Expression analysis of the NR5A2 gene and associations between its polymorphisms and reproductive traits in Jiaxing Black sows

Qiangqiang Chen\textsuperscript{a}, Jianfeng Cai\textsuperscript{a}, Wei Zhang\textsuperscript{b}, Lixia Xiao\textsuperscript{a}, Guoliang Liu\textsuperscript{c}, Haihong Li\textsuperscript{c}, Fen Wu\textsuperscript{a}, Qianqian Song\textsuperscript{d}, Kui Li\textsuperscript{a} and Jinzhi Zhang \textsuperscript{a} \textsuperscript{b} \textsuperscript{c} \textsuperscript{d} \textsuperscript{e} \textsuperscript{f} \textsuperscript{g}

\textsuperscript{a}College of Animal Sciences, Zhejiang University, Hangzhou, People’s Republic of China; \textsuperscript{b}Institute of Translation Medicine, School of Medicine, Zhejiang University, Hangzhou, People’s Republic of China; \textsuperscript{c}Zhejiang Qinglian Food Company Limited, Jiaxing, People’s Republic of China; \textsuperscript{d}School of Life Sciences, Shanghai Jiao Tong University, Shanghai, People’s Republic of China; \textsuperscript{e}Zhejiang General Station of Animal Husbandry Technology Promotion and Breeding Livestock Monitoring, People’s Republic of China

\textbf{ABSTRACT}

This study’s aim was to detect NR5A2 expression and explore association between single nucleotide polymorphism (SNP) of the NR5A2 gene with reproductive traits in Jiaxing Black (JXB) sows. The expression analysis showed that the expression level of pig NR5A2 mRNA in the ovary and liver was significantly higher \((p < 0.01)\) compared to other tissues. 10 SNPs were detected in the exons of NR5A2, and five SNPs \((g.136706A > T, g.84609G > A, g.136759G > C, g.138802T > C, g.138864A > G)\) met Hardy Weinberg equilibrium conditions. The total number of piglets born \((TBA)\) and number of still born piglets \((NSB)\) of sows with GG genotype at the \(g.136759G > C\) loci were higher than the CC genotype \((p < 0.05)\). For the \(g.138864A > G\) loci, individuals with the AA genotype had significantly higher TBA than those with the AG genotype \((p < 0.05)\). By combining genotypes, it was found that the H7/H8 diplotype had the best TBA performance. Moreover, the \(g.138864A > G\) and \(g.136759G > C\) loci had significant effects on the NR5A2 mRNA expression level in the ovary. The above results indicated that SNPs within NR5A2 exons of the NR5A2 gene have a certain effect on the reproductive traits of JXB pigs.

\textbf{KEYWORDS}

Jiaxing Black pig; NR5A2; reproductive traits; SNP

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\textbf{INTRODUCTION}

NR5A2 (Nuclear receptor subfamily 5, group A, member 2), also known as fetoprotein transcription factor (FTF) and liver receptor homolog-1 \((LRH-1)\), is an important orphan receptor belonging to the NR5A subfamily of nuclear receptors. LRH-1 was first extracted from the liver of mice, and subsequent studies have reported that LRH-1 is significantly more expressed in the ovary than in the liver or other tissues \((\text{Falender et al. 2003; Liu et al. 2003})\). LRH-1 was later isolated from zebrafish, rats, chickens, horses, frogs and humans. Studies have elicited mRNA expression of NR5A2 in the adult ovary, more precisely in granulocytes and luteal cells rather than follicular membrane cells and interstitial cells \((\text{Falender et al. 2003})\). Further research on the characterization of the imperative and indispensable function of LRH-1 and its extensive regulatory network in the initiation of ovulation has confirmed that LRH-1 is a nuclear receptor that can be targeted by agonists or antagonists, with a considerable potential for the regulation of fertility and the treatment of infertility \((\text{Bianco et al. 2019})\). Furthermore, LRH-1 depletion has been found to prevent ovulation, cumulus expansion, and luteinization, whereas uterine depletion of LRH-1 compromises decidualization and pregnancy \((\text{Meinsohn et al. 2019})\). Aromatase, encoded by Cyp19a1a, catalyzes the conversion of androgens into estrogens, which is crucial for female reproduction in mammals and other vertebrates \((\text{Shao et al. 2015})\). As a matter of fact, studies demonstrate that LRH-1 plays an essential role in upregulation of the cyp19a1a gene in the ovarian follicular cells during vitellogenesis, and the sequestration of LRH-1 in the cytoplasm may downregulate cyp19a1a expression in the mature ovary \((\text{Lu et al. 2014})\).

Relevant research indicates that despite the fact that NR5A2\(^{-/-}\) mice have normal follicular development, ovulation, and estrogen production, they exhibit an altered luteal function \((\text{Labelle-Dumais et al. 2007})\). However, LRH-1 may also be a multifunctional steroidogenic factor in ovarian physiology \((\text{Liu et al. 2003})\). Within the follicle, NR5A2 appears to be regulated by FSH and LH, whereas its expression in the corpus luteum is regulated by prolactin. Additionally, NR5A2 has been proven to control steroid hormone biosynthesis by regulating the expression of genes related to steroidogenesis, such as the steroidogenic acute regulatory protein \((\text{STAR})\) \((\text{Siriani et al. 2002})\), cholesterol side-chain cleavage \((\text{Cyp11a1})\) \((\text{Saxena et al. 2004})\), 17\(\alpha\)-hydroxylase \((\text{Liu et al. 2003})\), lyase \((\text{Cyp17})\) \((\text{Annicotto et al. 2003})\), 3\(\beta\)-hydroxysteroid dehydrogenase \((\text{3\(\beta\)-Hsd})\) \((\text{Siriani et al. 2002; Saxena et al. 2004})\), and 11\(\beta\)-hydroxylase \((\text{Cyp11b1})\) \((\text{Siriani et al. 2002})\). It is worth mentioning that a 3.5 folds decrease in the expression of LRH-1 within
the ovary of this kind of infertile mouse was detected (Freiman et al. 2001). Moreover, this conclusion is supported by in vivo experiments (mice). Further experimental results show that the reduced reproductive ability of NR5A2−/+ females arises from a reduction in circulating progesterone concentrations and can be remedied by exogenous progesterone supplementation (Labelle-Dumais et al. 2007). Thus, the high expression of NR5A2 in the gonads and its ability to regulate the expression of genes related to steroid synthesis point out the importance of NR5A2 on the reproductive function. The gene expression of NR5A2 in the mSSC5 (Mouse spermatogonial stem cells long-term propagated) was markedly higher in comparison to that in the mSSCsf (freshly isolated Mouse spermatogonial stem cells) (Bai et al. 2016). Thus, NR5A2 seems to promote the proliferation of mSSCsl. NR5A2 expression in mural granulosa cells of preovulatory follicles is essential for cumulus expansion; NR5A2 regulates cumulus expansion by controlling the expression of various genes (Bertolin et al. 2017). The correlation between NR5A2 and reproductive traits was confirmed by the results of the above experiments.

The current research on NR5A2 is focused on its link with human diseases such as intestinal disorders (Enteritis, intestinal cancer), breast cancer, and immune system diseases, diabetes, metabolic disease, and its regulating function on the liver. However, to the best of our knowledge, studies on NR5A2 in domestic animals are rare, let alone in pigs. Currently, related studies have discovered that different regions of the NR5A2 gene in Hu sheep exhibit polymorphisms related to the reproductive traits of Hu sheep (Li et al. 2015, 2019). Li et al. (2019) reported that nucleotide polymorphisms were associated with the litter size of Hu sheep based on amplified sequence of the NR5A2 promoter. Li et al. (2015) also found that single nucleotide polymorphisms (SNPs) in the coding region of the NR5A2 gene was associated with the reproductive performance of Hu sheep. Given the findings reported by the above studies, it is logic to conclude that there is a correlation between the NR5A2 gene and reproductive traits. Nonetheless, there is a limited amount of literature on the association of the NR5A2 gene with breeding traits in livestock. Moreover, in a recent study (Guo et al. 2020) reported that enhancing the expression of NR5A2 can stimulate progesterone synthesis in pigs. In early porcine embryo development, LRH1 inhibition upregulated the expression of the proapoptotic Bax and Casp3 genes, and decreased the expression levels of Bcl2, an anti-apoptotic gene (Guo et al. 2016).

To date, no research has been reported on NR5A2 gene polymorphisms related to pig reproductive traits. JXB pigs, a famous local breed in China, are known for their significant characteristics of high prolificacy, sexual precocity, high adaptation and roughage-resistance and so on (Wu et al. 2019). Based on these features, JXB pigs were used in this study as test animals to analyse the effect of the NR5A2 gene’s marker loci on their reproductive traits. In order to explore the association of SNPs of the NR5A2 gene with JXB pigs reproductive traits, and to find the molecular markers related to the reproductive traits of JXB pigs, the polymorphism loci within the NR5A2 gene in JXB pigs were detected by means of direct sequencing. Meanwhile, the link between different genotypes and diplotypes of a single polymorphic locus and the productive traits of JXB pigs was investigated.

Materials and methods

Animal and trait data collection

This experiment was conducted following the Chinese guidelines for animal welfare and approved by the animal welfare committee of Zhejiang University (Approval Number: 12969). All of the animal procedures were performed according to the Chinese guidelines for animal welfare and approved by Zhejiang University (Hangzhou, China). We chose JXB pigs fed by Zhejiang Qinglian Food Co., Ltd (Haining, China) as experimental subjects. Samples of ear tissues from 128 Jinxing sows were collected, soaked in 75% alcohol and stored at −20°C for DNA extraction. Eleven tissues (large intestine, kidney, lung, small intestine, heart, liver, longissimus dorsi, fat, ovary, hypophysis, hypothalamus) from five Jiaxing black sows were used for NR5A2 Tissue expression profile of the gene. Ovaries of Jiaxing black sows with g.138802T > C and g.138864A > G mutation loci were taken (five each of six genotypes). Data on the reproductive traits of 128 sows including the total number of piglets born (TBA), number of piglets born alive (NBA), Number of stillborn piglet (NSB), and litter weight at birth (LWB) were recorded and organized.

DNA and RNA extraction, cDNA synthesis

Fresh samples of tissues ear, large intestine, fat, heart, longissimus dorsi, kidney, liver, lung, ovary, small intestine, hypophysis and hypothalamus were frozen in liquid nitrogen immediately after being separated, then stored at −80°C. The total RNA content was isolated from the tissues using a TRIzol A+ Total RNA reagent (TIANGEN, Beijing, China) according to the instructions of manufacture. The amount and purity of the extracted RNA were measured by the NanoDropND2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the quality of the obtained RNA was checked by electrophoresis (Zhang et al. 2020). The Prime Script™ RT Reagent Kit with a gDNA Eraser (Takara Biotechnology Co. Ltd., Dalian, China) was used to convert RNA to cDNA following the manufacturer’s protocol.

Primer design, PCR amplification and sequencing

Genomic DNA was isolated from ear tissues using standard phenol/chloroform extraction and stored at −20°C. According to the Sus scrofa NR5A2 gene sequence (GenBank accession NC_010452), primers were designed by the Primer software version 5.0. These primers were used to amplify exon 1–10 (Table S1), with exon10 designed by 4 primers. The PCR reaction mixture (25 μL) consisted of a 12.5 μL 2 × Taq Master Mix, 1 μL of each primer, and 2 μL template DNA. PCR was carried out with an initial denaturing step at 94°C for 5 mins and then 35 cycles at 94°C for 40s, 55°C for 30 s and 72°C for 1 min; with a final extension at 72°C for 4 mins. After that, the PCR products were distinguished using 1% agarose gel electrophoresis with a 2000bp DNA marker.
All the PCR products were purified with an agarose gel DNA recovery kit (Tiangen, Beijing, China) and the purified products were sent to Qingke Yuxi Biotechnology Co., Ltd (Hangzhou, China) for sequencing. The sequencing results were analysed using the SeqMan software to spot polymorphism sites.

Relative expression of NR5A2 mRNA

The NR5A2 gene’s primer pairs included for real-time fluorescent quantitative PCR were designed using Primer-BLAST based on the pigs NR5A2 mRNA (NM_001267893.1). Primers were synthesized by Tsingke Biotech Co., Ltd. (Hangzhou, China).

Real-time PCR was performed using a StepOnePlus Real-Time PCR System (Applied Bio systems, Foster City, CA, USA) with a 20 µL PCR mixture including 2 µL of cDNA, 10 µL of 2 × SYBR Premix Ex TaqII, 0.4 µL ROX Reference Dye (50×) 0.8 µL of forward primer, 0.8 µL of reverse primer and 6 µL of nuclease-free water. The 2 × SYBR Premix Ex TaqII, ROX Reference Dye (50×) and nuclease-free water were contained in the TB Green Premix Ex Taq TM II (Takara, Dalian, China). The reactions were incubated in a 96-well optical plate (Applied Biosystems, American) at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 30 s. Each sample was analysed in triplicate. The mRNA expression levels were normalised to the mRNA expression of pig β-actin and calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical analysis

A phylogenetic tree of 8 species was constructed using the maximum likelihood method based on the nucleotide sequence of the NR5A2 gene established with the MEGA7.0 software, with chicken as an outgroup in birds. Genotype, allele frequencies and population parameters including heterozygosity (He), effective allele numbers (Ne), polymorphism information content (PIC), and Hardy-Weinberg equilibrium (HWE) were computed with Pop Gene (Version1.3.2). Linkage disequilibrium of the regions that are in accordance with Hardy-Weinberg equilibrium were explored using the HAPLOVIEW software (Version 4.2).

The relationship between SNPs and four reproduction traits (TBA, NBA, NSB, LWB) of 128 JXB sows was determined using the SPSS software (Version 20.0). The analytical results were expressed as the mean ± SD. The Phase software (version2.1) was used to analyse haplotypes and haplotype combinations. The correlation between diplotype and the reproduction traits in 128 JXB sows were also identified. HaploView was used to analyse the linkage disequilibrium (LD) and the general linear model (GLM) was as follows: $Y_{ijk}=\mu+P_i+G_j+M_k+N_h+e_{ijkh}$, where $Y_{ijk}$ is the individual observation of the reproduction traits, $\mu$ is overall population mean, $P_i$ is fixed effect of parity ($i = 1, 2$), $G_j$ is the effect of genotypes ($j = 1, 2, 3, 4, 5$), $M_k$ is the fixed effect of season, $N_h$ is the sire effect and $e_{ijkh}$ is the random residual error. The additive effects (a) of the NR5A2 gene were estimated as 1/2(BB-AA) and the dominance effect (d) as AB-1/2(AA + BB).

The relative expression data were checked for normality by the Kolmogorov–Smirnov test in the SPSS v20.0 software (IBM, Chicago, IL, USA), and logarithmic transformation (log2) was used to correct the non-normal distribution (Cao et al. 2019). Next, one-way ANOVA was implemented to evaluate the relative expression level of the NR5A2 gene among groups. Multiple comparison results of the groups were adjusted by Bonferroni correction.

Results

Phylogenetic relationships of NR5A2 proteins among 8 species

As illustrated in Figure 1, the NR5A2 proteins of all 8 species fell under two subgroups. All of the mammalian and zebrafish sequences clustered into one subgroup and the chicken sequences clustered into another subgroup. It can be seen that the gene is highly homologous in several of these species.

Polymerase chain reaction amplification of NR5A2 gene exons

All 10 exons of the NR5A2 gene were successfully amplified using JXB sows’ DNA and 13 pairs of primers. Since exon 10 sequence was too long, we split it into four smaller segments for amplification, which were in turn NR5A2-Exon10-a, NR5A2-Exon10-b, NR5A2-Exon10-c, and NR5A2-Exon10-d. The sizes of the amplified fragments were approximately consistent with the target ones which were 743, 1107, 683, 820 bp in length (Figure S1), respectively. Splicing of exons and introns were consistent with the GT-AG rule (Wu et al. 2019).

![Figure 1. Phylogenetic tree constructed based on the NR5A2 nucleotide sequence of 8 species.](image)

Note: Branches were labelled with species’ names. The number above each branch represented bootstrap values.
Identification of polymorphisms in the NR5A2 gene and genetic parameters analysis

In this study, the results of sequencing and sequence comparative analysis revealed that there were ten mutations within the NR5A2 gene of JXB sows (Figure S2). One SNP named g.84609G > A was identified in exon 9 and the remaining SNPs were present in exon 10, namely: g.135751G > T, g.138606G > T, g.136703T > A, g.136706A > T, g.136759G > C, g.138771C > T, g.138933C > T, g.138802T > C, g.138864A > G. Among those that result in synonymous mutations are g.135751G > T (Pro < Arg), g.138771C > T (Pro < Ser), g.138806G > T (Pro < Ser), g.136703T > A (Tyr < Cys), g.136706A > T (Phe < Tyr), g.136759G > C (Phe < Asp), g.138802T > C (Asn < Asp), g.138933C > T (Leu < Arg); The nonsense mutation is g.138933C > T (Ser < UAA). The gene heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC) of ten mutations were calculated, and the Chi-square test was used to determine whether or not the ten mutations in the population were in accordance with the Hardy-Weinberg equilibrium (HWE) (p > 0.05). The genotypic distribution of g.135751G > T, g.138806G > T, g.136703T > A, g.138771C > T and g.138802T > C deviated from the Hardy-Weinberg equilibrium (HWE) (p < 0.05), whereas the distribution of g.84609G > A, g.136706A > T, g.136759G > C, g.138802T > C, g.138864A > G followed the Hardy-Weinberg equilibrium (HWE) (p > 0.05), so we selected these mutations for further analysis.

Linkage disequilibrium analysis, haplotype and diplotype construction

D’/r2 linkage disequilibrium analysis indicated that five SNPs (g.135751G > T, g.138806G > T, g.136703T > A, g.138771C > T and g.138933C > T) exhibited a strong linkage disequilibrium (LD), thus constructing a 54 kb block1 (Figure 2). Using the Phase software, we identified 14 haplotypes (Table 2) and 17 haplotype combinations (Table S2). H8 was the most abundant haplotype (0.432) out of the 14 haplotypes, H8, H9, H11 and H12 accounted for 84% of the observations. Out of the 17 diplotypes, the frequencies of 12 diplotypes were lower than 0.03, which means that the remaining five diplotypes (H8H12, H8H11, H8H9, H6H8, and H7H8) were further analysed.

Association of NR5A2 polymorphisms with reproductive traits

The association of five NR5A2 SNPs with the reproductive traits of JXB sows is displayed in Table 3. At the g.136759G > C SNP, GG subjects produced more pigs per litter in terms of TBA and NBA compared to the CC sows (p < 0.05). Animal subjects with the AA genotype in g.13864A > G had higher NBA values than those with AG genotypes (p < 0.05) and no statistically significant difference was identified for the other traits including TBA, WPB and NDB. Besides, g.84609G > A, g.136706A > T, and g.138802T > C were not correlated with any trait. Further association analysis on diplotypes was delineated in Table 4. Sows with H8H11 diplotypes had lower TBA values than those with H7H8 diplotypes (p < 0.05). There were no statistically significant differences across the various diplotypes with respect to other traits such as TNB, NBA, NSB and LWB. Hence, as far as the TBA phenotype is concerned, the H7H8 diplotype can be regarded as the best diplotype in this population.

Relative expression levels of pig NR5A2 mRNA in 11 tissues

The relative expression results confirmed that NR5A2 mRNA was widely expressed in the large intestine, fat, heart, longissimus dorsi, kidney, liver, lung, ovary, small intestine, hypothalamus, and pituitary, but the level of expression was subject to tissue-specific regulation. The mRNA expression levels of pigs were significantly higher (p < 0.01) in the ovary and liver compared to other tissues (Figure 3).
NR5A2 mRNA relative expression with different genotypes in the ovary

The ovary was selected to detect the expression levels of different genotypes of the (g.136759G > C, g.138864A > G) loci that were significantly associated with reproduction traits, since it exhibited the highest NR5A2 mRNA relative expression levels. The results depicted that g.136759G > C and g.138864A > G were both significantly associated with the expression level of the NR5A2 gene. As laid out in Figure 4, for the g.136759G > C locus, ovaries with the GG genotype had higher ($p < 0.01$) NR5A2 mRNA expression levels than those with CC and GC genotypes. Similarly, ovaries with the AA genotype had higher ($p < 0.01$) NR5A2 mRNA expression levels than those with GG and AG genotypes in the g.138864A > G locus.

Table 2. Haplotypes based on the block 1 and frequencies in JXB sows.

| Haplotype | g.84609G > A | g.136706A > T | g.136759G > C | g.138802T > C | g.138864A > G | Frequency |
|-----------|--------------|--------------|--------------|--------------|--------------|-----------|
| H1        | G            | A            | G            | T            | A            | 0.024     |
| H2        | G            | A            | G            | T            | C            | 0.004     |
| H3        | G            | A            | G            | C            | A            | 0.016     |
| H4        | G            | A            | G            | C            | C            | 0.012     |
| H5        | G            | A            | C            | T            | C            | 0.008     |
| H6        | G            | T            | G            | T            | A            | 0.028     |
| H7        | G            | T            | C            | T            | A            | 0.036     |
| H8        | G            | T            | C            | C            | A            | 0.432     |
| H9        | A            | A            | G            | T            | C            | 0.136     |
| H10       | A            | A            | C            | T            | A            | 0.024     |
| H11       | A            | T            | G            | T            | A            | 0.108     |
| H12       | A            | T            | C            | T            | A            | 0.164     |
| H13       | A            | T            | C            | T            | C            | 0.004     |
| H14       | A            | T            | C            | C            | A            | 0.004     |

Discussion

Research on the physiological function of the NR5A2 gene is now well established. LRH-1, a member of the lone nuclear receptor superfamily, plays an important role in pigs’ early embryonic development (Guo et al. 2016). Sladitschek found that deletion of the NR5A2 gene severely impaired ectoderm differentiation in mice. The results (Labelle-Dumais et al. 2007) showed that during lutealization and natural gestation, NR5A2-/- mice experienced a significant decrease in progesterone production compared to wild type mice, which in turn led to a decrease in the fertility of the mice, undoubtedly indicating that this gene plays an important role in reproduction. Being two closely related homologs in the NR5A subfamily, SF-1 (NR5A1) and LRH-1 (NR5A2) (Fayard et al. 2004) have been previously found to be expressed in granulocytes (Falender et al. 2003). Both NR5A1 and NR5A2 play similar roles in the production of steroids in mouse granulocytes (Saxena et al. 2007). Recent studies have highlighted the potential application of the NR5A2 gene in the regulation of goat estrus, which implies that NR5A2 plays a key role in the goat ovary and is essential for luteinogenesis (An et al. 2018). Furthermore, C > G mutation was detected at the core promoter region-388 of the NR5A1 gene in Hu sheep, and the association analysis indicated that the number of second and third lambs of GC-type ewes was higher than that of CC-type ewes ($p < 0.05$) (Li et al. 2018). It was also found that NR5A2 mRNA levels were positively correlated with Hu sheep spawning rate and the number of lambs born. Two single nucleotide polymorphisms (T40C and T1419C) were detected in the NR5A2 coding sequence. Also, at the third litter and average litter size, ewes with the CC genotype at the T40C locus produced more lambs than ewes with the TT genotype. Li et al. (2019) amplified the NR5A2 gene’s promoter sequence and identified its core promoter region as −721 nt to −281 nt. An AT > G polymorphism of −700 nt was depicted in the core promoter region and a subsequent correlation analysis found that the second and average lambing numbers were significantly higher in GG ewes than in TG and TT ewes, with the third lambing number being significantly higher in GG ewes than in TT ewes.

In this study, 10 SNP loci were detected on the NR5A2 gene of JXB pigs, five of which were closely associated with their reproductive traits. According to the results obtained from the $\chi^2$ test, g.135751G > T, g.138806G > T, g.136703T > A,
Table 3. Association of five SNPs of the NR5A2 gene with reproductive traits in Jiaxing Black sows (MEAN ± SD).

| SNPs       | Traits/ Number | Genotypes (mean ± SD) |
|------------|----------------|-----------------------|
| g.84609G > A |                |                       |
| g.136706A > T |                |                       |
| g.136759G > C |                |                       |
| g.138802T > C |                |                       |
| g.138864A > G |                |                       |

Note: SNPs: single nucleotide polymorphism sites. TBA: total number of piglets born; NBA: number of piglets born alive; NSB: number of stillborn piglet; LWB: litter weight at birth; N: number of sows. Means with different lowercase superscripts differed significantly (p < 0.05); means with the same letter did not differ significantly (p > 0.05).

Figure 3. Relative expression levels of pig NR5A2 mRNA in 11 tissues with the average ΔCt value of heart as the calibrator.

Note: Data were shown as the mean ± SD for 5 pigs. Columns ** showed highly significant difference (p < 0.01).

Table 4. Association of diplotypes of the NR5A2 gene with reproductive traits in Jiaxing Black sows (MEAN ± SD).

| Diplotype | Traits (mean ± SD) |
|-----------|--------------------|
|           | TNB                |
|           | NBA                |
|           | LWB (kg)           |
|           | NSB                |
| H8H12     | 40                 |
|           | 13.10 ± 1.26b      |
|           | 12.51 ± 1.31       |
|           | 13.89 ± 2.05       |
|           | 1.37 ± 1.44        |
| H8H11     | 26                 |
|           | 12.51 ± 1.77b      |
|           | 12.06 ± 1.84       |
|           | 13.98 ± 2.78       |
|           | 1.16 ± 0.94        |
| H8H9      | 24                 |
|           | 12.70 ± 1.94b      |
|           | 11.89 ± 2.14       |
|           | 13.60 ± 2.30       |
|           | 1.39 ± 1.63        |
| H6H8      | 7                  |
|           | 13.43 ± 1.10b      |
|           | 12.76 ± 0.97       |
|           | 14.20 ± 2.72       |
|           | 2.04 ± 0.65        |
| H7H8      | 6                  |
|           | 14.06 ± 1.20b      |
|           | 13.32 ± 1.08       |
|           | 13.90 ± 1.50       |
|           | 1.54 ± 1.01        |

Notes: We deleted diplotypes with a diplotype frequency of less than 0.03, the frequencies of these 17 diplotypes have been summarised in Table S2 in the supplement. SNPs: single nucleotide polymorphism sites. TBA: total number of piglets born; NBA: number of piglets born alive; NSB: number of stillborn piglet; LWB: litter weight at birth; N: number of sows. Means with different lowercase superscripts differed significantly (p < 0.05); means with the same letter did not differ significantly (p > 0.05).
sows was significantly higher than that of H8H11 diplotype JXB sows, albeit no statistically significant discrepancy was detected relative to other diplotypes, which could be due to the low number of sows in some haplotype classes. In conclusion, based on this experiment’s results, the NR5A2 gene can be used as a target gene in livestock breeding.

The latest research articles report that NR5A2 is a downstream transcription factor of Notch signalling that regulates progesterone synthesis, and thus the NR5A2 gene is involved in suppressing Notch signalling to promote progesterone secretion by enhancing the expression of the NPC1 (NPC intracellular cholesterol transporter 1) and STAR (steroidogenic acute regulatory protein) (Guo et al. 2020). The NR5A2 gene core promoter-700T/G polymorphism produced a new transcription factor MTF-1 binding site that upregulated the transcriptional activity and mRNA expression of the NR5A2 gene in granulosa cells, thereby increasing the litter size of Hu sheep; presumably by regulating the steroid synthesis of NR5A2, which in turn affected ovulation and litter size (Li et al. 2019). This study is the first to report a correlation between the NR5A2 gene and reproductive performance in pigs, but further research is necessary to determine the exact molecular mechanism through which the polymorphism of the NR5A2 gene affects reproductive traits in pigs. All of the aforementioned evidence is enough to prove that NR5A2 polymorphisms may be used as a potential genetic marker to optimise the breeding of JXB pigs.

**Conclusion**

In a nutshell, this study demonstrated that the NR5A2 mRNA expression level in the ovary was the highest, and polymorphisms of the NR5A2 gene were significantly associated with the reproductive traits of Jiaxing Black pigs, which indicates that the NR5A2 gene can be used for molecular marker-assisted selection of reproductive traits in JXB pigs and to provide a theoretical basis to optimise the breeding of JXB pigs.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**ORCID**

Jinzhi Zhang http://orcid.org/0000-0002-8450-2352

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