

RESEARCH NOTE



Behavioural genetic differences between Chinese and European pigs

QINGPO CHU, TINGTING LIANG, LINGLING FU, HUIZHI LI and BO ZHOU*

College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

*For correspondence. E-mail: zhoubo@njau.edu.cn.

Received 29 July 2016; revised 17 November 2016; accepted 11 January 2017; published online 13 September 2017

Abstract. Aggression is a heritable trait and genetically related to neurotransmitter-related genes. Behavioural characteristics of some pig breeds are different. To compare the genetic differences between breeds, backtest and aggressive behaviour assessments, and genotyped using Sequenom iPLEX platform were performed in 50 Chinese indigenous Mi pigs and 100 landrace-large white (LLW) cross pigs with 32 SNPs localized in 11 neurotransmitter-related genes. The genetic polymorphisms of 26 SNPs had notable differences ($P < 0.05$) between Mi and LLW. The most frequent haplotypes were different in *DBH*, *HTR2A*, *GAD1*, *HTR2B*, *MAOA* and *MAOB* genes between Mi and LLW. The mean of backtest scores was significantly lower ($P < 0.001$) for Mi than LLW pigs. Skin lesion scores were greater ($P < 0.01$) in LLW pigs than Mi pigs. In this study, we have confirmed that Chinese Mi pigs are less active and less aggressive than European LLW pigs, and the genetic polymorphisms of neurotransmitter-related genes, which have been proved previously associated with aggressive behaviour, have considerable differences between Mi and LLW pigs.

Keywords. aggression; backtest; candidate genes; pigs; serotonin; SNP association.

Introduction

Behavioural characteristics of some pig breeds are different. For example, Danish Landrace pigs are shown to be more aggressive than Duroc pigs (Lund and Simonsen 2000). Chinese indigenous pigs display lower locomotion (Desautes *et al.* 1999), low behavioural reactivity (Mormede *et al.* 1984) and more docile character traits (Wattanukul *et al.* 1997; Li *et al.* 2008) than modern European pigs. Meishan sired gilts lie down more often, spend less time performing chain manipulation and drinker manipulation, and tend to be less active than Yorkshire sired gilts (Bergeron *et al.* 1996). Since modern commercial European pigs are aggressive, tail docking and teeth clipping are used routinely to prevent tail biting amongst pigs and reduce the harm of aggressive behaviour (Reese and Straw 2005; Sutherland *et al.* 2008).

Recording aggressive behaviour among pigs in a group is difficult since the pigs must be identified individually. Hessing *et al.* (1993) first used backtest to characterize aggression in pigs, but some researchers (Velie *et al.* 2009;

Rohrer *et al.* 2013) were unable to reproduce the same results. Aggressiveness can be investigated by direct or video observations, either by recording the total number of initiated and received attacks for each pig or by recording the identity and outcome of each dyadic encounter (Rydhmer and Canario 2014). During the first 24 h after mixing, most pigs are involved in many fights which lead to injuries such as skin lesions (Meese and Ewbank 1973). The accumulation of skin lesions are used as a predictor of individual aggressiveness in pigs (Turner *et al.* 2006a).

Pig behaviour studies have shown that aggression is a heritable trait (Lovendahl *et al.* 2005; Turner *et al.* 2008) and is associated with brain gene expression differences (Brunberg *et al.* 2013a, b). Also, there are quantitative trait loci (QTLs) with interesting effects on behavioural indices in pig (Desautes *et al.* 2002; Chen *et al.* 2009; Reiner *et al.* 2009). In humans and animals, aggressive behaviour is genetically related to neurotransmitter-related genes (Pavlov *et al.* 2012). For example, functional polymorphisms in the monoamine oxidase A (MAOA)

and serotonin transporter are associated with aggressive behaviour (Wendland *et al.* 2006; Buckholtz and Meyer-Lindenberg 2008).

Intense selection for pig growth traits may decrease animal welfare, e.g. by decreasing the capacity to adapt to stress. The challenge is to increase efficiency without decreasing animal welfare. Fortunately, the genetic tools that have been used to select production traits can also be used to decrease negative side effects of selection and improve traits important for animal welfare (Rydhmer and Canario 2014).

Chinese Mi pigs were originated in the lake Taihu basin in Jiangsu province (east China) and was a group of Taihu pig, including the Chinese Meishan and Erhualian pigs (Li and Enfield 1989). All these Taihu pig groups have similar characteristics (Fan *et al.* 2002). In China, some farmers have observed that some Chinese pigs are less aggressive than European pigs, but few scientific experiments have measured behavioural differences under the same management and environmental conditions. Thus, we conducted a controlled study of the different breeds in the same environmental conditions: (i) to demonstrate that Chinese pigs are less aggressive than European pigs; and (ii) to determine the behavioural genetic differences between Chinese Mi and European pigs.

Material and methods

Animals

In this study, the experiments were conducted at Jiangsu Yong-Kang Pig Breeding Farm, Changzhou, China. All procedures were reviewed and approved by animal care and use committee of Nanjing Agricultural University. A total of 50 Mi piglets from 15 litters (3–4 female piglets per litter) and 100 Landrace-large white (LLW) piglets from 30 litters (3–4 female piglets per litter) were used for backtest, aggressive behaviour assessments and genotyping.

Gene and SNP selection

The single-nucleotide polymorphisms (SNPs) of genes involved in neurotransmitter (Pavlov *et al.* 2012) were identified in the public SNP databases (<http://www.ncbi.nlm.nih.gov/SNP/>). Initially, we selected 40 SNPs of which only 32 were successfully determined in the 11 studied genes: monoamine oxidase A (MAOA) and B (MAOB), 5-hydroxytryptamine (serotonin) receptor 2A, 2B and 2C (HTR2A, HTR2B and HTR2C), solute carrier family 6 (neurotransmitter transporter) members 1 and 4 (SLC6A1 and SLC6A4), dopamine beta-hydroxylase (DBH), glutamate decarboxylase 1 (GAD1),

catechol-O-methyltransferase (COMT) and tryptophan hydroxylase 1 (TPH1) (table 1 in electronic supplementary material at <http://www.ias.ac.in/jgenet/>).

Genotyping

Genomic DNA was extracted from the ear tissue samples using a standard phenol/chloroform/isoamyl alcohol protocol (Isola *et al.* 1994). Genotyping was undertaken using the Sequenom iPLEX platform, according to the manufacturer's instructions (Oeth *et al.* 2005) (<http://www.sequenom.com>). The primers are shown in table 2 in electronic supplementary material. Samples were plated onto 26,384-well plates. Duplicate samples and negative controls were included to check genotyping quality. Only SNPs that were successfully genotyped in 80% samples were included in the analysis. The average rate of missing genotype and genotyping error were 0.05% and <0.01%, respectively, as reported by Sequenom iPLEX platform. The primary genotype data were analysed using the Typer 4.0 Analyser User Interface software (Sequenom, San Diego, USA) for cluster analysis. Samples with a call rate of $\leq 75\%$ were excluded from further analysis.

Allele frequencies for each SNP were calculated by allele counting, and the Hardy–Weinberg disequilibrium was tested. To measure linkage disequilibrium (LD), the two measures of LD (D' and r^2) and logarithm of the odds (LOD) were calculated using Haploview ver. 4.2 (Barrett *et al.* 2005). Analysis of haploblock partition defines the haploblock from the LD measure r^2 , initiating and extending a haploblock according to the pairwise and grouped r^2 value (Gu *et al.* 2005). Any SNPs in Hardy–Weinberg disequilibrium ($P < 0.001$) and/or with minor allele frequency (MAF) < 0.05 were excluded from the analysis of haplotype association.

Backtest assessments

The backtest assessments were performed twice at the age of 17 d. Here, each piglet was gently removed from its pen, individually put on its back and restrained in its supine position for 60 s. A trained assessor placed one hand loosely on the head of the pig, and placed the other hand loosely on the hind legs. During the 60 s testing period, each series of wriggles that the piglet made without a pause was counted as one escape attempt. The total number of escape attempts is the backtest score (BS) for each piglet. After the backtest assessments, a small ear tissue sample was collected from each experimental piglet.

Aggressive behaviour assessments

To compare the difference on aggressive behaviour between Mi and LLW pigs, skin lesions on the body were assessed

according to the method of [Welfare Quality \(2009\)](#) at two months of age. In the aggressive behaviour assessments, the pig's body was considered in five separate regions: ears, front (head to back of shoulder), middle (back of shoulder to hind-quarters), hind-quarters, and legs (from the accessory digit upwards). Each region was considered separately. According to [Welfare Quality \(2009\)](#), a lesion was defined as either a scratch (surface penetration of the epidermis) or a round lesion (penetration of the muscle tissue). The severity of lesions on the body and tail were assessed on a 16-point scale (1 = a group of small scratches, scratch >2.0 cm in length, 2 = parallel scratches up to 0.5 cm space between scratches, or a round lesion <2.0 cm in diameter; 5 = 2.0–5.0 cm diameter round lesion or >5.0 cm healed lesion; and 16 = a round, deep and opened lesion >5.0 cm diameter) ([Zhou et al. 2013](#)). Lesion scores for a pig were cumulated on an evaluation day ([Welfare Quality 2009](#)).

Further, the differences in aggressive behaviours between Mi and LLW were compared, then, immediately we performed a mixing test. For each breed, 18 pigs from six original pens (three pigs per pen) were randomly selected, to be mixed in three 3.0 × 3.0 m pens (six pigs per pen, one pig per original pen). All the experimental pens with solid concrete floors were in the same room for mixing, and equipped with a 2.0-m long feeder and two nipple drinkers to allow *ad libitum* access to feed and water. Behaviour was recorded using a digital video recording system (Hikvision network hard disk video recorder DS-7808HW-E1; Hikvision Digital Technology, Hang-Zhou, China) during the first 4 h of mixing. After 4 h of mixing, skin lesions on the body of Mi and LLW were assessed by a trained assessor according to the method of [Welfare Quality \(2009\)](#). Reciprocal aggression (fighting) behaviour is defined as aggressive interactions lasting more than 3 s in which both pigs were seen to be pushing, head knocking and/or biting each other ([D'Eath et al. 2009](#)).

Data analysis

Statistical calculations were performed with IBM SPSS 21 (IBM SPSS Statistics, Chicago, USA). Backtest data were analysed utilizing GLM procedure with fixed factors breed and genotype for each SNP. Backtest and aggressive behaviour assessment data were analysed using independent-samples *t*-test between Mi and LLW. Genotypes and allele carriage frequencies for each SNP were compared between the Mi and LLW pigs using the χ^2 test. Estimated haplotypes were constructed in Haploview ([Barrett et al. 2005](#)) and their frequencies were compared between the Mi and LLW pigs using a χ^2 with $P < 0.05$ considered statistically significant. Odds ratios (ORs) were estimated for haplotypes at each specific locus.

Results and discussion

Allele frequencies

In total, 50 Mi and 100 LLW pigs were genotyped using 45 candidate SNPs. In the first step of SNP validation, 28.8% of the total genotyped loci had a level of missing data higher than the accepted threshold of 80%. Among the remaining 32 loci, six loci were monomorphic in all the samples.

Except for six monomorphic loci in all the samples, 26 loci of 11 genes had significant differences ($P < 0.05$) in frequency between Mi and LLW. Six loci are located at exon, three loci are located at promoter and 17 loci are located at intron (table 1).

Previous studies have demonstrated that aggression is genetically related to neurotransmitter-related genes ([Pavlov et al. 2012](#)). In the current study, considerable allelic frequency differences of neurotransmitter-related genes were found for 26 loci from a total of 32 loci between Mi and LLW. Polymorphisms of neurotransmitter-related genes are linked to the concentration of 5-hydroxytryptamine (serotonin) in the brain ([Buckholtz and Meyer-Lindenberg 2008](#)), and could influence the aggressive behaviour ([Vage et al. 2010](#); [Pavlov et al. 2012](#)). For example, *MAOA* gene is the most promising candidate gene for aggression ([Buckholtz and Meyer-Lindenberg 2008](#); [Takahashi et al. 2012](#)). Therefore, the considerable differences of genetic polymorphisms in neurotransmitter-related genes probably were part of reasons for the notable differences in aggressive behaviours between Mi and LLW.

Haplotype analysis

The D' and r^2 values are shown in figure 1; and figure 1 in electronic supplementary material, respectively. As shown in pairwise comparison on SNPs in *DBH* gene, a haploblock including two SNPs was found in LLW but not in Mi. In chromosome X, there was a haploblock constituted by three bright red boxes in Mi, but no haploblock in LLW. A haploblock was found in both Mi and LLW in *HTR2A* gene at chromosome 11. A big haploblock with a length of 37 kb including four SNPs were found in both Mi and LLW in *GADI* gene at chromosome 15. In total, three haploblocks were found in both Mi and LLW.

The most frequent haplotypes (MFH) were different between Mi and LLW in *DBH*, *HTR2A*, *GADI*, *HTR2B*, *MAOA* and *MAOB* (table 2). For each gene or chromosome, the average per cent of MFH was 0.72 and 0.58 in Mi and LLW, respectively. The maximum value of LOD was 21.06 and 27.92 in Mi and LLW, respectively. No haplotype was identified in complete association with the phenotypes, suggesting a complex inheritance of aggression.

Table 1. The major allele frequencies of SNPs genotyped in neurotransmitter-related genes in Chinese Mi and LLW pigs.

Gene	SNP ID	SNP-allele major/minor	Mi pig		LLW pig		χ^2 test*
			MAF	P_{HWE}	MAF	P_{HWE}	P
<i>MAOA</i>	rs81499537	T/C	0.23	0.897	0.38	0.191	0.042
	rs81242206	C/T	0.00	–	0.00	–	–
	rs81242207	T/C	0.08	0.820	0.49	0.012	0.000
	rs81220383	C/T	0.06	0.901	0.20	0.041	0.022
	rs80875407	T/C	0.00	–	0.00	–	–
<i>MAOB</i>	rs80895596	T/C	0.00	–	0.00	–	–
	rs81261165	G/A	0.00	–	0.00	–	–
	rs10719981	T/C	0.10	0.073	0.30	0.393	0.000
<i>HTR2C</i>	rs45432250	G/C	0.15	0.072	1.00	–	0.000
	rs81473455	G/T	0.00	–	0.00	–	–
<i>HTR2A</i>	rs81473456	C/G	0.08	0.989	0.12	0.636	0.029
	rs81219944	A/G	0.02	0.989	0.80	0.733	0.000
<i>HTR2B</i>	rs81305923	C/G	0.08	0.824	0.37	0.898	0.000
	rs81304824	C/T	0.08	0.824	0.40	0.906	0.000
	rs81333780	T/C	0.00	–	0.18	0.000	0.002
	rs81219577	G/A	0.06	0.997	0.32	0.928	0.000
	rs81001546	C/T	0.10	0.723	0.76	0.928	0.000
<i>SLC6A4</i>	rs80885453	G/A	0.00	–	0.00	–	–
	rs81220218	G/T	0.08	0.956	0.57	0.914	0.000
<i>SLC6A1</i>	rs80841574	A/G	0.46	0.970	0.67	0.857	0.006
<i>DBH</i>	rs80837467	G/A	0.36	0.841	0.68	0.670	0.000
	rs80980072	C/G	0.08	0.974	0.70	0.329	0.000
	rs45435444	C/T	0.35	0.072	0.79	0.942	0.000
	rs45435433	G/A	0.36	0.691	0.68	0.160	0.000
<i>GADI</i>	rs45435447	C/T	0.06	0.997	0.14	0.459	0.003
	rs80971411	G/C	0.27	0.539	0.95	0.277	0.000
	rs80845328	C/T	0.48	0.988	0.06	0.994	0.000
	rs80983628	G/T	0.49	0.989	0.06	0.994	0.000
<i>COMT</i>	rs81210452	T/C	0.48	0.954	0.06	0.994	0.000
	rs80894601	C/G	0.09	0.850	0.39	0.654	0.000
<i>TPHI</i>	rs81357983	A/G	0.06	0.002	0.28	0.127	0.000
	rs81357984	C/T	0.07	0.862	0.20	0.281	0.013

* χ^2 test for the difference on allele frequency between Chinese Mi and LLW pigs. Differences were considered significant at $P < 0.05$.

A nonrandom frequency distortion of haplotypes for the three genes (*HTR2A*, *MAOA* and *DBH*) with multiple SNPs was discovered using Haploview by forming haplotypes (table 3) when comparing Mizhu and LLW. The MFH in the Mizhu ('protective') and in the LLW ('risk') for aggressive behaviour were identified for *HTR2A* (risk GTG, protective CCG), *MAOA* (risk CCT, protective CTT) and *DBH* (risk ATAC, protective GCGC). Some other haplotypes of low frequency were also detected (others). There were significant differences ($P < 0.001$) between the frequency of 'protective' and 'risk' and between the frequency of 'protective' and 'others' in *HTR2A*, *MAOA* and *DBH*. The odds ratio of behaviours with 'risk' haplotype compared with 'protective' haplotype varied from 12.65 (*MAOA*) to 23.20 (*DBH*). There were also significant differences ($P < 0.05$) between the frequency of 'risk' and 'others' and between the frequency of 'protective' and 'others' in *HTR2A*, *MAOA* and *DBH*, and the odds ratio varied from 0.01 to 4.74.

Backtest

The mean of backtest scores (BS) was significantly lower ($P < 0.001$) for Mi (1.56 ± 0.16) than LLW pigs (4.36 ± 0.19 , figure 2) and the range of BS for Mi and LLW piglets at 17 d of age were 0–4 and 2–8, respectively.

SNPs: rs45435433, rs45435444, rs80837467 and rs80980072 were localized to *DBH*; rs81219944 to *HTR2A* and rs81001546 to *HTR2B* gene, significant differences ($P < 0.05$) on BS were observed among their different genotypes (figure 2 in electronic supplementary material). These differences were verified by the same trend in Mi and/or LLW pigs. SNPs rs81499537 in *MAOA* and rs80841574 in *SLC6A1*, significant differences ($P < 0.05$) on BS were also observed between two of their three genotypes, but they were not verified by a significant difference between genotypes in Mi or LLW pigs.

As compared to modern European breeds, Chinese pig breeds, such as Meishan and Erhualian, display very different stress responses (Meuniersalaun et al. 1991) and

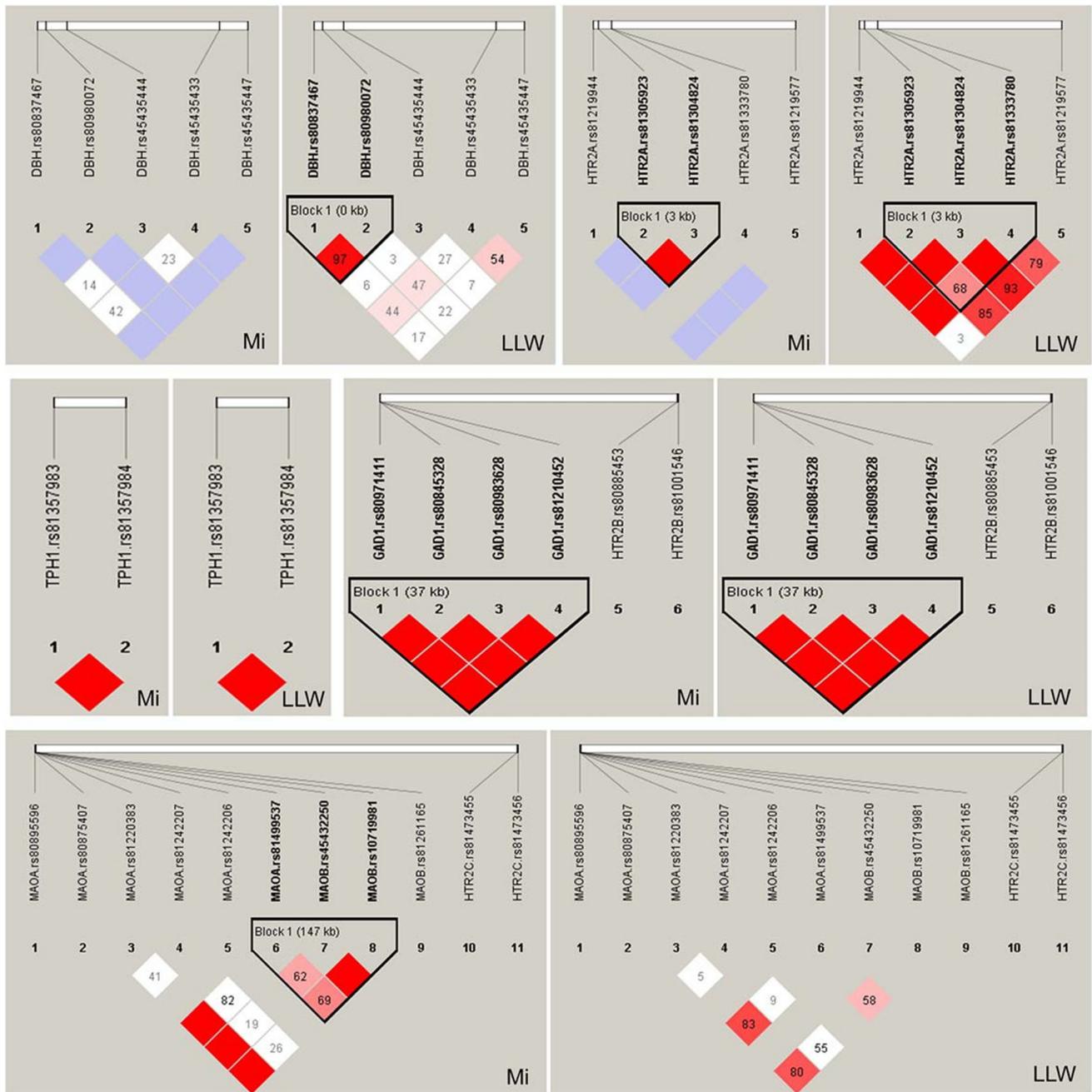


Figure 1. Comparison of LD (D') between Mi and LLW pigs. Pairwise LD (D' and LOD) and haplotype plots are shown for SNPs of *DBH*, *TPH1*, *MAOA*, *MAOB*, *HTR2C*, *HTR2A*, *GAD1* and *HTR2B* genes: white boxes, $D' < 1$ and LOD < 2 ; blue boxes, $D' = 1$ and LOD < 2 ; shades of pink/red boxes, $D' < 1$ and LOD ≥ 2 ; and bright red boxes, $D' = 1$ and LOD ≥ 2 .

are more docile and fight less (Wattanukul *et al.* 1997). A previous study demonstrated that the mean of BS at 3, 10 and 17 d of age was significantly lower in Erhualian piglets than in Pietrain piglets (Li *et al.* 2008), which is consistent with our study.

A previous study showed that the extent of LD differed significantly between European and Chinese breeds, extending up to 400 kb in Europe and up to 10 kb in China (Amaral *et al.* 2008). However, in our results, a big

haplotype (37 kb) including four SNPs in *GAD1* was determined in both Mi and LLW. Amaral *et al.* (2008) also found that Chinese pigs had a greater level of haplotype diversity and shared high levels of frequent haplotypes with large white Landrace and Duroc. In contrast, our study shows that many major haplotypes of SNPs were fixed in Mi or LLW pigs. The different results suggest that although some haplotypes in Chinese and European pig breeds were common because of past introgression from

Table 2. The MFH and their percent, the value of D' between the two loci and the log of the likelihood odds ratio of SNPs using Haploview in the genes of neurotransmitter-related genes.

Chromosome	Gene	MFH		D'		LOD	
		Mi	LLW	Mi	LLW	Mi	LLW
1	<i>DBH</i>	GCCGC (0.39)	AGTAC (0.47)	0.14–1.00	0.04–0.97	0.00–1.69	0.00–27.92
2	<i>TPHI</i>	AC (0.91)	AC (0.52)	1.00	1.00	0.07	3.13
11	<i>HTR2A</i>	ACCTG (0.90)	GCCTA (0.25)	1.00	0.04–1.00	0.04–9.55	0.01–25.41
15	<i>GADI</i>	GTTG (0.48)	CCGT (0.95)	1.00	1.00	5.21–21.06	2.01–4.09
	<i>HTR2B</i>	GC (0.90)	GT (0.76)	–	–	–	–
	Chr 15	GTTGCG (0.40)	CCGTGT (0.70)	1.00	1.00	5.21–21.06	2.01–4.09
X	<i>MAOA</i>	TTCTCT (0.71)	TTCCCT (0.31)	0.42–1.00	0.06–0.83	0.94–2.29	0.01–8.11
	<i>MAOB</i>	GTG (0.86)	CTG (0.70)	1.0	–	8.39	–
	<i>HTR2C</i>	GC (0.98)	GC (0.88)	–	–	–	–
	Chr X	TTCTCTGTGGC (0.66)	TTCCCTCTGGC (0.25)	0.19–1.00	0.06–0.83	0.22–8.39	0.01–8.55

Chinese pig breeds (Amaral et al. 2008), but also there were notably different genotypes for neurotransmitter-related genes.

A previous study found that behavioural characteristics could affect growth and carcass traits of pigs (Rohrer et al. 2013). Terenina et al. (2013) found that *MAOA* polymorphisms affect the growth rate, lean content and intramuscular fat in the study of association with stress neuroendocrine and production traits using large white and Meishan pigs, which indicates that there were significant differences of *MAOA* gene polymorphisms between the two highly divergent breeds in growth and carcass performances (Bidanel et al. 1993). Further, previous studies showed that active pigs tend to grow faster and have a leaner carcass (van Erp-van der Kooij et al. 2003; Velie et al. 2009), which would result in selection of aggressive pigs. Turner et al. (2006b) did not only found significant genetic or phenotypic correlations between lesions and growth rate or backfat depth by a postmixing aggressiveness trial. It is therefore, technically possible to select for a reduced aggressive behaviour without substantially inhibiting genetic progress in growth rate or backfat depth through antagonistic genetic relationships.

Aggressive behaviour assessments

At the same age and environments, skin lesion scores on the ears ($P < 0.001$), front ($P < 0.001$), middle ($P < 0.001$) and hind-quarters ($P < 0.001$) and legs ($P = 0.003$) were greater in LLW pigs than Mi pigs (figure 3; figure 3 in electronic supplementary material). During the first 4 h after mixing, no fighting behaviour lasted more than 3 s in Mi, but 15 fighting behaviours that lasted at least for 30 s were observed in LLW, where two fighting behaviours lasted more than 20 and 10 min, respectively. After 4 h of mixing, skin lesions on the ears ($P < 0.001$), front ($P < 0.006$) and middle ($P < 0.009$) regions, except for hind-quarters ($P = 0.205$) and legs ($P = 0.887$) regions, were increased significantly in LLW pigs. However, in Mi pigs, skin lesions on the ears ($P = 0.984$), front ($P = 0.834$), middle ($P = 0.768$) and hind-quarters ($P = 0.054$) and legs ($P = 0.065$) regions were not increased after 4 h of mixing (figure 3; figure 4 in electronic supplementary material).

We performed the aggressive behaviour assessments to compare the aggression between Mi and LLW pigs in the same environments. Skin lesion scores were considerably lower in Mi pigs than in LLW pigs at two months of age. Further, skin lesions did not increase in Mi after mixing, but notably increased in LLW. Mixing of unfamiliar pigs is often associated with a period of intense and physically damaging aggression lasting ~24 h (Ewbank 1976). However, physically damaging aggression was so weak that no apparent injuries were observed in Mi. During the first

Table 3. The distribution (frequency) of haplotypes in the Mizhu and LLW pigs, and calculated ORs for aggressive behaviour for having the most frequent haplotype in Mizhu pigs (protective) or the most frequent haplotype in LLW pigs (risk), alternatively haplotypes of lower frequency (others).

Gene	Pig	Haplotypes number* (frequency)			OR (95% CI)	P	
		Risk	Protective	Others			
<i>HTR2A</i>	LLW	70 (0.35)	58 (0.29)	72 (0.36)	Risk versus protective Risk versus others	13.73 (6.16–30.62) 0.12 (0.02–1.00)	<0.001 0.021
	Mizhu	8 (0.08)	91 (0.91)	1 (0.01)	Protective versus others	0.01 (0.00–0.07)	<0.001
<i>MAOA</i>	LLW	62 (0.31)	58 (0.29)	80 (0.40)	Risk versus protective Risk versus others	12.65 (5.11–31.33) 2.97 (1.14–7.74)	<0.001 0.021
	Mizhu	6 (0.06)	71 (0.71)	23 (0.23)	Protective versus others	0.24 (0.13–0.42)	<0.001
<i>DBH</i>	LLW	94 (0.47)	14 (0.07)	92 (0.46)	Risk versus protective Risk versus others	23.20 (9.67–55.64) 4.74 (2.32–9.66)	<0.001 <0.001
	Mizhu	11 (0.11)	38 (0.38)	51 (0.51)	Protective versus others	0.20 (0.10–0.41)	<0.001

* Numbers are counted as allele, including homozygous and heterozygous combinations.

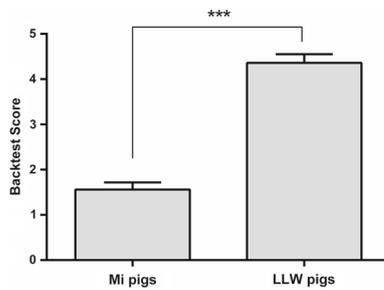


Figure 2. The mean of BS in Mi and LLW piglets. Difference of BS between Mi and LLW was significant, *** $P < 0.001$.

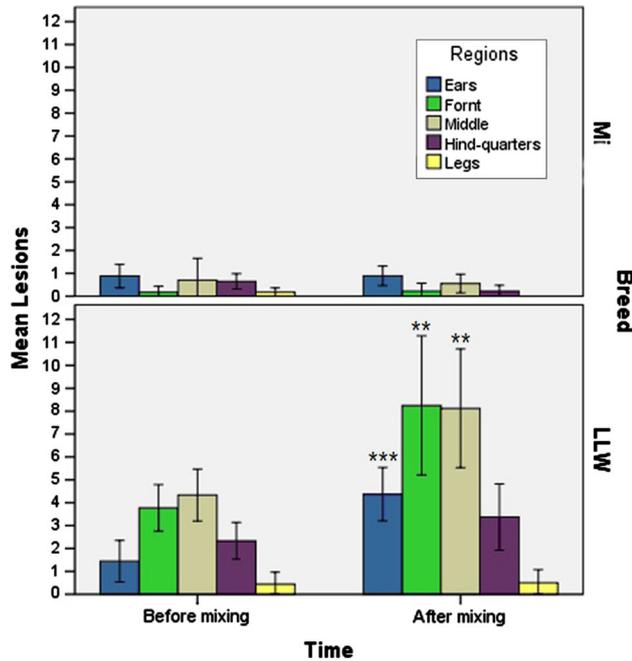


Figure 3. Comparison of the number of skin lesions for Mi ($n = 18$) and LLW ($n = 18$) nursery pigs before and after 4 h of mixing.

4 h of mixing, reciprocal aggressive behaviour was repetitiously observed in all the three pens of LLW pigs, but no fighting behaviour in Mi. These results show that Chinese Mi pigs are less aggressive than LLW pigs at the same age and environment, which could be related to different genetic backgrounds.

In conclusion, our studies have confirmed that Chinese Mi pigs are less active and less aggressive than European LLW pigs, and the genetic polymorphisms of neurotransmitter-related genes, which have been proved previously associated with aggressive behaviour, have considerable differences between Mi and LLW pigs. Therefore, Chinese Mi pigs could be used as research animals for genetics of aggressive behaviour in pigs.

Acknowledgements

We thank staff of Jiangsu Yong-Kang Pig Breeding Farm for their help in this work. This research was supported by National Natural Science Foundation of China (31672465) and Agro-Scientific Research Foundation in the Public Interest (201003011).

References

- Amaral A. J., Megens H. J., Crooijmans R. P., Heuven H. C. and Groenen M. A. 2008 Linkage disequilibrium decay and haplotype block structure in the pig. *Genetics* **179**, 569–579.
- Barrett J. C., Fry B., Maller J. and Daly M. J. 2005 Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265.
- Bergeron R., Gonyou H. W. and Eurell T. E. 1996 Behavioral and physiological responses of Meishan, Yorkshire and crossbred gilts to conventional and turn-around gestation stalls. *Can. J. Anim. Sci.* **76**, 289–297.
- Bidanel J. P., Caritez J. C., Gruand J. and Legault C. 1993 Growth, carcass and meat quality performance of crossbred pigs with graded proportions of meishan genes. *Genet. Select. Evol.* **25**, 83–99.
- Brunberg E., Jensen P., Isaksson A. and Keeling L. J. 2013a Behavioural and brain gene expression profiling in pigs

- during tail biting outbreaks – evidence of a tail biting resistant phenotype. *PLoS One* **8**.
- Brunberg E., Jensen P., Isaksson A. and Keeling L. J. 2013b Brain gene expression differences are associated with abnormal tail biting behavior in pigs. *Genes Brain Behav.* **12**, 275–281.
- Buckholtz J. W. and Meyer-Lindenberg A. 2008 MAOA and the neurogenetic architecture of human aggression. *Trends Neurosci.* **31**, 120–129.
- Chen C. Y., Guo Y. M., Yang G. C., Yang Z. Q., Zhang Z. Y., Yang B. et al. 2009 A genome wide detection of quantitative trait loci on pig maternal infanticide behavior in a large scale white Duroc x Erhualian resource population. *Behav. Gene.* **39**, 213–219.
- D'Eath R. B., Roehe R., Turner S. P., Ison S. H., Farish M., Jack M. C. et al. 2009 Genetics of animal temperament: aggressive behaviour at mixing is genetically associated with the response to handling in pigs. *Animal* **3**, 1544–1554.
- Desautels C., Bidanel J. P., Milant D., Iannuccelli N., Amigues Y., Bourgeois F. et al. 2002 Genetic linkage mapping of quantitative trait loci for behavioral and neuroendocrine stress response traits in pigs. *J. Anim. Sci.* **80**, 2276–2285.
- Desautels C., Sarrieau A., Caritez J. C. and Mormede P. 1999 Behavior and pituitary-adrenal function in Large White and Meishan pigs. *Domes. Anim. Endocrinol.* **16**, 193–205.
- Ewbank R. 1976 Social hierarchy in suckling and fattening pigs: a review. *Livest. Prod. Sci.* **3**, 363–372.
- Fan B., Wang Z. G., Li Y. J., Zhao X. L., Liu B., Zhao S. H. et al. 2002 Genetic variation analysis within and among Chinese indigenous swine populations using microsatellite markers. *Anim. Gene.* **33**, 422–427.
- Gu S., Pakstis A. J. and Kidd K. K. 2005 HAPLOT: a graphical comparison of haplotype blocks, tagSNP sets and SNP variation for multiple populations. *Bioinformatics* **21**, 3938–3939.
- Hessing M. J., Hagelsø A. M., Van Beek J., Wiepkema R., Schouten W. and Krukow R. 1993 Individual behavioural characteristics in pigs. *Appl. Anim. Behav. Sci.* **37**, 285–295.
- Isola J., DeVries S., Chu L., Ghazvini S. and Waldman F. 1994 Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. *Am. J. Pathol.* **145**, 1301–1308.
- Li L. A., Xia D., Bao E. D., Wei S., Xiao J. S., Bao J. W. et al. 2008 Erhualian and pietrain pigs exhibit distinct behavioral, endocrine and biochemical responses during transport. *Livest. Sci.* **113**, 169–177.
- Li M. D. and Enfield F. D. 1989 A characterization of Chinese breeds of swine using cluster-analysis. *J. Anim. Breed. Genet.* **106**, 379–388.
- Lovendahl P., Damgaard L. H., Nielsen B. L., Thodberg K., Su G. S. and Rydhmer L. 2005 Aggressive behaviour of sows at mixing and maternal behaviour are heritable and genetically correlated traits. *Livest. Prod. Sci.* **93**, 73–85.
- Lund A. and Simonsen H. 2000 Aggression and stimulus-directed activities in two breeds of finishing pig. *Pig J.* **45**, 123–130.
- Meese G. and Ewbank R. 1973 The establishment and nature of the dominance hierarchy in the domesticated pig. *Anim. Behav.* **21**, 326–334.
- Meuniersalaun M. C., Gort F., Prunier A. and Schouten W. P. G. 1991 Behavioral-patterns and progesterone, cortisol and prolactin levels around parturition in European (Large-White) and Chinese (Meishan) sows. *Appl. Anim. Behav. Sci.* **31**, 43–59.
- Mormede P., Dantzer R., Bluth R. and Caritez J. 1984 Differences in adaptive abilities of three breeds of Chinese pigs. Behavioural and neuroendocrine studies. *Genet. Sel. Evol.* **16**, 85–102.
- Oeth P., Beaulieu M., Park C., Kosman D., del Mistro G., van den Boom D. et al. 2005 iPLEX assay: Increased plating efficiency and flexibility for mass array system through single base primer extension with mass-modified terminators. (<http://128.135.75.36/iPLEXAppNote.pdf>).
- Pavlov K. A., Chistiakov D. A. and Chekhonin V. P. 2012 Genetic determinants of aggression and impulsivity in humans. *J. Appl. Genet.* **53**, 61–82.
- Reese D. and Straw B. E. 2005 Teeth Clipping — Have You Tried to Quit? *Nebraska Swine Rep.* **33** (http://digitalcommons.unl.edu/coopext_swine/33).
- Reiner G., Kohler F., Berge T., Fischer R., Hubner-Weitz K., Scholl J. et al. 2009 Mapping of quantitative trait loci affecting behaviour in swine. *Anim. Genet.* **40**, 366–376.
- Rohrer G., Brown-Brandl T., Rempel L., Schneider J. and Holl J. 2013 Genetic analysis of behavior traits in swine production. *Livest. Sci.* **157**, 28–37.
- Rydhmer L. and Canario L. 2014 Behavioral genetics in pigs and relations to welfare. In *Genetics and the behavior of domestic animals* (ed. T. Grandin and M. J. Deesing), 2nd edition, pp. 397–434. Academic Press, San Diego, USA.
- Sutherland M. A., Bryer P. J., Krebs N. and McGlone J. J. 2008 Tail docking in pigs: acute physiological and behavioural responses. *Animal* **2**, 292–297.
- Takahashi A., Quadros I. M., De Almeida R. M. and Miczek K. A. 2012 Behavioral and pharmacogenetics of aggressive behavior. *Curr. Top. Behav. Neurosci.* **12**, 73–138.
- Terenina E., Babigumira B. M., Le Mignon G., Bazovkina D., Rousseau S., Salin F. et al. 2013 Association study of molecular polymorphisms in candidate genes related to stress responses with production and meat quality traits in pigs. *Domest. Anim. Endocrinol.* **44**, 81–97.
- Turner S. P., Farnworth M. J., White I. M. S., Brotherstone S., Mendl M., Knap P. et al. 2006a The accumulation of skin lesions and their use as a predictor of individual aggressiveness in pigs. *Appl. Anim. Behav. Sci.* **96**, 245–259.
- Turner S. P., Roehe R., Mekki W., Farnworth M. J., Knap P. W. and Lawrence A. B. 2008 Bayesian analysis of genetic associations of skin lesions and behavioural traits to identify genetic components of individual aggressiveness in pigs. *Behav. Genet.* **38**, 67–75.
- Turner S. P., White I. M. S., Brotherstone S., Farnworth M. J., Knap P. W., Penny P. et al. 2006b Heritability of post-mixing aggressiveness in grower-stage pigs and its relationship with production traits. *Anim. Sci.* **82**, 615–620.
- Vage J., Wade C., Biagi T., Fatjo J., Amat M., Lindblad-Toh K. et al. 2010 Association of dopamine- and serotonin-related genes with canine aggression. *Genes Brain Behav.* **9**, 372–378.
- van Erp-van der Kooij E., Kuijpers A. H., van Eerdenburg F. J. C. M. and Tielen M. J. M. 2003 Coping characteristics and performance in fattening pigs. *Livest. Prod. Sci.* **84**, 31–38.
- Velie B. D., Maltecca C. and Cassady J. P. 2009 Genetic relationships among pig behavior, growth, backfat, and loin muscle area. *J. Anim. Sci.* **87**, 2767–2773.
- Wattanukul W., Sinclair A. G., Stewart A. H., Edwards S. A. and English P. R. 1997 Performance and behaviour of lactating sows and piglets in crate and multisuckling systems: A study involving European White and manor meishan genotypes. *Anim. Sci.* **64**, 339–349.
- Welfare Quality 2009 *Welfare quality assessment protocol for pigs (sows and piglets, growing and finishing pigs)*, pp. 53–54. Lelystad, The Netherlands.
- Wendland J. R., Lesch K. P., Newman T. K., Timme A., Gachot-Neveu H., Thierry B. et al. 2006 Differentialfunctional

variability of serotonin transporter and monoamine oxidase a genes in macaque species displaying contrasting levels of aggression-related behavior. *Behav. Genet.* **36**, 163–172.

Zhou B., Yang X. J., Zhao R. Q., Huang R. H., Wang Y. H., Wang S. T. *et al.* 2013 Effects of tail docking and teeth clipping on the physiological responses, wounds, behavior, growth, and backfat depth of pigs. *J. Anim. Sci.* **91**, 4908–4916.

Corresponding editor: INDRAJIT NANDA