A38096 A38096 perlecan precursor - human	-2.89
homologue to GP 16551866 dbj BAB71190. unnamed protein product	-2.88
similar to GP 14017907 dbj BAB47474. KIAA1845 protein	-2.87
homologue to GP 16041098 dbj BAB69716. hypothetical protein	-2.86
similar to GP 15207801 dbj BAB62909. testicular protein kinase 2	-2.86
homologue to GP 12655011 gb AAH01353.1 katanin p80	-2.85
dihydrolipoamide acetyltransferase [Sus scrofa]	-2.84
homologue to GP 11125678 emb CAC15497. dJ591C20.4.1	-2.84
BAI1-associated protein 1 [Sus scrofa]	-2.83
similar to GP 15277241 dbj BAB63334. Testis expressed gene	-2.83
nerve growth factor B	-2.83
NADH/NADPH thyroid oxidase p138-tox [Sus scrofa]	-2.82
homologue to PIR S71363 S71363 probable ATP-binding cassette transporter	-2.81
homologue to GP 11181624 gb AAG32665.1 thioredoxin interacting factor	-2.80
homologue to GP 17105409 gb AAH13920.1 Unknown	-2.80
homologue to GP 14043208 gb AAH07592.1 transcriptional regulator protein	-2.77
homologue to GP 14017837 dbj BAB47439. KIAA1810 protein	-2.77
similar to GP 7023394 dbj BAA91948.1 unnamed protein product	-2.76

similar to GP 7023394 dbj BAA91948.1 unnamed protein product	-2.76
similar to GP 14329676 emb CAC40651. centaurin beta	-2.76
similar to GP 12653099 gb AAH00316.1 Similar to RAB2	-2.76
homologue to GP 13879374 gb AAH06664.1 Similar to RIKEN cDNA 3110039B05 gene	-2.76
homologue to GP 14790013 gb AAH10854.1 Similar to amyloid beta	-2.76
similar to GP 12654685 gb AAH01181.1 Unknown	-2.76
similar to GP 12841758 dbj BAB25339. putative	-2.75
homologue to GP 13491980 gb AAK28027.1 c-Jun binding protein JBP	-2.74
homologue to PIR S05432 OGBO7 cytochrome-c oxidase	-2.74
homologue to GP 2959864 emb CAA05321.1 synaptogyrin 1b	-2.73
similar to SP P20616 SG2_BOVIN Secretogranin II precursor of class II subfamily of Interferon-alpha	-2.72
brachyury [Sus scrofa]	-2.72
homologue to GP 10435513 dbj BAB14606. unnamed protein product	-2.70

Table 3. Differentially expressed genes from microarray mapped to immune related pathways

Pathway	Gene Symbol	GenBank accession
Cytokine-cytokine receptor interaction and	TGFB1	NM_000660
	INHBB	NM_002193
	IL17B	NM_014443
	EPO	NM_000799
	IL10	NM_000572
	LTA	NM_000595
	IFNA6	NM_021002
	PDGFRB	NM_002609
Cytokines and Inflammatory Response	TGFB1	NM_000660
	IL10	NM_000572
	LTA	NM_000595
Antigen processing and presentation	CALR	NM_004343
	HSPA9	NM_004134
	PSME2	NM_002818
	LTA	NM_000595
	IFNA6	NM_021002
	CD74	NM_004355
Leukocyte transendothelial migration	RAC1	NM_006908
	MYL6	NM_079423
	MAPK13	NM_002754
	CLDN6	NM_021195
	VCL	NM_003373
	GNAI2	NM_002070
Jak-STAT signaling pathway	PIAS1	NM_016166
	EPO	NM_000799
	BCL2L1	NM_138578
	IL10	NM_000572
	IFNA6	NM_021002
Toll-like receptor signaling pathway	RAC1	NM_006908
	MAPK13	NM_002754
	LBP	NM_004139
	IFNA6	NM_021002
Natural killer cell mediated cytotoxicity	RAC1	NM_006908

	NFATC4 GZMB IFNA6	NM_004554 NM_004131 NM_021002
Complement and coagulation cascades	MBL2 A2M VWF	NM_000242 NM_000014 NM_000552
T cell receptor signaling pathway	NFATC4 IL10	NM_004554 NM_000572

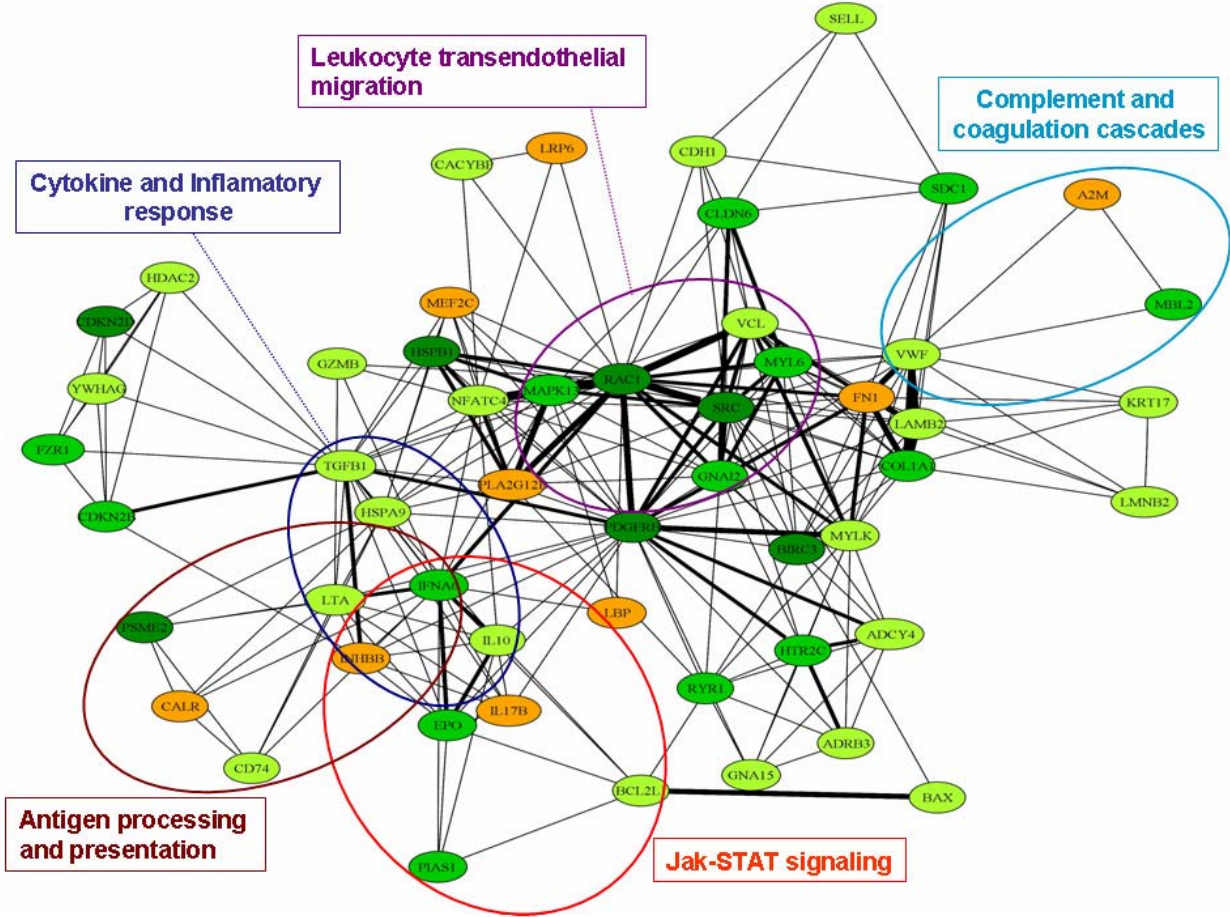


Figure 1 Differentially genes from microarray analysis were mapped to several immune related pathways including Jak-STAT signaling, antigen processing and presentation, cytokine and inflammatory response and complement and coagulation cascades. Green indicates down-regulated genes while red codes for up-regulated genes.