Towards the genetic dissection of the complex maternal infanticide behaviour using a white Duroc × Erhualian pig F2 design

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Congying Chen

Born in Jiangxi, P. R. China

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1. Name of referee: Prof. Dr. Dr. Bertram Brenig

2. Name of co-referee: Prof. Dr. Christoph Knorr

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To my family

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1. **C.-Y. Chen**, C. L. Gilbert, G.-C. Yang, Y.-M. Guo, A. Segonds-Pichon, J.-W. Ma, B. Brenig, C. Sargent, N. Affara, L.-S. Huang. (2008) Maternal infanticide in sows: Incidence and behavioural comparisons between savaging and non-savaging sows at parturition. *Applied Animal Behaviour Science,* 109: 238-248.

2. **C.-Y. Chen**, C. Sargent, C. Quilter, J. Ren, N. Affara, B. Brenig, L.-S. Huang. (2008) Molecular characterization of porcine progesterone receptor membrane component 2 (*PGRMC2*) gene and its association with sow maternal infanticide. *Mammalian genome* (Submitted)

3. **C.-Y. Chen,** Y.-M. Guo, B. Brenig, N. Affara, L.-S. Huang. (2008) A genome wide detection for quantitative trait loci on pig maternal infanticide behaviour in a large White Duroc × Erhualian resource population. (submitted)

4. G.-C. Yang, J. Ren, Y.-M. Guo, N.-S. Ding, **C.-Y. Chen** and L.-S. Huang. (2006) Genetic evidence for the origin of an IGF2 quantitative trait nucleotide in Chinese pigs. *Animal Genetics*, 37: 179-180.

5. G.-L. Xu, J. Ren, N.-S. Ding, Y.-M. Guo, **C.-Y. Chen**, H.-S. Ai and L.-S. Huang. (2006) Genetic analysis of the *KIT* and *MC1R* genes in Chinese indigenous pigs with belt coat colour phenotypes. *Animal Genetics*, 37 : 518-519.

6. R.-R. Chen, A. E. Day, J. Ren, **C.-Y. Chen**, H.-S. Ai, N.-S. Ding, J.-W. Ma, Y.-M. Guo, K. W. Siggens, K. M. Harvey, G.J. Evans and L.-S. Huang. (2005) Characterization of three SNPs and localization of the porcine sperm adhesion molecule (SPAM1) gene to chromosome 18 by hybrid panel mapping. *Animal Genetics*, 36: 273-275.

Abstract

Aggressive behaviour by newly farrowed sows towards their own offspring, known as savaging or maternal infanticide, occurs commonly in the domestic pig, with a significant impact upon both the agricultural economy and animal welfare. In this thesis, maternal behaviours were in detail recorded in 288 F_2 sows of a designed large White Duroc × Erhualian resource population from 5 h before to 24 h after parturition using real time 1:0 sampling. Genetic background of maternal infanticide was investigated by combination of quantitative trait locus mapping and candidate gene analysis. Savaging sows were defined as those with an apparently deliberate attack on one or more piglets resulting in the death by biting of at least one piglet. The incidence of savaging was, 10.7% in gilts (n = 103) and 5.3% at the second farrowing (n = 94) in farm 1; farm 2, 14.6% (n = 48) and 6.25% (n = 16), respectively; farm 3, 6.8% (n = 44) at the second farrowing and 3.2% (n = 31) at the third farrowing; farm 4, 15.7% (n = 70) at the first farrow. The incidence of savaging tended to be higher in gilts (P = 0.058) although some savaging gilts were harvested for the microarray gene expression study before their second litters. There was no effect of the different farms on incidence of savaging. Prepartum nest building behaviours were not a predictor for savaging, but savage sows had a greater frequency of posture change from before parturition through the expulsive phase. This restlessness included an increase in rearing behaviour and a reduced ability to lie down carefully without endangering piglets.

After finishing the phenotype analysis, we performed the whole genome scan for QTL mapping for maternal infanticide behaviour. All 288 F_2 sows together with their 19 F_0 grandparents and 68 F_1 parents were genotyped for 183 microsatellite markers. A QTL analysis was performed using a composite regression interval mapping method. When only additive effect was considered, a 5% genome-wide threshold QTL was detected on SSC16 for sows at the second farrow. Suggestive QTLs were identified on SSC9 for sows at the first farrow, on SSCX for sows at the second farrow, and on SSC2 for sows at the third farrow. When additive, dominant and imprinting effects were considered, one QTL was detected on SSC16 for sows at the second farrow. When the additive and dominant effects were considered, only a suggestive QTL was found on SSC3 for sows at the first

farrow. QTLs on SSC2 and SSCX were consistent with the results from different Western commercial lines. According to comparative maps of human and pig, some interesting candidate genes were found on several of the detected QTL regions.

In this thesis, we also studied the progesterone receptor membrane component 2 (*PGRMC2*) as a candidate gene for sow maternal infanticide. We described the chromosomal mapping, isolation and molecular characterization of the porcine *PGRMC2* gene and analyzed the association of *PGRMC2* polymorphisms with sow maternal infanticide. *PGRMC2* was assigned to pig SSC8 by radiation hybrid mapping and the most significantly linked marker is CL344180 at a distance of 19.57cR. *PGRMC2* has two different transcripts in pigs. The full-length cDNA of the large transcript is 1858 bp long and contains a 669-bp open reading frame (ORF) encoding a protein of 223 amino acids with a calculated molecular mass of 23.771 kDa and a theoretical isoelectric point of 4.77. The transcription initiation site of the small splice variant is located 115 bp downstream of that of the large transcript. The shorter transcript encodes a protein of 170 amino acids. The deduced porcine *PGRMC2* proteins show high homology with those of other mammals. Similar to the other mammalian orthologs, the porcine *PGRMC2* gene consists of 3 exons with sizes of 446 bp, 156 bp and 1259 bp. The 5' UTR lengths of the two transcripts are 25 bp and 70 bp respectively and both transcripts have the same 1161 bp 3' UTR. The promoter sequence is GC-rich and lacks a typical TATA box. Several putative cis-regulatory DNA-motifs, which represent potential binding sites for transcription factors like NF-1, AP-2, Sp1, T-Ag, glyco and RBS, were identified in the 208-bp upstream genomic region. Five single nucleotide polymorphisms (SNPs) were revealed in introns and the 3' UTR of the porcine *PGRMC2* gene. A family based association study of haplotypes and SNPs with sow maternal infanticide using the transmission disequilibrium test (TDT) showed that allele A of exon 3 c-G>A and allele T of intron 2 g-1578A>T more frequently transmitted to infanticide sows. But this transmission trend was not found on any haplotypes. RT-PCR results indicate that *PGRMC2* is expressed ubiquitously in pigs and that the large splice variant exists in all 18 tested tissues. Both transcripts were identified in the hypothalamus and liver.

Introduction

The purpose of modern pig production is to obtain high return at low costs. Efficient pig production depends on a number of factors such as a rapid growth rate and a high reproductive rate. The number of piglets produced per sow and per year is the economically most important reproductive trait for the pig producer (Palmø, 1999). Over the last several decades, pig breeding program was focused on selection of large litter size and now many attentions have also paid to the number of born alive and litter size at weaning. So it is important to reduce the stillbirth rate and mortality before weaning. The National Animal Health Monitoring System of America indicated that pre-weaning mortality rate in USA amounted to 11% in 2001 (Lay, 2002). This was attributed to very complex causes. Under the standard feeding condition, more than 50% of pre-weaning mortality was due to being overlain by the sow and another anomaly was due to savaging of piglets by the sow which was also defined as maternal infanticide (Lay, 2002). Piglet mortality before weaning within commercial pig production is about 10% in Europe. The main reason that causes this loss is sows' behaviours and maternal ability around the partiturition. So maternal behaviours not only influence the survival rate of piglets before weaning but also bring a big problem to piglets' welfare. Especially the maternal infanticide of sows causes big economic loss to pig production industry. Maternal ability must be improved so that the sow can take care of her piglets successfully. Furthermore, in modern piglet production, characterised by larger herds and a lower degree of supervision, the sow has greater responsibility for rearing her piglets.

In humans, extreme behaviours in women during and after pregnancy were sometimes occurred. These behaviours occur in a wide range from mild depress to severe puerperal psychosis (Jones *et al.*, 2001). There are some common features between sow maternal infanticide and women puerperal psychosis (Quilter *et al.*, 2007). So pig may be used as a model for human puerperal psychosis. The understanding to the aetiology of sow maternal aggression from neuroendocrine and genetic background can provide some clues to human puerperal psychosis. The following chapters outline the progress in understanding and studying of maternal behaviours around farrowing, especially maternal infanticide in recent years.

1 Maternal behaviours around farrowing

Maternal behaviours around farrowing differ greatly between individual sows (Fraser, 1990). It depends on species (genetic background), environment, experience and internal motivation. There is perhaps more species diversity in maternal behaviour than in any other behaviour. Current knowledge of mechanisms controlling maternal behaviour in the pig is elusive. In modern pig industry, the sows are always housed in the farrowing crate during birth to increase the piglet survival. These crates are common in pig keeping and restrict freedom of movement for sows. Under this restrictive condition, some maternal behaviours that are intrinsic to pigs can not be exhibited. Jarvis *et al.* (2004) studied the different effects of the crates and pens with/without straw on maternal behaviours during 8 hour period after the expulsion of the first piglet and discovered that the sows in pens were more active during the first 2 h than the gilts in crates. Gilts in crates spent longer sitting throughout the 8 h period and tended to show more savaging to their piglets. In recent years, considering the sow welfare, the farrowing crate is already discarded or even prohibited by law in some European countries (e.g. Sweden and Switzerland). Although many aspects of maternal behaviours are general common in predators, maternal behaviours are unique. In natural or semi-natural condition, maternal behaviour of free-ranging domestic pigs around farrowing is a behavioural sequence, from the start of isolation to nest building, lie down, push out piglets and the last of nursing and maternal caring.

Approximately 24 h before farrowing, the sows will leave the flock and find a suitable site for nest building (Jensen, 1986). Sows housed in pens, indoors also perform nest building behaviour, such as gathering straw, pawing on the floor. This behaviour has not been modified along with the domestication of pig (Jensen, 1986). The signal that initiates this behavioural cascade probably results from fetal maturation (Gilbert, 2001). An endocrine pathway involves in the generation of most of the prepartum behavioural components. The onset of nest building is associated with a periparturient decline in progesterone, an increase in prolactin and a major rise in plasma concentration of PGF2α on the day before parturition (Gilbert *et al.*, 2001; Algers and Uvnäs-Moberg, 2007). The nest building behaviour stops at about 4 hours before the onset of parturition and oxytocin level increases dramatically at about 6 hours before the onset of parturition

(Castrén *et al*., 1993). Whether the increase in oxytocin release directly affect the central nervous system or whether oxytocin acts to stops the nest building remains unknown (Algers and Uvnäs-Moberg, 2007).

The behavioural trend of sows near parturition tends towards impassivity. Sows spend more time in lying down and occupying the nest. There are some variabilities on the duration of parturition, from 26 to 505 minutes (van Dijk *et al*., 2005). Duration of the expulsive stage has significant association with breed but no effect of parity on the duration of the expulsive stage was found (van Dijk *et al*., 2005). The maternal behaviour after expulsion is characterised by passivity and unresponsiveness to the piglets (Jarvis *et al*., 1999). The duration of maternal lateral lying in the first day after parturition is high (Cronin and Smith, 1992), at which time piglets are often located near the udder. The sows and piglets stay at the nest during the farrowing day and the day after, and they keep away from the group until the piglets are about ten days old (Jensen and Redbo, 1987). Sows can not recognize their piglets until about one day after parturition. This facilitates the foster of the piglets between litters. The nursing frequency during the first 24 hours is about one per hour and a teat order is settled during the first day and stays constant until weaning. With the piglets growing, the number of nursing decreases (Algers, 1993).

Maternal caring behaviours during the period of lactation are important for reducing the piglets' mortality. More than half of the liveborn mortality occurred during the first four days after parturition. The main reasons that cause the pre-weaning loss of piglets are malnutrition through unsuccessful suckling behaviour, and crushing of piglets by the sow. More piglets survived from the farrowing crates where the sows are restricted than from the open systems before weaning, and piglets grow more quickly in open systems (Marchant *et al.*, 2000). Although the mortality rates of piglets in farrowing crates are lower than in pens, the death of piglets due to crushing still amount to 4.8% to 18% (Svendsen and Bengtsson, 1982; English *et al*., 1978). Fraser (1990) divided crushing into two distinct behaviour patterns: posterior crushing, piglets were trapped beneath the sow's hind quarters; and ventral crushing, piglets were crushed under the udder and rib cage. Farrowing crates prevent posterior but not ventral crushing. Malnourished piglets spend more time near the sows in suckling and are more vulnerable to crushing (Fraser, 1990). Rolling movement from lying on the side to lying on the other side, lying down

quickly without carefulness and sitting behaviour put piglets at higher risk for crushing. There seems to be large individual difference in some behaviours, for example, McGlone *et al.* (1991) showed that the heritability for sitting frequency was 0.4 in slaughter pigs. So selection could be carried out successfully to decrease sow sitting behaviour. The degree of sow's reaction to piglet screaming or other stimuli from piglet also determined fewer or more crushed piglets. Some individuals have very strong reply to piglet's distress calls by letting the piglet escape, while others appear to be completely unaffected (Hutson *et al.*, 1991; Wechsler and Hegglin, 1997).

2 Maternal infanticide–an extreme form of failure maternal behaviour

Among piglets born alive, crushing was the most frequent cause of death (2.1%), followed by deaths due to diarrhoea (1.7%), anaemia (1.2%), savaging (1.1%) and losses of small weak piglets (0.9%) (Spicer *et al.*, 1986). Savaging, also called maternal infanticide, is an extreme form of failure maternal behaviour during farrowing. Failure to establish normal maternal bonds occurs in individuals of many species. In some animals this may simply show as abandonment or refusal to care for offspring. An extreme behaviour, usually associated with litter bearing species, involves active maternal aggression towards newborns including infanticide. This behaviour may be defined as an active attack of piglets using the jaws that results in serious or fatal bite wounds. It is most often seen in the first day after birth. Treatment of affected sows has not advanced beyond the limited benefits conferred by sedation, as suggested in an early report of treatment of 428 sows by Lewis and Oakley (1970). This approach cannot prevent the behaviour before it is administered or guarantee no return after recovery from sedation. This savaging behaviour has been observed in domestic sows (Harris *et al.*, 2003) causing both significant economic losses to the pig industry and problems of animal welfare.

Savaging of sows towards their newborn offspring has been described in large surveys of commercial pigs in Europe with an incidence of 8% (Knap and Merks, 1987) or 7 to 12% (van der Steen *et al*., 1988). Harris and Gonyou (2003) investigated the piglet-directed aggression at the first farrowing of 6625 crossbred gilts on seven commercial farms. Their results showed that 5.3% gilts displayed some degree of

aggression towards their piglets, while 2.9% killed piglets. And 0.6% piglets were killed by savaging. There was marked variations among farms in the reported incidence of savaging. Savaging deaths represented on average 11.2% of total reported pre-weaning mortality. They also revealed that the number of piglets per litter killed by savaging was reduced by 40% if there h continuous lights in the farrowing house. Additionally, animals that savaged piglets as gilts were more likely to savage during their second parity (Harris and Gonyou, 2003). In their earlier study, Harris *et al.* (2001) found that 33.3% individuals showed some offspring-directed aggression in farmed wild pig females and parturition lasted longer in savaging sows.

It has been indicated that savaging sows are more fearful of humans. Sows that readily interacted with humans were non-savaging and more protective of their litters. Fear of the piglets, lack of experience during adolescence, and the pain associated with parturition, have all been implicated in savaging behaviour. However, the definitive cause(s) of savaging remains elusive (Lay *et al*., 2002). The sows do not have any clues to show that they will be savaging before parturition. But some studies indicated that savaging sows were more restless than non-savaging sows in the day before birth (Jarvis *et al.,* 1999, 2001; Appleyard *et al.*, 2000) and within 24 hours of birth (Ahlstrom *et al*., 2002; Jarvis *et al.*, 2004).

In the case of maternal infanticide to piglets, the aetiology is poorly understood and we have no idea about what the internal regulator may be at present. Van der Steen *et al*. (1988) indicated that the year, season and feeding level had no effect on the incidence of savaging in gilts. But maternal experience may be a factor causing savaging. Gilts show more frequency of savaging than sows (Randall, 1972; English *et al*., 1978; Harris *et al.*, 2003). Endocrine and genetic component also play important roles on savaging.

3 The role of neuro-endocrine in maternal behaviours and infanticide

Neuro-endocrine plays an important role in sow's reproduction and maternal behaviours. Sow parturition is a complex process along with changes of a series of hormones in the body. For example, the levels of estrogen, PGF2a and prolactin are increased and progesterone is decreased. Although the detailed active mechanism of neuro-endocrine on animal reproduction and maternal behaviours is unknown at present,

a accumulated knowledge has supported the hormonal regulation of maternal behaviour (Algers and Uvnäs-Moberg, 2007).

3.1 Estrogen and progesterone

Estrogen and progesterone have been shown to be important in the initiation of maternal behaviour. In primates (Pryce *et al*., 1988), rodents (Fahrbach *et al*., 1984) and sheep (Kendrick and Keverne, 1991) estrogen and progesterone have been linked to the expression of maternal behaviour and the successful bonding of the mother with her young. It is therefore possible that factors which interfere with the production of circulating sex steroids could be causally linked to the performance of poor maternal behaviour in sows and consequently piglet-directed aggression. McLean *et al.* (1998) studied the relationship between sex steroid concentration and maternal aggression in gilts and discovered that there was considerable variation on estrogen between individuals on prepartum. Pre-farrowing oestradiol concentrations positively related to dominance rank value during pregnancy. Estrogen is known to have generally excitatory effects (Herzog, 1999; Smith *et al*., 2002) and considerable regulatory influence on systems mediating anxiety and mood (Fink *et al*., 1996). It exerts its effect on target cells by interacting with specific intracellular estrogen receptors (ERs) or by non-genomic action through membrane receptors. Gilts savaging to piglets showed a higher pre-farrowing oestradiol to progesterone ratio, significantly higher levels of oestradiol post-partum and had the tendency to exhibit lower progesterone levels on the days leading up to farrowing. De Passille *et al*. (1993) also indicated that elevation of progesterone concentrations in post-partum induced a reduction of maternal care in the sow. Maternal aggression appeared to be negatively related to aggression during pregnancy, but it was not reflected in plasma concentrations of sex steroids around parturition (McLean *et al.*, 1998).

There are more studies on effects of progesterone and estrogen to maternal behaviours in rodents. In the rat, the natural variation in maternal care is associated with differential expression of estrogen receptor alpha in medial preoptic area (MPOA) of the hypothalamus and these variations can be transmitted to her female offspring (Champagne *et al.*, 2003). Schneider *et al.* (2003) used the progesterone receptor (PR)

knockout and PR blockade mice to study the neuroendocrine mechanisms of male infanticide. Compared to 74 percent of the control mice committed infanticide, progesterone receptor gene deficient mice were found a complete lack of infanticide in the resulting litters born. Additionally, deficient mice showed significantly more paternal care than the controls by frequently contacting pups and retrieving them to nests. Progesterone, and not testosterone, may be key in specifically controlling infant-directed aggression in male mice. And paternal behaviour may be based on the same biological mechanism as maternal behaviour. In ewe, Kendrick and Keverne (1991) indicated that maternal behaviour was only stimulated by exposure to oestradiol in combination with progesterone.

3.2 Oxytocin

Oxytocin (OT) is a nonapeptide abundantly expressed in specific magnocellular cell groups in the paraventricular and supraoptic nuclei of the hypothalamus and secreted mainly from the posterior pituitary gland. Oxytocin is involved in several different aspects of maternal behaviour and physiology of mammals. In pigs, it is essential for milk ejection reflex (Algers *et al*., 1990). The termination of nest building and the initiation of parturition in sows are also associated with elevated concentration of oxytocin (Castrén *et al*., 1993). There exists a link on maternal infanticide and plasma oxytocin. Plasma oxytocin is reduced in savaging sows. A likely explanation is that the savaging sows suckle their offspring less than normal animals, so teat stimulation, and hence OT secretion, is reduced (Affara *et al.*, personal communication).

The relationship between oxytocin and maternal behaviours has been clarified more clearly in rat although the results from different studies remain controversial. Oxytocin induces the general maternal behaviours, such as pup grooming (Argiolas and Gessa, 1991). Neumann *et al.* (2000) revealed that the actions of intracerebral oxytocin included independent effects on the responses of the hypothalamo-pituitary-adrenal axis and oxytocin systems to stressors and the anxiety-related behaviour which are modulated by the reproductive state of the animals in rats. Consiglio *et al.* (2005) indicated that OT exhibited an inhibitory role on maternal aggression in rats at least in the central amygdaloid nucleus and bed nucleus of stria terminalis. The effects of OT within the

Central Nucleus of the Amygdala (CeA) and paraventricular nucleus of the hypothalamus (PVN) on maternal aggressive behaviour might be related to its regulation of local amino acid release (Bosch *et al.*, 2007). However, compared to predictions from neuropharmacological studies, many of the expected behavioural deficits have not shown in oxytocin knockout mice (Pedersen *et al*., 1992). In Nishimori's study (Nishimori *et al*., 1996), oxytocin-deficient females did not display any changes in their maternal behaviour towards their pups, but all offspring died shortly after birth because of the dam's inability to nurse. But controversially in Ragnauth's study, oxytocin can inhibit infanticide in mice. Oxytocin gene knockout mice showed 100% infanticide while wild-type mice were only 16% infanticide and 50% maternity (Ragnauth *et al.*, 2005).

The actions of oxytocin is mediated via the oxytocin receptor (OTR), the OTR knockout mice showed normal parturition but exhibited the defects in lactation and maternal nurturing (Takayanagi *et al.*, 2005). Female rats that showed more maternal care to offspring had significantly higher oxytocin receptor levels in the medial preoptic area, the lateral septum, the central nucleus of the amygdala, the paraventricular of the hypothalamus, and the bed of the stria terminalis (Champagne *et al.*, 2001).

3.3 Prolactin (PRL)

Prolactin is not only synthesized in the pituitary gland, but also in the central nervous system, the uterus, the immune system and the associated tissues of conception. Its biological actions are not limited solely to reproduction because it has been shown to control a variety of behaviours and even plays a role in homeostasis (Dutt *et al.*, 1994). Prolactin is a neuromodulator of behavioural and neuro-endocrinal stress coping in the rat. The level of prolactin and prolactin receptor in intracerebroventricular influences the anxiety-related behaviour on the plus-maze (Torner *et al.*, 2001). Prolactin promotes the rapid onset of maternal behaviour, also influences nursing behaviours in sows and maternal responsiveness in rodents positively. The inhibition of prolactin release induces the severely disruption of maternal behaviours, at least in hamster (McCarthy *et al*., 1994). Down-regulating the expression of prolactin receptor through intracerebroventricular administration of the prolactin receptor antagonist disrupts parturition and impairs maternal behaviour through the modulation of prepartum

prolactin (Nephew *et al.*, 2007). Homozygous mutant and heterozygous mutant nulliparous female mice on prolactin receptor show a deficiency in pup-induced maternal behaviour (Lucas *et al.*, 1998). Moreover, primiparous heterozygous females exhibit a profound deficit in maternal care when challenged with foster pups.

The role of prolactin on porcine maternal behaviour is not clear. But maternal killing of piglets is thought to take place before suckling. Moreover, high level of prolactin is associated with reduced level of dopamine. It is well known that low level of dopamine will induce depression.

3.4 Other hormones

Besides the hormones described above, other hormones like PGF2α, growth hormone (GH) and Corticotropin-releasing hormone (CRH) could be also associated with maternal behaviour. GH shows a number of PRL-like activities. In steroid-treated rats, GH treatment stimulated a more rapid onset of maternal behaviour (Bridges and Millard, 1988). Prostaglandin F_2 alpha induced the nest building behaviour, but it is not involved in subsequent maternal behaviour (Gilbert *et al.*, 2001). CRH is released by stressors and produces stress-like behavioural effects. The effect of CRH on inhibition of maternal behaviour in rat is associated with dose and mothering experience. Higher doses of CRH significantly increased pup-killing in naïve rats. In contrast, CRH produced no pup-killing in rats with mothering experience (Pedersen *et al.*, 1991).

3.5 The regulation chain of hormones for maternal ability

Effects of stress such as fearfulness or novelty-induced anxiety on farrowing and reproduction behaviours may be mediated by the close association between the hypothalamic-pituitary-adrenocortical (HPA) axis and the hypothalamic – pituitary - gonadal axis. During late gestation, activation of the HPA axis in response to stressors may result in a lower secretion of oestrogen and progesterone as well as abnormal elevations of prolactin levels. Low levels of oestrogen and progesterone before farrowing may influence the preparation of the central nervous system (CNS) for triggering effects (after farrowing) of increased oestrogen concentration on maternal behaviour. The progress of parturition and triggering maternal behaviour need the increase in oxytocin

during farrowing and in oestrogen after farrowing. But this increase may also be inhibited by activation of the HPA axis. So the initiation of maternal behaviour during the first period of following is dependent on the well-timed secretion of oestrogen, progesterone, prolactin and oxytocin, and may thus be adversely affected by stressors such as fear or anxiety.

4 Genetic background of maternal behaviours

In addition to environment, experience and hormonal status, a strong genetic background contributes to maternal behaviours including maternal infanticide in pigs. But very few studies on genetic background of domestic animal behaviours have been reported. In pigs, sexual behaviour, sitting behaviour and fear of humans are determined genetically to some extent. Aggressiveness towards piglets (maternal infanticide) is the only type of maternal behaviour for which the genetic variation has been thoroughly analysed (Knap and Merks, 1987; van der Steen *et al*., 1988).

Evidences for a genetic contribution to maternal infanticide come from the following. In addition to Knap and Merks (1987) who reported daughter-dam heritability estimates of 0.4-0.9, van der Steen *et al*. (1988) estimated the heritability of maternal aggressive behaviour of sows at parturition and discovered that estimates of heritability differ from daughter/sire (0.12 - 0.25) compared to daughter/dam (0.5 - 0.9). The high daughter/dam heritability may suggest one or a few responsible genes. They also showed that the effects were additive. The heritabilities of some maternal behaviours around farrowing are shown on Table 1. Except for maternal infanticide, the heritabilities for other maternal behaviours which were estimated from different sow populations are comparatively low. An intermediate heritability for performed aggression in sows at mixing suggests that this aggressive behaviour is a heritable trait. Whether it exits a relationship with aggression to piglets or not is unknown. Estimated heritabilities that were recorded under field conditions and based on questionnaires to the farmers in Norwegian (N) and Finnish (SF) nucleus herds for sows' reaction to piglets screaming when handled, sows' fear of humans and sows' aggressive behaviour towards humans were higher than corresponding heritabilities based on behavioural tests presented in other studies (Gäde *et al.*, 2007; Løvendahl *et al.*, 2005; Grandinson *et al.*, 2003). And the field recording of maternal

Table 1: Heritabilities of maternal behaviours around farrowing in different populations

Sow's behaviour	Heritability	Animal	Reference
Maternal infanticide	0.4-0.9	Dutch Landrace (DL), Duroc and (Duroc*DL) sows	Knap and Merks (1987)
Maternal infanticide	0.12 - 0.25[c] 0.5-0.9	923 first litters	Van der Steen *et al*., (1988)
reaction to a piglet scream when handled (maternal ability)	0.06	741–1335 sows	Grandinson *et al*., 2003
reaction to her piglets being handled	0.01	741–1335 sows	Grandinson *et al*., 2003
aggression towards the stockperson	0.08	741–1335 sows	Grandinson *et al*., 2003
performed aggression at mixing	F_A1=0.17, F_A2=0.24[a]	835 sows	Løvendahl *et al*., 2005
reaction to a piglet scream	0.08	835 sows	Løvendahl *et al*., 2005
received aggression	F_R1=0.06 F_R2=0.04	835 sows	Løvendahl *et al*., 2005
maternal ability	0.05 (0.01)[b]	32 nucleus multiplier herds	Gäde *et al*., 2007
crushing of piglets	0.03 (0.01)[b]	32 nucleus multiplier herds	Gäde *et al*., 2007
savaging of piglets	0.02 (0.02)[b]	32 nucleus multiplier herds	Gäde *et al*., 2007
attitude to people	0.06 (0.03)[b]	32 nucleus multiplier herds	Gäde *et al*., 2007
group behaviour	0.07 (0.06)[b]	32 nucleus multiplier herds	Gäde *et al*., 2007
reaction to piglet screaming when handled	0.16(N) 0.12 (SF)	Norwegian (N) and Finnish (SF) nucleus herds	Vangen *et al*., 2005
fear during routine management	0.14(N) 0.17 (SF)	Norwegian (N) and Finnish (SF) nucleus herds	Vangen *et al*., 2005
aggression towards humans	0.11 (N)	Norwegian (N) and Finnish (SF) nucleus herds	Vangen *et al*., 2005

Note: a: F_A1: mild aggressions performed; F_A2: severe aggressions performed;
b: heritability of multiplier herds in bracket
c: 0.12 - 0.25: daughter/sire, 0.5 - 0.9: daughter/dam

behaviour with questionnaires was one way of detecting genetic variation between sows and seem to work in a large scale under field conditions (Vangen *et al.*, 2005). These comparably high heritabilities are very encouraging for selection of good maternal ability of sows. Furthermore, from Vangen *et al.* (2005) study, it appears that by letting the farmers judge behaviour over a longer period of time, instead of making a single test, the environmental variation can be reduced, and a more reliable estimate of the sow's temperament can be made.

The other evidences for genetic contribution to maternal behaviours are from the studies of different pig breeds and other species, e.g. mouse and rat. In mice, different strains have significant variations on aggressive behaviour. Shoji and Kato (2006) compared the maternal and non-maternal behaviours of primiparous females in five inbred strains of mice and revealed strain-specific variations in maternal behaviour. It has been shown that for sows with equivalent metabolic statuses and husbandry systems, there is still a great variation in maternal behaviour (Fraser, 1990; van der Steen *et al*., 1988). This suggests that variations in maternal behaviour are due to genetic background. Chinese indigenous pig breeds, such as Meishan, show lower incidence of aggressive behaviour towards their offspring than European white sows. Meishan sows spend less time standing with fewer posture changes during lactation when compared to sows of a synthetic white line (Sinclair *et al*., 1998). Furthermore, van der Steen and de Groot (1992) discovered that Meishan sows possess good maternal characteristics with lower piglet mortality and even heavier piglets at weaning. Because of good maternity, Meishan piglets have 5% advantage in survival rate compared to Large White piglets.

To improve the sow's maternal abilities in the absence of humans, selection for good maternal ability may be useful. Knol (2001) showed that selection for increased piglet survival as a direct effect has been successful. Verheijen's study (2001) on sows with differing estimated breeding values for mothering ability also showed that selection for mothering ability can improve piglet survival, without negative effects on other litter features. Although Gäde *et al.* (2007) suggested that it may be difficult to genetically improve the maternal behaviour because of low heritability, studies from Vangen *et al*. (2005) indicated that the maternal behaviours were heritable. Considering the high heritability of maternal infanticide which was consistently reported in two studies (van de

Steen *et al.*, 1988; Knap and Merks, 1987), it is necessary and possible to reduce the incidence of sow maternal infanticide through selection. Forde (2002) discovered that gilts having a high level of fear of humans were more likely to savage their piglets than those who showed aggressive towards the stockperson. If there was an underlying genetic basis, selection against fear of humans in sows would be beneficial for the piglets although it could lead to a higher level of aggression to the stockperson.

5 Quantitative trait loci (QTL) mapping for animal maternal behaviour traits

Quantitative traits, such as body weight and length in pigs, show a continuous distribution of phenotypic values and are usually controlled by multiple genes and influenced by environmental factors. A quantitative trait locus (QTL) is defined as a chromosomal region that harbours one or more genes affecting a quantitative trait (Geldermann, 1975). Most behavioural variations are quantitative in nature, such as differences in aggression and mood. Individual differences in almost any behaviour can be attributable in part to quantitative genetic variation (Plomin *et al*., 1994). The method for QTL mapping at present is a whole genome scan approach using random genetic markers covering the genome to identify genomic regions that harbor QTL in pedigrees. Three essential items must be needed for all QTL mapping: resource population, markers, statistic methods and software. Both experimental and commercial pedigrees are utilized to map quantitative trait loci in domestic animals. In pigs, the main populations used in these studies are F_2 crosses between different breeds or lines which are highly divergent for the traits of interest. Two significant linkage maps of pig were published by the mid-1990s with the development of international PiGMaP gene mapping project. The largest contained over 1,200 microsatellites (Archibald *et al.*, 1995; Rohrer *et al.*, 1996).

The main statistic methods for QTL mapping are interval mapping and linkage analysis. Most analysis are carried out with online software of QTL express (http://qtl.cap.ed.ac.uk/). Maternal infanticide as binary trait has been confirmed the feasibility of QTL mapping in intercrosses and backcrosses using interval mapping (Peripato *et al.*, 2002; Visscher *et al*., 1996; Rebai, 1997; McIntyre *et al*., 2001). Bayesian mapping is also used for quantitative trait loci mapping of complex binary traits.

The initial QTL scan in pigs used the F_2 design by generally crossing European Wild Boar with a commercial breed (Andersson *et al.*, 1994) or crossing the exotic Chinese Meishan breed with a commercial breed. Since then, more than 1675 QTL representing 281 different traits have been identified (Table 2).

Table 2: Number of QTL by Pig Trait Types (cited from Hu *et al.*, 2005 and Pig QTLdb (http://www.animalgenome.org/QTLdb/)

Trait types	**Number of QTL**	**Trait types**	**Number of QTL**
Anatomy	553	Endocrine	4
Behavioural	22	Enzyme Activity	1
Chemical	18	Fat Composition	64
Coat color	2	Fatness	403
Conductivity	25	Feed Conversion	8
Conformation	8	Feed Intake	16
Defects	18	Flavor	19
Digestive Organ	10	Growth	224
Disease Resistance	7	Immune Capacity	8
Litter Size	21	Odor	5
Meat color	69	PH	58
Reproductive Organ	33	Texture	65
Reproductive Traits	9	Stiffening	3

From the QTL database, comparatively few QTLs (22 QTLs) for pig behaviours have been mapped. These behavioural QTLs were included in five published papers. Sixteen of 22 QTLs for behaviours are those for behavioural and neuro-endocrinal responses to a "novel environment" stress in a F_2 experimental cross between Meishan and Large White pig breeds (Désautés *et al.*, 2002), and the other six QTLs were about stressor response. These QTLs were mapped in the experiment of the highest degree of heterozygosity which was produced by mating the three genetically diverse purebred founder groups Meishan, Pietrain and European Wild Boar (Pierzchala *et al.*, 2003).

No QTL on maternal behaviours of pigs at present has been reported in QTLs

database. But Quilter *et al.* (2007) mapped 4 QTLs for maternal infanticide behaviour on SSC2, SSC10 and SSCX using an affected sib pair via whole-genome linkage analysis with 80 microsatellite markers covering the 18 porcine auto chromosomes and the X chromosome in 11 different commercial lines (Table 3). In mice, many QTLs for numerous behaviours have been mapped (Flint, 2003). But there are only two QTLs reported for maternal performance for offspring survival by interval mapping in an F_2 intercross of inbred mouse strains composed of 241 females.

6 Progress on research of genes responsible for maternal behaviours

The main aim of QTL mapping is to discover the quantitative trait gene (QTG) and quantitative trait nucleotides (QTNs) for interesting traits and eventually elucidate the genetic mechanisms for the formation of trait variations. But there are few successful stories on the identification of gene(s) underlying a trait, even less in behaviours. The reasons are mainly as follows: firstly, there are many QTLs for a trait, each has small effects and acts in unexpectly complex way. Secondly, the resolution of the QTL mapping is poor, especially when we are working with inbred strains. For a behavioural QTL explaining 4% of the trait variance, the 95% confidence interval will be about 40 cM when mapped in an F_2 with 400 animals (Darvasi and Soller, 1997). There are few published mapping data that have mapped QTL to a centimorgan (Mott *et al*., 2000; Talbot *et al*., 1999). Flint (2003) indicated that even when a fine mapping was conducted successfully, the identification of QTNs was still difficult. The small effect of each QTL shows the subtle changes of gene function or expression. The QTNs can lie in the regulatory region and require functional characterization. If regulatory regions locate on many tens of kilobases away from the gene they influence (Higgs *et al*., 1990), the confirmation of QTNs will be more difficult.

Table 3: QTL mapping on mammalian maternal behaviours

Behaviour	Animal	Marker	Statistics method	QTL location	Reference
Maternal infanticide	119 affected sib pairs from 11 different lines	80 SSR covering auto chromosome and X chromosome	Non-parametric linkage test	SSCXq2.1-2.2	Quilter *et al.*, 2007
Maternal infanticide	119 affected sib pairs from 11 different lines	80 SSR covering auto chromosome and X chromosome	Non-parametric linkage test	SSCXq2.4	Quilter *et al.*, 2007
Maternal infanticide	119 affected sib pairs from 11 different lines	80 SSR covering auto chromosome and X chromosome	Non-parametric linkage test	SSC10 (25-80cM)	Quilter *et al.*, 2007
Maternal infanticide	119 affected sib pairs from 11 different lines	80 SSR covering auto chromosome and X chromosome	Non-parametric linkage test	SSC2 (20-100cM)	Quilter *et al.*, 2007
Maternal Performance for Offspring survival	241 females of an F_2 intercross of inbred mouse strains	96 microsatellite markers	interval mapping	D2Mit17 + 6 cM	Peripato *et al.*, 2002
Maternal Performance for Offspring survival	241 females of an F_2 intercross of inbred mouse strains	96 microsatellite markers	interval mapping	D7Mit21 + 2cM	Peripato *et al.*, 2002

As described above, it is difficult from QTL to QTN to traits of maternal behaviours. Neither QTG (Quantitative Trait Gene) nor QTN on maternal behaviours were identified through the classic fine-mapping approach. But in rodents, there are some reports about association of some genes with the variations of maternal behaviours by using targeted disruption or expression. *Mesoderm-specific transcript* (*Mest,* also known *Peg1*) is the first imprinted gene with a proposed function in the regulation of mammalian behaviour. Lefebvre *et al.* (1998) produced the *Mest* deficient mice through targeted disruption and discovered that *Mest* deficient females showed abnormal maternal behaviour and impaired placentophagia, a distinctive mammalian behaviour. *Neuronal nitric oxide synthase* (*nNOS*) is another gene associated with maternal behaviours. Nelson *et al*. (1995) found that deletion of the *nNOS* gene resulted in increased aggression in male mice. In Gammie and Nelson's study (1999), compared to wild-type mice, female mice with targeted disruption of the neuronal nitric oxide synthase gene exhibited significant deficits in maternal aggression and in terms of percentage displaying aggression.

Some hormones that play important roles in maternal behaviours around parturition and their receptor genes are also indicated to be important to maternal behaviours, e.g. Affara *et al.* (2005) found that the A/G polymorphism at position 929 in *estrogen receptor 2* (*ESR2*) had a possible association with sow maternal infanticide (unpublished data). In mice, natural variations in maternal care are associated with expression of hormone receptor genes. Good maternal care mice (high licking/grooming mothers) showed increased *ERα* expression in the medial preoptic area (MPOA) (Champagne *et al.*, 2003). Increased levels of pup licking/grooming and arched back-nursing (high LG-ABN mothers) showed increased *GABA(A)* (*gamma-aminobutyric acid*, *A*) receptor subunit alpha1 mRNA levels in the medial prefrontal cortex (Caldji *et al.*, 2003). Female rats that were more maternally responsive to pups and that showed increased levels of pup licking/grooming have significantly higher oxytocin receptor levels in the medial preoptic area, the lateral septum, the central nucleus of the amygdala, the paraventricular nucleus of the hypothalamus, and the bed nucleus of the stria terminalis (Francis *et al.*, 2000; Champagne *et al.*, 2001).

Corticotropin-releasing factor, an activator of fear and anxious can inhibit maternal aggression to the intruders in mice, but not other maternal behaviours (Gammie *et al.*,

2004). Whether this maternal aggression to intruders has some relationship with maternal aggression to infants is unknown. In addition, to complex behaviour traits, some immediate early genes may be also important to maternal behaviour around parturition. For example, mice lacking the immediate early gene *fosB* show a defect in nurturing (Brown *et al.*, 1996).

7 Progesterone receptor membrane component 2 (*PGRMC2*) as a candidate gene of porcine maternal infanticide behaviour

Progesterone plays an important role in maternal behaviours as described above. Progesterone receptor blockade during late pregnancy in mice leads to abhorrent maternal behaviour including infanticide (Wang *et al.*, 1995). Progesterone may exert its action in living cells by several ways: firstly, the classic genomic pathway. In classic genomic pathway, progesterone needs to get into the nucleus and interact with intracellular progesterone receptors to alter gene expression followed by protein synthesis (Cato *et al*., 2002). Intracellular progesterone receptors (PRs) are expressed as two protein isoforms, PR-A and PR-B. Both proteins arise from the same gene and are members of the nuclear receptor superfamily of transcription factors (Conneely *et al.*, 2002). Secondly, non-genomic effects that are transmitted by membrane receptors and currently poorly characterized (Losel *et al*., 2003). The time required to fully activate classic genomic pathway is comparatively long. Effects are usually seen after hours or even days. In contrast to the genomic pathway, non-genomically mediated phenomena often occur with very short time, even within seconds (Losel *et al.*, 2003). The non-genomic effects don't depend on gene transcription or protein synthesis that is associated with intracellular progesterone receptors and involve steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins. The mechanism of rapid steroid signaling is not uniform. A classification of non-genomic rapid steroid effect is shown in Figure 1.

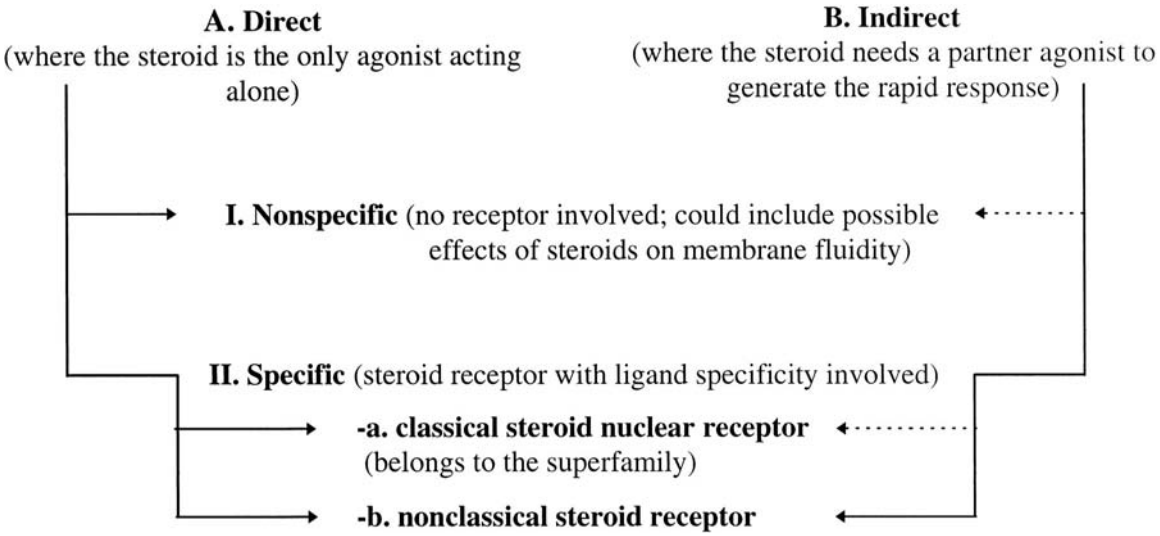

Figure 1. Classification of non-genomic steroid actions. Dotted arrows indicate a hypothetical category with no example yet known. Other arrows indicate examples for categories with given examples (cited from Falkenstein *et al.*, copyright 2000, The Endocrine Society).

Many actions of progesterone are modulated by non-genomic mechanisms. Membrane progesterone receptors (PRs) in human sperms are responsible for the rapid, non-genomic activation of Ca^{2+} and Cl^- channels, leading to the acrosomal reaction (Luconi *et al.*, 1998). Non-genomic progesterone effects have also been described in conjunction with sexual behaviour. In the central nervous system, various steroids synthesized in the adrenal glands and gonads modulate neuron excitability. These neuroactive steroids, such as estradiol or progesterone for which neuroactive properties have been described (Paul and Purdy, 1992) influence the reaction to stress, brain plasticity, vigilance, mood and so on (Rupprecht and Holsboer, 1999). Steroid hormones also induce rapid neuronal survival via non-genomic mechanisms (McEwen and Alves, 1999). And potent anesthetic and anxiolytic actions of sex steroids largely depend on non-genomic modulation of the *g-amino butyric acid type A* (*GABA-A*) receptor by acting on brain cells (Gee, 1988).

Gerdes *et al.* (1998) cloned two human putative steroid binding membrane proteins (mPR), termed Hpr6.6 (*PGRMC1*) and *Dg6* (*PGRMC2*). *PGRMC1* locates on the human X chromosome and *PGRMC2* on human chromosome 4. Of the progesterone binding membrane proteins, the human *PGRMC1* and *PGRMC2* gene were cloned and well characterized (Losel *et al.*, 2005 and Bernauer *et al.*, 2001). The human mPR gene is

composed of three exons separated by two introns. The 5'-region lacks a typical TATA box, but has high homology to a transcription initiator consensus sequence. The proximal region is GC-rich, and a CpG island spans the putative transcription start site (Bernauer *et al*., 2001). Several upstream regulatory DNA motifs were identified, including AP2, NF-AT, C/EBP and Ahr/Arnt (Bernauer *et al*., 2001). Both *PGRMC1* and *PGRMC2* contain cytochrome b5-like Heme/Steroid binding domain and belong to the cytochrome b5 family, membrane-associated progesterone receptor (MAPR) protein subfamily. Besides proteins that bind to steroids, this family also includes heme binding domains from a diverse range of proteins. The deduced protein of human *PGRMC2* gene contains 223 amino acids and has an N-terminal hydrophobic region. It has no N-glycosylation site. *PGRMC2* shares about 50% identity overall with *PGRMC1*. Including a highly conserved 58-amino acid sequence, they share 68% identity in their C termini (Gerdes *et al.,* 1998). Full-length cDNA sequence of the porcine *PGRMC1* gene from vascular smooth muscle cells has been described (Falkenstein *et al.*, 1996). However, little is known about structure of *PGRMC2* in pigs so far.

In summary, due to increasing concerns on animal welfare in the world, improvement of animal welfare becomes more and more important. Mapping of QTL for maternal infanticide and further analysis of this QTL region will help to identify candidate genes involved in sow maternal infanticide. The identification of candidate genes or genetic markers related to maternal infanticide will lead to the possibility of developing genetic diagnosis of high risk animals. In turn, this would allow removal of such animals from the breeding herds. Secondly, it will contribute to our understanding of the neural mechanisms underlying maternal aggression. Furthermore, because maternal infanticide in pigs has many features in common to a postnatal psychosis in humans, pig can be used as a model of human postnatal illness (Quilter *et al.*, 2007). Identification of gene or genetic markers related to maternal infanticide can provide the useful clues to understand and therapy of human postnatal illness.

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Curriculum vitae

Family Name**: Chen**

First Name: **Congying**

Sex: **Male**

Date of birth**: Oct. 19, 1975**

Place of Birth: **Jiangxi, P. R. China**

Marital Status**: Married**

Parents: **Bikuo Chen and Minxiu Deng**

Nationality**: P. R. China**

Education

2006.12—: Institute of Veterinary Medicine, University of Göttingen
Postgraduate student
Major: Molecular genetics in livestock

2005.1—2006.11: University of Göttingen, University of Cambridge, Jiangxi Agricultural University
Cooperated postgraduate student
Major: Molecular genetics in livestock

2002.9—2004.12: Lecturer in Animal genetics and breeding

1999.9—2002.7: Postgraduate student for master degree in Animal Genetics and Breeding
College of Animal Science and Technology
Jiangxi Agricultural University
Supervisor: Prof. Huang Lusheng and Prof. Chen Jingbo

1995.9---1999.7: Undergraduate student in Animal Nutrition
College of Animal Science and Technology
Jiangxi Agricultural University