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Gene Expression Profiling in Porcine Maternal Infanticide: A Model for Puerperal Psychosis

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The etiology of mental disorders remains largely unclear. Complex interactions between genetic and environmental factors are key to the development of such disorders. Puerperal psychosis is the most extreme form of postnatal mood disorder in women. Similarly, parturition in the pig can trigger extreme behavioral disturbances, including maternal infanticide. In this study, we have used a targeted cDNA microarray approach using the pig as a model to understand the genes and genetic pathways that are involved in these processes. Two subtracted cDNA libraries from porcine hypothalamus were constructed, which were enriched for genes that were over-expressed and under-expressed in the aberrant behavioral phenotype, compared to the matched control. In addition to this, a normalized library was constructed from hypothalamus and pituitary samples taken from pigs in a variety of reproductive states. The libraries were partially sequenced and combined represented approximately 5,159 different genes. Microarray analysis determined differences in gene expression between hypothalamus samples from nine matched pairs of infanticidal versus control animals, using a common reference design. Microarray analysis of variance (MAANOVA) identified 52 clones as being differentially expressed ($P \leq 0.002$) in the infanticide phenotype, a second analysis with friendly statistics package for microarray analysis (FSPMA) identified 9 genes in common to MAANOVA, and a further 16 genes. A rapid cross-species screen onto a human oligonucleotide array confirmed 3 genes and highlighted 61 more potential candidates. Some of these genes and the pathways in which they are involved were also implicated in a parallel QTL study on maternal infanticide.

KEY WORDS: puerperal psychosis; maternal infanticide; aggression; microarray; pig model

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INTRODUCTION

Parturition is a period of substantial and rapid biological and psychological change. In domestic pigs, maternal (infanticidal) aggression is a well-recognized phenomenon, recently shown to be part of a wider behavioral phenotype [Chen et al., 2008]. Affected sows have a severe impact upon animal welfare and the agricultural economy. Suboptimal environments, particularly insufficient space to interact freely with new-born piglets, results in higher rates of infanticide [Jarvis et al., 2004]. The frequency of occurrence in primiparous sows has been estimated in large surveys of commercial pig farms to be 8% [Knap and Merks, 1987] and 7–12% [Van der Steen et al., 1988]. General behavior shown in these animals and factors affecting it are discussed in more detail elsewhere [Quilter et al., 2007]. However, porcine maternal behavior seems to be mostly influenced by genetic predisposition and the sows' previous experiences. Aggressive infanticide has been seen more frequently in primiparous sows (gilts) than more experienced sows [Van der Steen et al., 1988]. However, it should be taken into consideration those gilts that show aggression may be culled from the herd, which would in turn lower the incidence of aggression in older sows. Epidemiological analyses have clearly shown that aggressive infanticide has a strong heritable component, with daughter-dam heritability estimates reported to be as high as 0.4–0.9 [Knap and Merks, 1987] and 0.47–0.87 [Van der Steen et al., 1988]. This therefore suggests that a genetic predisposition to aggressive infanticide exists that can be ameliorated by experience. Identification and understanding of the maternal infanticide phenotype is therefore of utmost importance to the agricultural community with the ultimate aim of putting in place measures to reduce maternal aggression in the sow.

It is our hypothesis that pigs can be used as a model for human postnatal illness, particularly puerperal psychosis, as maternal infanticidal aggression in pigs has many features in common [Quilter et al., 2007]. Childbirth in women can be a trigger for a wide range of psychotic disorders ranging from mild "baby blues" to severe episodes of psychotic illnesses [Jones et al., 2001]. Puerperal psychosis is the most extreme form of postnatal mood disorder, occurring in 1 in 1000 births. The etiology of this condition is unclear but family studies have shown that pregnant women with pre-existing bipolar disorder

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(also known as manic depression) have an increased risk [Jones and Craddock, 2001]. It has been suggested that puerperal episodes identify a more familial subtype of bipolar disorder [Jones and Craddock, 2002]. Furthermore, family studies consistently demonstrate a difference in the risk of puerperal psychosis in individuals with a first degree relative with bipolar disorder and puerperal psychosis compared to those without [Jones and Craddock, 2001]. The general behavior presented by women with this condition is discussed in more detail elsewhere [Quilter et al., 2007].

Puerperal psychosis develops rapidly after childbirth and it is thought that hormonal mechanisms are likely to be the trigger for this condition. Variation at the serotonin transporter gene (5-HTT) has been shown to influence susceptibility of bipolar patients to puerperal psychosis [Coyle et al., 2000], which is also interesting as 5-HTT expression is influenced by oestrogen, the concentration of which falls dramatically at parturition [McQueen et al., 1997]. This rapid reduction in the level of oestrogen also reduces its antidopaminergic effect exposing supersensitive dopamine receptors, which may also act as a trigger for psychosis [Jones et al., 2000].

Determination of the differences in levels of gene transcription between two states (condition under study and control) may help to understand the underlying cause. DNA microarray studies are currently the most common mRNA profiling technique used to study psychiatric disorders such as schizophrenia and bipolar disorder [reviewed by Iwamoto and Kato, 2006]. However, there are no previous microarray studies specifically targeting puerperal psychosis. Due to the complex genetic component of this disease, a microarray study would be useful to identify genetic pathways affected by the disease, as these will be important for the development of new drugs in the treatment of puerperal psychosis. Using an animal model such as the pig overcomes having to use post-mortem brain material or peripheral blood from human patients, the limitations of which are reviewed elsewhere [Iwamoto and Kato, 2006]. Furthermore, the animal model permits access to the key tissue (hypothalamus) at the time of an aggressive episode and thus a direct examination of the pathophysiology as it occurs.

In the pig, microarray studies have mainly been limited to meat production [Kim et al., 2006], and reproduction [Gladney et al., 2004]. However, one group has investigated changes in global gene expression of the frontal cortex in early weaned and socially isolated pigs, a brain region involved in cognitive function and behavior organization. They found that social isolation may impact on expression of genes involved in the regulation of neuronal function, development, and protection [Poletto et al., 2006]. Furthermore, several behavioral microarray studies have been carried out on brain samples from rodents. These include identification of genes involved in stress vulnerability and depressive behavior [Pearson et al., 2006], different behavioral models in mice [Letwin et al., 2006] and genes stably regulated by maternal care [Weaver et al., 2006].

In this study, it was our goal to identify genes and/or genetic pathways with significant impact on maternal (infanticidal) aggression in pigs and relate this to human psychiatric conditions, particularly to postnatal illness. We used a targeted cDNA microarray approach. Three libraries (two subtracted and one normalized) were constructed primarily from porcine hypothalamic material, the reason for this being that the majority of cross-species studies show that parturition and maternal behavior are regulated by neuroendocrine systems that originate in and are coordinated by hypothalamic nuclei, particularly the medial preoptic area (MPO), paraventricular nucleus (PVN), supraoptic nucleus (SON), and associated projections. These brain areas are responsive to peripheral stimuli such as sex steroids, oxytocin, prostaglandin F2α, and prolactin (PRL), which have been well characterized in pigs and implicated in maternal behavior [Ellendorff et al., 1979; Taverne et al., 1982; Gilbert et al., 1994]. The pituitary gland was included in the normalized library as many genes from this tissue are also important for maternal behavior. For example, PRL is produced by the pituitary gland and it has been suggested that exposure to PRL during pregnancy helps to stimulate the immediate onset of maternal behavior at parturition [Bridges et al., 1985]. These libraries were used to make our porcine cDNA array. In addition, a rapid cross-species screen of a fully characterized human oligonucleotide chip was carried out as this offered the opportunity to interrogate an expanded gene set. These arrays were used to determine the differences in gene expression between hypothalamic cDNA from maternal infanticidal pigs and their matched control counterparts. Results were analyzed using two different analysis of variance (ANOVA) methods: Microarray analysis of variance (MAANOVA v2.0) (www.jax.org/staff/churchill/labsite/software/anova/index.html) and a friendly statistics package for microarray analysis (FSPMA) [Sykacek et al., 2005] and verified by quantitative real-time PCR (Q-RT-PCR).

MATERIALS AND METHODS

Animals

Samples were collected from three farms with a known history of maternal infanticide amongst their stock. Animals were collected (1) in a variety of reproductive states for the normalized library and (2) nine matched pairs of pigs with maternal (infanticidal) aggression and their paired controls. The nine pairs of test animals all came from the same farm (six primiparous, two second parity, one third parity). Infanticidal animals were defined as sows that killed two or more of their offspring by biting them to death, usually within 24 hr of birth. Paired control animals were matched (1) genetically and (2) samples collected as near to the same time after the birth of the first piglet (BFP) as their matched pair (Supplementary Table I). Rearing and parturition environments were also matched between pairs. All sows were housed during pregnancy in social groups in strawed yards, and transferred to parturition (farrowing) crates during the week before birth. Most farrowing crates were provided with shredded paper as bedding although straw was present in a few cases. All had a solid floor with slats to the rear.

Sample Collection

Pigs were killed humanely, in accordance with UK law, by intravenous injection with 1 ml/10 kg body weight of the veterinary euthanasia agent quinalbarbitone sodium 400 mg/ml and cinchocaine hydrochloride 25 mg/ml (Somulose: Arnolds Veterinary Products, Shrewsbury, UK). The brain was then removed as quickly as possible (within 20 min of death) and placed in a resin mould, which had guidelines for cutting out a consistent sized block of tissue containing the hypothalamus (Fig. 1). This tissue was then roughly chopped and put into a sterile collection tube containing RNA later (Qiagen, Crawley, UK). The pituitary was also removed, chopped, and put straight into RNA later. The samples were transported on ice and stored at -20°C until needed. All utensils were cleaned with RNA-ZAP (Ambion, Warrington, UK) before use to make them RNase free and gloves were worn throughout.

RNA Isolation

RNA was obtained from each sample separately and the whole sample was processed because it was likely to contain many different types of neurones and structures. By processing the whole block of tissue the differences seen in expression levels could not be due to different cell types being represented

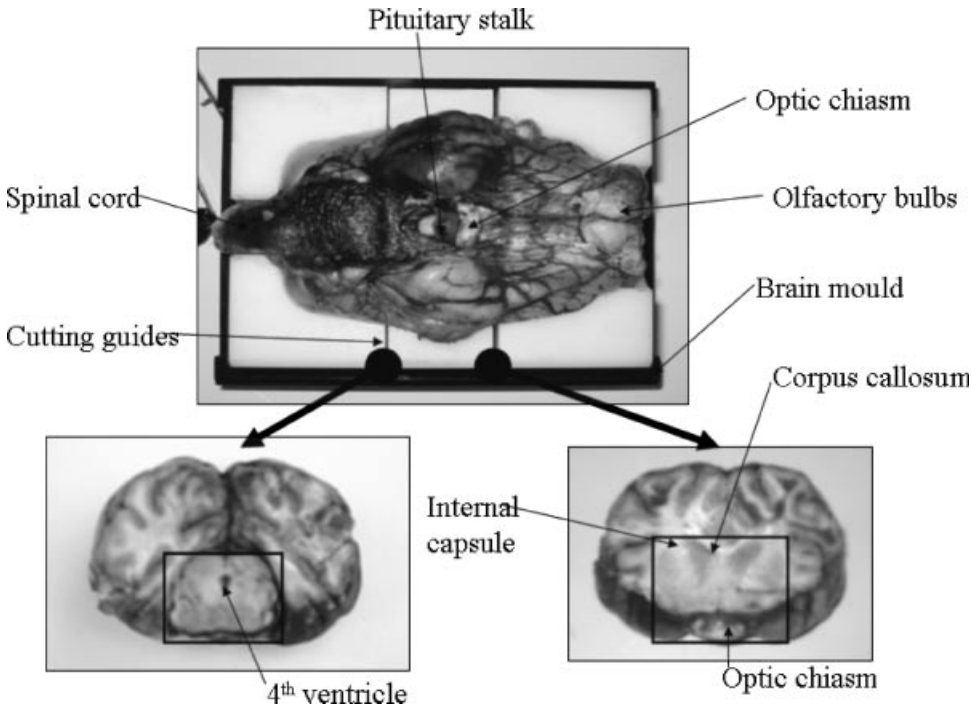

Fig. 1. Shows a pig brain in the brain mould used to make a consistent section of hypothalamus. Cutting guidelines are shown and the square box indicates the section of hypothalamus taken.

in different samples. RNA was extracted (due to the large amount of tissue) using the Qiazol lysis reagent from a commercial kit specifically designed for extraction of RNA from high lipid containing tissues (RNeasy lipid, Qiagen). The total RNA was then further purified using the RNeasy filter binding system (Qiagen). RNAs extracted from the same original block were then pooled. The RNA quality was assessed with the Lab-On-A Chip System (Agilent, Stockport, CA). Some of the RNA extracted from each sample was used to make a pool of infanticidal RNA and a pool of control RNA. From this Poly-A+ mRNA was obtained using the Oligotex Affinity Purification kit (Qiagen) for library production. The remaining RNA was kept for hybridization to the array.

cDNA Library Production

Two subtracted libraries were constructed from the hypothalamus samples of two pairs of pigs showing maternal infanticide and matched paired control counterparts and these were enriched for genes that were over-expressed and under-expressed in the infanticide phenotype. A normalized library was constructed from hypothalamus and pituitary samples from pigs in a variety of reproductive states in order to obtain the optimum number of expressed genes. Animals used for all three libraries are detailed in Supplementary Table II.

The libraries were made using the Clonetech PCR-Select cDNA subtraction kit. For the first subtraction, a pool of maternal infanticidal aggressive hypothalamus cDNA was used as the tester cDNA and a pool of matched control non-aggressive hypothalamic cDNA samples as the driver. This selected for genes that were over-expressed in the maternal infanticide samples compared to the control samples. The second subtraction used pooled control hypothalamus cDNA as the tester and pooled maternal infanticide hypothalamus cDNA as the driver. This enriched in genes over-expressed in the control samples compared to the maternal infanticide samples, that is, genes that are under-expressed in the maternal infanticide samples. For the normalized library the same pool of RNA of animals in a variety of reproductive states were used to make two pools of tester, the aim being that highly abundant sequences would hybridize to each other and reduce the redundancy of the library. Nested PCR was used to amplify the normalization products.

Double stranded cDNA was prepared from 2×2 μg of each pool of poly A+ mRNA, using the Promega Universal RiboClone system. Double stranded cDNA was digested to completion with 20U *Rsa1* (Roche, Burgess Hill, Switzerland) for 2 hr at 37°C (1.5 hr normalized library), and used to perform the suppression subtractive hybridization (SSH) procedure according to the Clonetech PCR-Select protocol. An 8 hr incubation was used for the first hybridization and 16 hr for the second hybridization for the subtracted libraries (8 hr for both hybridizations for the normalized library). SSH efficiency was tested by semi-quantitative PCR of housekeeping genes ribosomal protein S7 (RPS7) and glyceraldehyde 3-phosphate dehydrogenase (G3PDH). The degree of subtraction for both genes was ~10 PCR cycles. The subtracted PCR products were ligated into pGEM-T Easy plasmid vectors (Promega, Southampton, UK) and subcloned into *E. coli* (XL-10-Gold supercompetent cells, Stratagene, Bath, UK). Colonies were picked using a BioPick colony picker robot (BioRobotics, Cambridge, UK). In total, 1,824 colonies were picked from the over-expressed library, 2,208 from the under-expressed library and 6,144 from the normalized library

Array Construction

PCR was used to amplify the library inserts. Clones were spotted in triplicate on the array and in a randomized manner. In addition to the libraries, 63 candidate genes identified from the literature of related conditions in humans and rodents, were also added to the array. Primers were designed from available porcine or conserved regions of mammalian sequence (NCBI) using Primer 3 (http://www.es.embnet.org/cgi-bin/primer3_www.cgi). PCR products were spotted onto the array in triplicate. Clones from Arabidopsis genes were also included

as negative controls. All printing was carried out by the microarray facility in the Pathology Department, University of Cambridge, using a BioRobotics Microgrid II arrayer.

Library Sequencing

Single-pass sequencing was performed on about 1100 clones using a vector directed primer. Sequences were then submitted to the nr database (NCBI) using default parameters http://www.ncbi.nih.org/blast/. Each clone was assigned an accession number match to a gene with the most information and high similarity score. Where possible pig matches were collected but human information was also collected when pig matches were uninformative. Any sequence with no hit was searched against the expressed sequence tags (EST) database (est-others) using the *Sus scrofa* parameter. Ascribed gene function was determined using PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed and Online Mendelian Inheritance in Man (OMIM) http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=omim&itool=toolbar. In an attempt to identify whether the genes identified were part of relevant pathways the data were analyzed using pathway express http://vortex.cs.wayne.edu/projects.htm#pathway-express.

Microarray Hybridization

Standard quality control hybridizations were carried out to confirm array quality [Ellis et al., 2004]. The cDNA array was then used to investigate the difference in gene expression of hypothalamus samples from nine animals with maternal (infanticidal) aggression and nine matched non-aggressive controls, which represented nine biological replicates. Each RNA sample (20 ng) was reverse transcribed into cDNA and labeled with either Cy3 or Cy5 according to the indirect amino allyl protocol used by Ellis et al. [2004]. Four technical replicates were carried out for each sample, which included two dye swaps, using a common reference design, that is, a repeated dye swap reference design [Wu et al., 2003] (Fig. 2). Using a reference design allowed each slide to be directly compared to another and also accommodated the addition of samples as they were being collected throughout the study. After hybridization at 55°C for 19 hr, cover slips were removed from slides in $1 \times$ SSC/0.1%SDS, washed successively in $1 \times$ SSC/0.1%SDS for 5 min, $1 \times$ SSC for 5 min, dipped in ddH_2O and dried by gentle centrifugation.

In addition to the cDNA array, the nine pairs of matched samples were also hybridized to a human oligonucleotide chip (Ilumina). This includes 22,800 oligonucleotides representing approximately 17,000 genes. (Mitochondrial genes are not included on this array.) Each RNA sample (1000 ng) was labeled using the SMART protocol as modified by Petalidis et al. [2003]. Porcine Cot-1 was used for blocking and a modification of the hybridization protocol used by Ellis et al. [2007] for this array was carried out but with an increased hybridization time of 48 hr. This time two technical replicates were carried out for each sample, which included one dye swap, using a common reference design. Supplementary Figure 1 shows the log 2 of the mean signal of all the slides against the number of oligonucleotides on the array. This shows a relatively normal distribution with only a slight skew to the left, giving evidence that the cross-species hybridization has worked well.

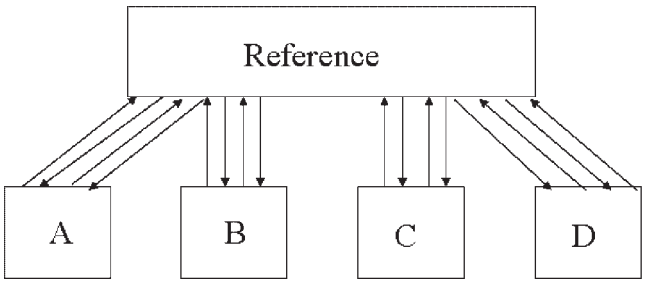

Fig. 2. Diagrammatic representation of a repeated dye swap design for microarray hybridization.

Spot intensities for both arrays were measured with an Axon 4001A scanner (GenePix, Wokingham, UK) and spotfinding and quantification of the resulting fluorescent data were carried out using Bluefuse software (BlueGnome, Cambridge, UK). Unreliable data, that is, damaged/misshapen spots were excluded manually.

Data Analysis

Data from both arrays were normalized using a global loess regression. Two different mixed model ANOVA packages were used to analyze the data, for both arrays. The first, MAANOVA allowed for biological effects: phenotype, breed, and family and technical effects: array and spot to be taken into account, and was used to obtain an overall result from all the maternal infanticide animals against all the control animals [Wu et al., 2003]. The second, FSPMA [Sykacek et al., 2005], a mixed model ANOVA program written in R language, identified differentially expressed genes from each individual pair separately and in addition, from the four Large White animals analyzed as a group ($P < 0.005$ corrected by false discovery rate [Hochberg and Benjamini, 1990]). Following normalization, data from technical replicates were averaged for both analysis methods and tables of clones were produced ranked by P-value of an F statistic.

For the MAANOVA analysis, in order to choose an appropriate P-value, an estimation of false discovery rate was considered according to Stekel [2003]. Genes with a $P \leq 0.002$ were identified as being significantly differentially expressed with an acceptable false discovery rate. For the FSPMA analysis, each pair, the four Large White analysis and the MAANOVA results were compared. In order to reduce the number of genes up for consideration, genes in common to ≥ 3 of these analyses with a P-value ≤ 0.05 were proposed as candidate genes. By carrying out both analyses we hope to identify as many candidate genes as possible, which can be put forward for further analysis.

Quantitative Real–Time RT-PCR

Nine genes (PRL, RPL23, CGA, GNAI1, HSPA8, DRD2, HTR2C, OPRM1, and TTR), which were differentially expressed on the cDNA microarray, were also selected for further investigation using Q-RT-PCR. In addition, PRLR was also tested. Ten primer pairs were designed so that one pair spanned an intron boundary to control for DNA contamination (Supplementary Table III). Q-RT-PCR was carried out using the QuantiTect SYBR Green RT-PCR kit (Qiagen) according to the manufacturer's instructions. Reactions were run on a BioRad iCycler (BioRad, Hemel Hempstead, UK) using the following cycling conditions: 50°C for 30 min followed by 95°C for 15 min followed by 40 cycles of 94°C (15 sec)/55°C (30 sec)/72°C (30 sec)/77°C (15 sec). A melting curve was also examined for specificity of the RT-PCR product. Genes were tested in triplicate across the nine pairs of animals used for microarray analysis. Starting concentration of sample from each animal was 9 ng. Proteasome (prosome, macropain) 26S subunit, non-ATPase, 2 (PSMD2) was used as a control gene because it showed no variation in expression between samples on the array, and is one of the most constantly expressed housekeeping genes in human tissues [Hsiao et al., 2001]. This gene

was chosen because 16S ribosomal RNA, which is often used as a housekeeping gene, showed differential expression on the array (pig FSPMA) and it has also been published that other commonly considered housekeeping genes (e.g., β-actin and GAPDH) exhibit considerably variable expression between tissue types [Hsiao et al., 2001]. Quantification of relative gene expression levels was carried out using a comparative C_t method ($2^{-\Delta\Delta C_t}$) [Livak and Schmittgen, 2001]. This allows quantification of a template by comparing expression levels of the candidate gene to the control gene (PSMD2).

RESULTS

Libraries

The normalized library was found to have about 50% redundancy and the subtracted libraries about 40% each. The total number of clones spotted in triplicate that includes the three libraries, candidate genes and all controls was 11,232, which corresponds to an estimated 909 (over-expressed), 1,178 (under-expressed) and 3,072 (normalized) genes from the libraries. However, sequencing of the libraries is not complete so gene diversity may be higher.

It was interesting to note that there was an abundance of PRL clones in the over-expressed library and mitochondrial genes, particularly NADH dehydrogenase genes and ATPase 6/8, were abundantly represented in the under-expressed library.

Microarray Analysis of Gene Expression

Porcine array. For the MAANOVA analysis, 52 clones were identified as being significantly differentially expressed in maternal infanticide animals compared to control animals, at a threshold of $P \leq 0.002$. This gives a number of clones with a reasonable false discovery rate of 43% [P-value × number of genes on the array/observed number of genes × 100, i.e., $(0.002 \times 11234/52) \times 100$]. Two genes (COX1 and ND2) were duplicated and six failed to sequence. The FSPMA analysis, which looked at ≥3 analyses with a P-value ≤0.05, identified 8 genes in common to the MAANOVA analysis (although not all with a $P \leq 0.002$) and a further 16 candidate genes. Two genes were common to five or more analyses (TTR and PRL). Genes were prioritized based on their potential involvement in the phenotype and are summarized in Table I. In addition, 7 more relevant genes from the MAANOVA analysis are included in Table I (in bold), which had less stringent P-values (between 0.002 and 0.01). The complete list of genes, including P-value ($P \leq 0.002$ MAANOVA) and/or number of analyses in common (≥3 analyses with a P-value ≤0.05 FSPMA) (see Methods) is found in Supplementary Table IV.

Human array. For the MAANOVA analysis of the human oligonucleotide array 25 human oligonucleotides were identified as being significantly differentially expressed at a threshold of $P \leq 0.002$. FSPMA identified a further 76 human oligonucleotides as being significantly differentially expressed ≥3 analyses with a P-value ≤0.05. Of these identified oligonucleotides, 12 (MAANOVA) and 53 (FSPMA) matched relevant porcine sequences when blasted against the nr or EST-others (*S. scrofa*) databases (NCBI) (Supplementary Table V). The putative gene functions of pig sequences were confirmed via Unigene and it was checked that any remaining anonymous pig sequences BLASTed back to the relevant human gene. Three of these 64 genes were in common to the porcine array. Priority genes are summarized in Table II. The complete list of genes, including P-value ($P \leq 0.002$ MAANOVA) and/or number of analyses in common (≥3 analyses with a P-value ≤0.05 FSPMA) (see Methods) is found in Supplementary Table VI.

Quantitative Real Time PCR Analysis of Gene Expression

The genes PRL, RPL23, CGA, GNAI1, HSPA8, OPRM1, and DRD2 were chosen to verify that significant differential gene expression on our array analyzed by MAANOVA was reliable. Figure 3 compares the fold change of the genes calculated by Q-RT-PCR to the fold changes of these genes on the array from the MAANOVA analysis. PRLR, which was not picked up by the analysis and therefore may not be present on our array, is also included. This gene is of interest due to it being the receptor for PRL. In addition, direction of gene expression for three of the most significant differentially expressed genes from the FSPMA microarray analysis (PRL, TTR—both pig and human, HTR2C—human) is compared to the Q-RT-PCR results (Table III).

DISCUSSION

We have identified interesting candidate genes for maternal (infanticidal) aggression in pigs from our microarray analyses and the validity of our results have been confirmed by Q-RT-PCR (Fig. 3). In general, the fold changes were small on our arrays but we consider this to be due to the fact that our samples were made up of the whole hypothalamus, and it is possible that genes of interest may only be present in a small subset of cells. The changes seen by both approaches are comparable both in fold change and direction of expression. For the three genes illustrated in Table III the human sequences of the oligonucleotides for PRL, TTR, and 5HTR2C on the human array were not a complete match to the pig sequence and may account for differences seen. Due to the small changes in gene expression on the arrays the few discrepancies seen between the pig array and QRT-PCR results are largely explained by the fact that QRT-PCR is a more sensitive technique and has allowed us to pick up some subtle fold changes. While no single change in gene expression could be exclusively attributed to infanticidal behavior, the involvement of several neural pathways has been clearly implicated. There has been a tendency amongst stock-keepers to regard this behavior as an unpreventable vice that occurs spontaneously and to cull affected sows. Our data suggest that the phenotype is actually akin to a psychiatric disorder (puerperal psychosis) with parallels in other species, including humans, and this should inform future strategies to predict, treat, or prevent this distressing phenomenon.

Puerperal psychosis has a strong link to bipolar disorder (manic depression) and patients also suffer from delusions and hallucinations, which may be similar to some symptoms of schizophrenia [Jones and Craddock, 2001]. This suggests that any connection between orthologs of our identified genes to human psychiatric behavior or brain disorders will be of interest. Below, we discuss in more detail some of the key candidate genes, which are of particular relevance to an aggressive/infanticidal phenotype.

Prolactin Regulation

One of the most important genes identified as being differentially expressed by both analyses was PRL. PRL increases dramatically at lactation, which is stimulated by suckling; hence we would have expected to see significantly lower levels in our infanticidal animals, whose remaining offspring were usually removed before suckling occurred, than controls. When all infanticidal animals were considered as a group by the MAANOVA analysis an overall down-regulation of PRL was found, which was also confirmed by the Q-RT-PCR suggesting an effect from removal of piglets rather than a cause of aggression. But when the pairs were considered separately by

TABLE I. Priority Differentially Expressed Genes Identified From the Porcine Array

Gene symbol	Gene name	Source	Associated disorder/description
TTR	Transthyretin (prealbumin, amyloidosis type I)	FSPMA	Alzheimer's, depression
POU1F1	POU domain class1 transcription factor 1	FSPMA	Regulates PRL
16S	16S ribosomal RNA	FSPMA	Bipolar and schizophrenia
HNRPA2B1	Heterogeneous nuclear ribonucleoprotein A2/B1	FSPMA	Alzheimer's, brain cancer, and Down syndrome brain activity
HSPA8	Heat shock 70 kDa protein 8	FSPMA	MAPK pathway and Alzheimer's
ND4	NADH dehydrogenase subunit 4	FSPMA	Bipolar and schizophrenia
PTS	6-Pyruvoyltetrahydropterin synthase	FSPMA	Null-mice associated with low dopamine and serotonin
ZNF248	Zinc finger protein 248	FSPMA	Chromosome 10 locus and Alzheimher's
c17orf25	Chromosome 17 open reading frame 25	MAANOVA	May be linked to with anxiety in mice, Alzheimer's and autism
RFX5	*Homo sapiens* regulatory factor X, 5	MAANOVA	Cognitive and motor deficits, binds MHC II promoter
STMN1	Stathmin 1/oncoprotein 18	MAANOVA	Multiple sclerosis and Alzheimer's
CANX	Calnexin	MAANOVA	Linked to heritable neuropathies
YY1	YY1 transcription factor	MAANOVA	Stress related pathways, in neuronal degeneration, genomic imprinting
TSPAN3	Tetraspanin 3	MAANOVA	Signaling genes associated with depression
ND2	NADH dehydrogenase subunit 2	MAANOVA	Bipolar and schizophrenia
COX1	Cytochrome *C* oxidase subunit I	MAANOVA	Bipolar and schizophrenia
ATP8	ATP synthase F0 subunit 8	MAANOVA	Bipolar and schizophrenia
CCL25	Chemokine (C–C motif) ligand 25	MAANOVA	Neuroinflammation associated with Alzheimer's and Down syndrome
MBP	Myelin basic protein	MAANOVA	Schizophrenia and bipolar
XIST	X (inactive)-specific transcript	MAANOVA	X inactivation-gender differences for some neuropsychiatric disorders
DRD2	Dopamine receptor D2	MAANOVA	Mood disorders
PCMT1	Protein-L-isoaspartate (D-aspartate) *O*-methyltransferase	MAANOVA	Hyperactivity in mice
FRZB	Frizzled-related protein	MAANOVA	FRZ3 associated with schizophrenia
GRIN1	Glutamate receptor, ionotropic, *N*-methyl-D-aspartate 1	MAANOVA	Schizophrenia
ATP6	ATP synthase F0 subunit 6	MAANOVA	Bipolar and schizophrenia
FTH1	Ferritin heavy-chain	MAANOVA	Neurodegenerative diseases
CNN3	Calponin 3, acidic	MAANOVA	Involved in neuronal and glial plasticity.
PCDH20	Protocadherin 20	MAANOVA	Cadherin-related neuronal receptor
POMC	Proopiomelanocortin (POMC) gene	MAANOVA	Bind opiod receptor-regulates dopamine
ITM2B	Integral membrane protein 2B	MAANOVA	Early onset dementia, Alzheimer's
MAPK1	Mitogen-activated kinase-1	MAANOVA	Alzheimer's
GLYATL2	Glycine-*N*-acyltransferase-like 2	MAANOVA/FSPMA	Glycine associated with GRIN1 and schizophrenia
ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, cardiac muscle	MAANOVA/FSPMA	Mitochondrial F1 complex
CGA	Pituitary glycoprotein hormone alpha subunit	MAANOVA/FSPMA	Glycoprotein hormones
PRL	Prolactin	MAANOVA/FSPMA	Maternal behavior
POU3F3	POU domain, class 3, transcription factor 3	MAANOVA/FSPMA	Oligodendrocytes-schizophrenia
GNAI1	Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	MAANOVA/FSPMA	Linked to dopamine receptors
CRSP3	Cofactor required for Sp1 transcriptional activation, subunit 3	MAANOVA/FSPMA	Required for MAPK-mediated activation of Elk-1

the FSPMA analysis, 13 clones representing PRL matched the criteria of being in common to $\geq$3 of these analyses with a *P*-value $\leq$0.05 on the porcine array. In one pair, PRL was up-regulated for all 13 clones in the infanticidal animal (3028). However, in other pairs results were less clear. Discrepancies may be down to the relatively small changes in gene expression. The more sensitive Q-RT-PCR technique confirmed up-regulation in animal 3028 and indicated up-regulation in the infanticidal animals of three other pairs (5844/5845, 2169/365, 1301/1299). In addition, when the four Large White animals were considered as an infanticidal group on the porcine array (FSPMA) overall up-regulation was seen, which was confirmed by Q-RT-PCR. This suggests that in some infanticidal animals there definitely is a significant increase in PRL. PRL was also seen to be abundantly represented in the subtracted library specific for over-expressed genes in the infanticide phenotype, which supports this result. The reason why this gene (and others) may show differences in gene

TABLE II. Priority Differentially Expressed Genes Identified When Porcine cDNA was Hybridized to the Human Oligonucleotide Array

Gene symbol	Gene name	Source	Associated disorder/description
MRPS18A	Mitochondrial ribosomal protein S18A	FSPMA	Mediates dysregulation of neuronal differentiation in Down syndrome
HTR2C	5-Hydroxytryptamine (serotonin) receptor 2C	FSPMA	Depression, anorexia, Prader Willi
POU1F1	POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1)	FSPMA	Confirmation
SSSCA1	Sjogren's syndrome/scleroderma autoantigen 1	FSPMA	Antibody for Sjogren syndrome, neuropsychological impairment in some patients
TIMM23	Translocase of inner mitochondrial membrane 23 homolog (yeast)	FSPMA	Mediates importation of mitochondrial preproteins
CDK5	Cyclin-dependent kinase 5	FSPMA	May have a pivotal role in regulating higher cognitive functions and neurodegenerative diseases
CIB1	Calcium and integrin binding 1 (calmyrin)	FSPMA	May play a role in the pathogenesis of Alzheimer's
DNAJC15	DnaJ (Hsp40) homolog, subfamily C, member 15	FSPMA	Inactivation may play a role in development of pediatric brain tumors
FSHB	Follicle stimulating hormone, beta polypeptide	FSPMA	Member of the pituitary glycoprotein hormone family, enables ovarian folliculogenesis
GABRA1	Gamma-aminobutyric acid (GABA) A receptor, alpha 1	FSPMA	Receptor for the major inhibitory neurotransmitter in brain, binds neurosteroids, epilesy, schizophrenia
HEBP2	Heme binding protein 2	FSPMA	Placental protein, induction of necrotic cell death and mitochondrial permeabilization
ID3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	FSPMA	Expressed in mouse brain. ID2 associated with neural activity and cognitive functions
IDH3G	Isocitrate dehydrogenase 3 (NAD+) gamma	FSPMA	Encodes the gamma subunit of the NAD(+)-dependent isocitrate dehydrogenase, regulates tricarboxylic acid cycle
ILK	Integrin-linked kinase	FSPMA	Activation of p38 MAP kinase is involved in ILK-mediated signal transduction
KCNIP1	Kv channel interacting protein 1	FSPMA	May regulate neuronal excitability, in response to changes in intracellular calcium
Magmas	Mitochondria-associated protein involved in granulocyte-macrophage colony-stimulating factor signal transduction	FSPMA	Mitochondria-associated protein involved in signal transduction
NDUFA12	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12	FSPMA	Myc-induced mitochondrial protein involved in human cancer cell lines
NDUFB6	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17 kDa	FSPMA	Nuclear gene encoding subunit of complex 1(NADH) of mitochondrial respiratory chain
PITX2	Paired-like homeodomain transcription factor 2	FSPMA	Mutations in PIX2 associated with occular defects
PRL	Prolactin	FSPMA	Confirmation
TFPI2	Tissue factor pathway inhibitor 2	FSPMA	Placental protein associated with tumor and inflammation
TPM3	Tropomyosin 3	FSPMA	Regulates neuronal size and shape
TTR	Transthyretin (prealbumin, amyloidosis type I)	FSPMA	Confirmation
TUBA3	Tubulin, alpha 3	MAANOVA	Mutations result in abnormal lamination of brain, associated behavioral deficits, brain specific expression
PDE1B	Phosphodiesterase 1B, calmodulin-dependent	MAANOVA	May participate in learning, memory, and regulation of phosphorylation of DARPP-32 in dopaminergic neurons
C10orf57	Chromosome 10 open reading frame 57	MAANOVA	Nearby Alzheimer's locus
BCL2L1	BCL2-like 1	MAANOVA	Regulates the electrical and osmotic homeostasis of mitochondria
CUTL2	Cut-like 2 (*Drosophila*)	MAANOVA	Predominent expression in cytoplasm of neurons in thalamus and limbic system
NDN	Necdin homolog (mouse)	MAANOVA	Imprinted gene associated with Prader Willi, may suppress growth in postmitotic neurons
marf09	Membrane-associated ring finger (C3HC4) 9	MAANOVA	Protein ubiquitination
OPRS1	Opioid receptor, sigma 1	MAANOVA	This gene encodes a receptor protein that interacts with a variety of psychotomimetic drugs

expression between pairs may be down to the likelihood that this is a polygenic disorder with not all animals or breeds sharing identical genetic contributions.

High levels of PRL have been previously associated with aggression and hostility [Kellner et al., 1984]. For two of these pairs, the infanticidal animals (2169, 1301) only left one survivor indicating that they were particularly aggressive (no information for 3028 and 5844). Significant differential expression of PRL does not appear to correlate with the time that the sample was taken or breed of pig, although more

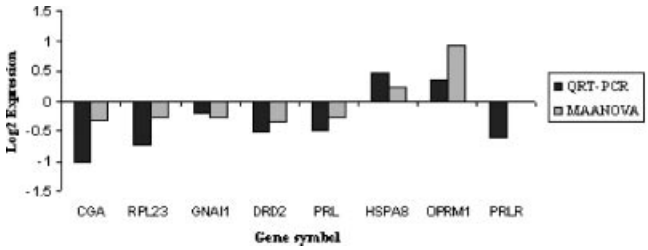

Fig. 3. Comparison of Q-RT-PCR fold change with array (MAANOVA) results for key candidate genes.

replicates would be required to confirm this. Pairs 3028/5572 and 2169/365 are mother daughter pairs but the other two pairs are full sisters. Although MAANOVA does take into account many effects such as breed it does not account for the differences in the time of sampling thus adding animals together may sometimes mask results. Unfortunately this is the main disadvantage of using 'real' farm animals as opposed to an expensive experimental animal herd. By using both analysis methods, our aim was to identify as many candidate genes as possible, which can be put forward for further analysis.

PRL was also significantly differentially expressed from the human oligonucleotide array (FSPMA). When pairs were analyzed separately up-regulation of PRL was seen in the infanticidal animals of two pairs (3028/5572, Y47/Y19) the latter of which was also aggressive in her first litter. However, some pairs were not in agreement with the pig array, which may be explained by the fact that the human PRL oligonucleotide showed a match for 52/70 nucleotides, which could lead to some mismatching to the porcine cDNA. In addition, the cDNA from the array matched exon 2 and part of intron 3 whereas the oligonucleotide matched part of exon 5.

We consider that PRL is particularly important as it holds a central position in the regulation of a variety of behaviors, including maternal aggression, feeding, stress, and fertility. It is controlled by a negative feedback loop where PRL stimulates hypothalamic dopamine release, which in turn suppresses PRL. POU domain, class 1, transcription factor 1 (POU1F1), which also regulates PRL was differentially expressed on both pig and human arrays (FSPMA). PRL affects maternal aggression against an intruder via oxytocin, low levels of which are associated with this phenotype [Ragnauth et al., 2005]. Oxytocin, thought to originate from centrally projecting PVN neurons of the hypothalamus, is one of the key neuropeptides regulating the initiation and maintenance of maternal behavior [Pedersen and Boccia, 2002]. In the ovaries, PRL via the prolactin receptor (PRLR; long form) regulates the oestrogen receptors Esr1 and Esr2 via the Jak2-Stat5 pathway [Frasor and Gibori, 2003]. Although not detected by our arrays, we also investigated the expression of the PRLR by Q-RT-PCR and found it to be down-regulated overall. A four-gene micronet, which promotes social recognition and hence helps control aggression in mice, has been proposed involving the oestrogen receptors, oxytocin and the oxytocin receptor [Kavaliers et al., 2004]. Although results between pairs are not consistent on our arrays, perturbation of at least one gene in this network is seen in all comparisons. In addition, the oestrogen receptors are present on porcine chromosome 1 where a QTL (not significant) was indicated in a parallel QTL study [Quilter et al., 2007].

Dopamine Regulation

One of the key genes implicated by the porcine MAANOVA analysis was dopamine receptor 2 (DRD2). MAANOVA detected an overall down-regulation of DRD2, which was

TABLE III. A Comparison of Array and Q-RT-PCR Direction of Gene Expression for PRL, TTR, and HT2RC (Shaded Areas Show Agreement; Numbers Represent Numbers of Clones; ns, Not Statistically Significant)

Gene	Platform	LW 3028/5572	Landrace Y47/Y19	LW 5221/5219	LW 5844/5845	Pairs LW 5587/5585	Hamp/LW 1311/1313	Hamp/LW 1301/1299	Hamp/LW 2167/358	Hamp/LW 2169/365	4 × LW
PRL (13 clones significant)	Human array	UP	UP	DOWN	None	None	None	None	None	None	None
	Pig array	UP(13)	DOWN(1)	None	None	DOWN(4)	UP(3)/ DOWN(3)	UP(5)/ DOWN(4)	DOWN(5)/UP(1)	DOWN(1)	UP(4)
	QRT_PCR (ex4/5)	UP	DOWN	None	UP	DOWN	(DOWNns)	(UPns)	(DOWNns)	UP	None
	QRT_PCR (ex2/3)	UP	DOWN	DOWN	UP	DOWN	DOWN	UP	DOWN	UP	UP
TTR (1 clone)	Human array	UP	None	None	None	None	None	None	DOWN	None	UP
	Pig array	UP	UP	None	None	None	UP	DOWN	DOWN	None	None
	QRT_PCR	UP	(UPns)	DOWN	None	DOWN	UP	DOWN	None	None	None
HTR2C	Human array	None	None	UP	None	UP	None	None	DOWN	None	UP
	QRT_PCR	UP	DOWN	UP	UP	None	UP	DOWN	None	DOWN	UP
Time point/hr		16	16	16	8	10	13	22	24	10	19.5

confirmed by the Q-RT-PCR. DRD2 negatively regulates PRL secretion so this is in agreement with up-regulation of PRL. In turn, there is a negative feedback regulation of PRL on dopamine secretion, that is, increased PRL will lead to decreased dopamine levels (Fig. 4). Too little dopamine is associated with depression and social withdrawal (negative symptoms of schizophrenia) whereas too much dopamine can cause hallucinations and paranoia (positive symptoms of schizophrenia) and mania [Carter, 1998]. In fact, DRD2 has been one of the most extensively investigated genes in neuropsychiatric disorders, has been implicated in schizophrenia, posttraumatic stress disorder, movement disorders, and migraine [reviewed by Noble, 2003] and almost all antipsychotic drugs are dopamine antagonists. Interestingly, in patients given antagonist antipsychotic medication, increased PRL secretion has been found in those with the DRD2*A1 allele, which reflects increasingly tight DRD2 binding [Young et al., 2004].

Dopamine secretion is also regulated via pro-opiomelanocortin (POMC), which was significantly down-regulated (MAANOVA). Endorphins are cleaved from POMC a precursor hormone highly expressed in the hypothalamus and bind to the microopioid receptor (OPRM1). OPRM1 was seen to be significantly up-regulated in the infanticidal animals on the human array (MAANOVA), which was confirmed by Q-RT-PCR. This up-regulation may be in response to the lack of endorphins, but the reduced activity of OPRM1 would in turn reduce dopamine secretion [Kas et al., 2004] (Fig. 4). As with the oestrogen receptors, the OPRM1 gene is on SSC1, where a potential QTL was identified [Quilter et al., 2007].

5HTR2C was found to be differentially expressed in the infanticidal animal of three pairs and the 4xLW analysis from the human array (FSPMA). Q-RT-PCR confirmed up-regulation in the infanticidal animal for pair (5221/5219) and for the 4xLW analysis. It also indicated up-regulation in the infanticidal animal for three other pairs (3028/5572, 5844/5845, 1311/1313). Serotonin regulates dopamine release via 5HTR2C and 5HTR3 receptors and drugs that decrease 5HTR2C and increase 5HTR3 mediated dopamine release have been found to alleviate depression [Dremencov et al., 2006]. Up-regulation of 5HTR2C may therefore be associated with increased depression and a decrease in dopamine secretion (Fig. 4). The human 5HTR2C oligonucleotide only matched porcine sequence at 56/70 nucleotides, which may account for differences between the array and Q-RT-PCR results. Both oligonucleotide and Q-RT-PCR primers match to exon 6 (although different parts) of the 5HTR2C gene. Heisler et al. [2002] found that 5-HT systems also activate POMC (proopiomelanocortin) neurones and can be linked to anorexia and variation in the membrane protein 5-HTT (neurotransmitter transporter, serotonin) has actually been associated with susceptibility to bipolar affective puerperal psychosis [Coyle et al., 2000]. 5HTR2C was also a prime candidate for maternal aggression in a parallel QTL study, which detected a QTL in Xq, syntenic to where this gene maps in humans [Quilter et al., 2007].

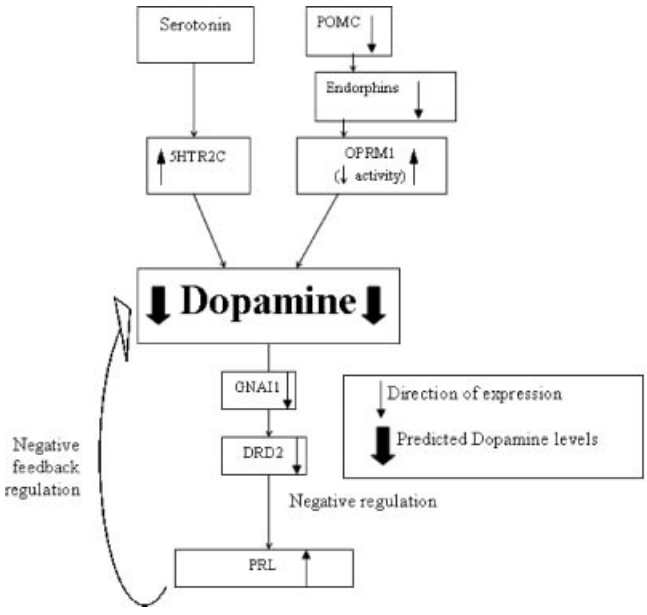

Fig. 4. Flow diagram representing the expression of key genes indicated from our microarray studies and their combined impact on reducing dopamine levels, which is associated with depression.

Neuroimaging and microarray studies have provided evidence for myelin and oligodendrocyte abnormalities in schizophrenia and mood disorders [Uranova et al., 2007]. Rosin et al. [2005] suggest that dopamine DRD2 and DRD3 receptor activation may play an important role in oligodendrocyte protection against oxidative glutamate toxicity and oxygen–glucose deprivation injury. The down-regulation of DRD2 seen in our infanticidal pigs may lead to loss of this neuroprotective effect.

There are many links between dopamine receptors and other genes highlighted by our microarray analysis. Dopamine receptors are also known to modulate NMDA glutamate receptor-mediated functions through direct protein–protein interactions. GRIN1, the receptor for glutamate, is the major excitatory neurotransmitter and showed differential gene expression (MAANOVA) on our porcine array. It is highly expressed in the brain and is involved with learning, memory, some aspects of behavior and schizophrenia [Begni et al., 2003]. GLYATL2 a glycine transferase was also differentially expressed (porcine MAANOVA and FSPMA). Glycine receptor alpha 2 was a candidate gene from our parallel QTL study [Quilter et al., 2007].

There is strong experimental evidence that dopamine demonstrates neurotoxic properties and dopamine-oxidized metabolites have been shown to inhibit the mitochondrial respiratory system. Ben-Shachar et al. [2004] suggest an additional mechanism for dopamine toxicity, which involves mitochondrial complex I inhibition. This would support the array data discussed below.

G Proteins and MAPK Signaling Pathway

There is a large body of evidence that dysfunction of central serotonergic and noradrenergic, and dopaminergic systems, all of which act via G proteins (Gi, Gs, Gq) is involved in the etiology of human depression [Sidhu and Niznik, 2000]. Adenylate cyclase inhibiting G alpha protein (GNAI1 or Gi) was shown to be differentially expressed by both porcine analyses. Q-RT-PCR confirmed down-regulation of this gene.

GNAI1 is also linked to the MAPK signaling pathway, which in turn is linked to Alzheimer's disease (AD). HSPA8 [FSPMA and MAANOVA (less significant)], MAPK1 (MAANOVA) and ILK (human FSPMA) are also part of this pathway. Q-RT-PCR confirmed up-regulation of HSPA8. In fact many genes that are differentially expressed on both our porcine and human arrays have a link to AD, a neurodegenerative disorder with marked behavioral disturbances. Neuroinflammation, which may exacerbate neurodegeneration, is found in such conditions and it is thought that neuroinflammation may activate adhesion molecules such as CD45 [implicated in a parallel QTL study Quilter et al., 2007]. The ERK and MAPK pathways are then activated which induce proinflammatory gene expression leading to the production of cytokines and chemokines.

Oestrogen Related Pathways

In addition to the previously discussed link of PRL to oestrogen regulation another interesting gene found to be differentially expressed in five pairs (porcine FSPMA) and three pairs of animals (human FSPMA) was transthyretin (TTR). TTR is a major transporter of thyroid hormones and vitamin A. It binds to the Alzheimer beta peptide and may have a neuroprotective effect. Altered levels of TTR have been described in the cerebral spinal fluid of psychiatric patients and TTR knock out mice are associated with reduced depression [Sousa et al., 2004]. The increased expression of TTR seen in three of our infanticidal animals therefore suggests an association with a depressive phenotype. This was confirmed by Q-RT-PCR, in 6/9 pairs. Up-regulation was also seen with the human array; however there were some differences between arrays, which may be explained by the fact that the TTR oligonucleotide only matched porcine sequence at 56/70 nucleotides. Both cDNA and oligonucleotide match to exon 3 on the TTR gene.

TTR also has a direct relationship with oestrogen, as expression increases in oestrogen replacement therapy patients and both ESR 1 and 2 receptor proteins are present in the choroid plexus where TTR is abundantly expressed [Tang et al., 2004]. Over expression of TTR therefore correlates with increased levels of oestrogen, and high ratios of circulating oestrogens to progesterone in late pregnancy have been associated with increased aggression toward piglets [McLean et al., 1998]. This may be exacerbated by the fact that we also saw overall down-regulation of PRLR, which leads to a corresponding fall in progesterone [Johnson and Everitt, 2000].

Mitochondrial Pathways

Intriguingly, several mitochondrial genes were shown to be differentially expressed in both porcine analyses (ATP6, ATP8, COX1 and ND2 (MAANOVA), 16S, ND4 (FSPMA), and ATP5A1 nuclear gene both analyses). Mitochondrial genes were not present on the human array but several mitochondrial-associated proteins were found to be differentially expressed. Mitochondria play a crucial role in ATP production through oxidative phosphorylation (OXPHOS), are involved in amino acid, lipid, and steroid metabolism, play a role in intracellular calcium buffering, are main producers of reactive oxygen species (ROS) and are regulators of apoptosis. Mitochondria contain copies of their own genome (mtDNA) inherited solely through the mother, which encodes ribosomal RNAs, transfer RNAs and subunits of the respiratory chain. In addition, nuclear DNA encodes further subunits of the respiratory chain. Brain tissue has high aerobic activity, requiring high mitochondrial content and is therefore more likely to be affected by mitochondrial disease. Mitochondrial dysfunction has been observed in a wide range of brain disorders, including schizophrenia, bipolar disease, dementia, Alzheimer's disease, epilepsy, and Parkinson's disease which have underlying pathophysiological mechanisms in common,

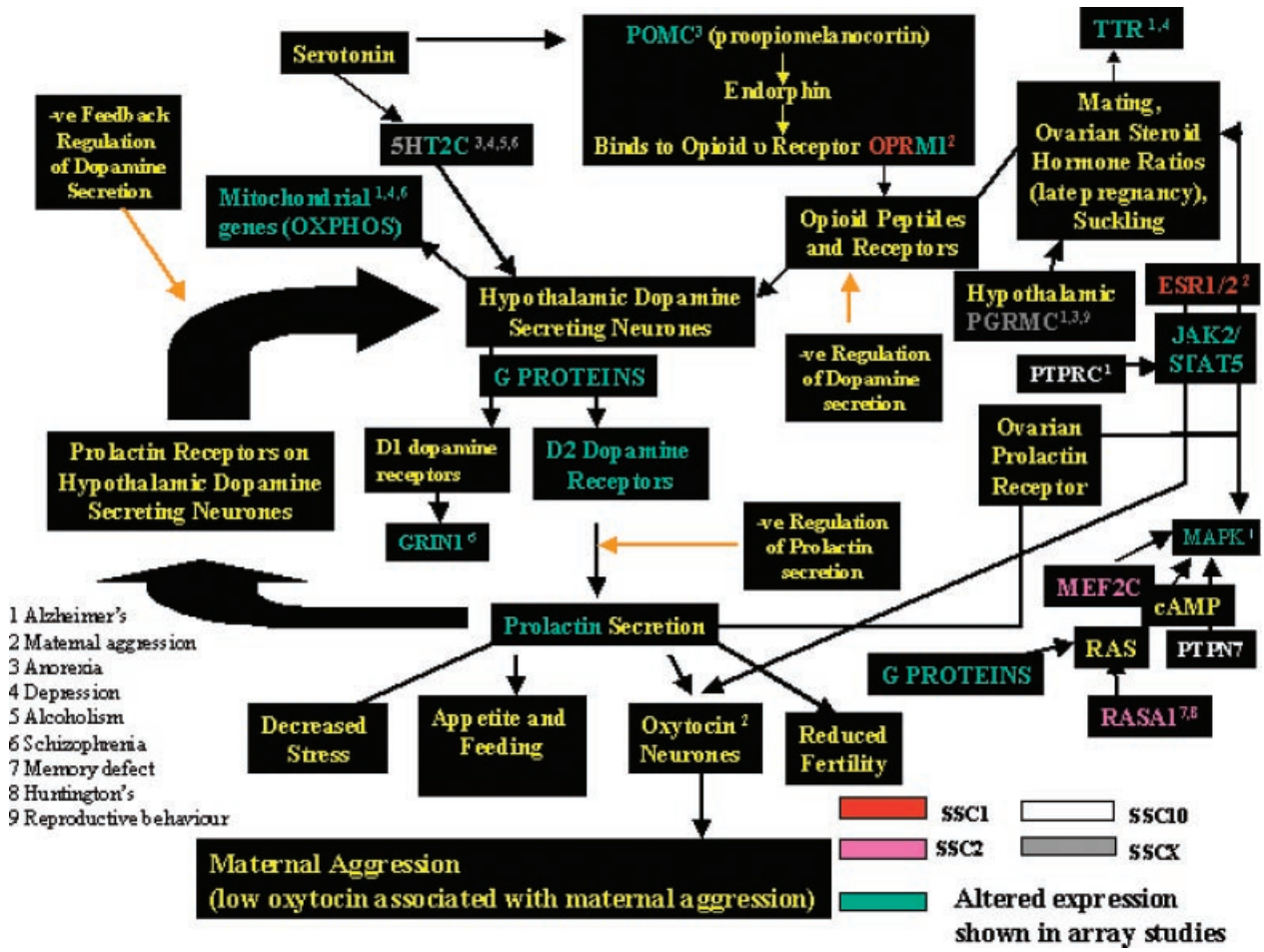

Fig. 5. Overall summary of gene networks implicated in porcine maternal infanticide from both microarray and QTL studies (green indicates genes implicated from array results and red, pink, white, and gray indicates genes from porcine chromosome QTL regions reported in Quilter et al. [2007]).

namely altered energy metabolism, ROS production and the accumulation of mitochondrial DNA (mtDNA) damage [reviewed by Pieczenik and Neustadt, 2007]. Alterations in the OXPHOS system correlates with reduced ATP levels in the frontal and left temporal lobes of schizophrenia patients. In particular, activities of complexes I and IV have been shown to be impaired in schizophrenia implicating ND2, ND4, and COX1 as good candidates from our analysis [Ben-Shachar, 2002]. COX7C (cytochrome *C* subunit VIIc), which is part of the terminal component of the mitochondria respiratory chain was a candidate from our parallel QTL study [Quilter et al., 2007]. Seelan and Grossman [1997] showed that COX7C is the second nuclear gene of COX to be regulated by transcription factor YY1, a nuclear target for stress-related signaling pathways in neuronal degeneration [Korhonen et al., 1997] that was found to be differentially expressed on our porcine array (MAANOVA).

Other Genes

POU3F3 was differentially expressed in both porcine analyses and is connected to oligodendrocytes, abnormalities of which are associated with mood disorders [Uranova et al., 2007]. In a recent study, this gene was also shown to be close to a single nucleotide polymorphism (SNP), found to have strong association with bipolar disorder [The Wellcome Trust Case Control Consortium, 2007]. In fact other genes highlighted from this large study are related to our results: GABRA1 is common to both studies; the WTCCC had SNPs close to NDUFAB1, NDUFV2, and NDUFA12 and we have differential expression of NDUFA12 and NDUFB6, all components of mitochondrial complex I; the WTCCC had a SNP in GRM7 a metatropic glutamate receptor and we have GRIN1 an ionotropic glutamate receptor. The fact that we are finding genes in pathways common to such an extensive study on bipolar disorder provides more evidence that the results are a good representation of genes contributing to the porcine maternal infanticide and the human puerperal psychosis phenotypes.

SUMMARY

Overall our microarray analysis has given us an interesting network of genes expressed in the hypothalamus of maternal infanticide pigs. We have focused on the genes most relevant to the infanticidal phenotype such as those affecting PRL and dopamine regulation but many other genes may also be relevant to the phenotype as summarized in Tables I and II. It is important to appreciate that array analysis will detect gene expression changes in genes that have both a primary and secondary impact on the phenotype. Thus one may expect to detect changes in gene expression in causative genes (where these affect promoter regions or mRNA stability) and in genes in pathways regulated by causative genes. This may also explain why different analyses identified different genes. The cross-species experiment to the human array confirmed differential expression in genes involved with PRL regulation (POU1F1), TTR, and several mitochondria associated genes, implicating that these are important candidates for maternal infanticide. In addition, OPRM1 and 5HTR2C detected by the cross-species study were also candidates from a parallel QTL study and are key genes involved in dopamine regulation. The QTL study also implicated genes involved in the MAPK and JAK/STAT pathways as well as mitochondrial linked genes [Quilter et al., 2007]. We hypothesize that many of these genes are inter-linked and Figure 5 summarizes these relationships.

Our analysis has provided a workable set of genes, worth further investigation. It would be useful to identify SNPs within or close to these candidate genes, which could be tested for an association with the infanticide phenotype seen in pigs. Any confirmed associations could be extended to an analysis of the orthologous genes in patients with puerperal psychosis.

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