

Association of Single-Nucleotide Polymorphisms of *MDR1* and *OPN* Genes with Reproductive Traits in Different Breeds of Sows

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ABSTRACT

The objective of this study was to investigate the single-nucleotide polymorphisms (SNP) of the *Multiple Drug Resistance Gene 1* (*MDR1*) and the *Porcine Osteopontin* (*OPN*) gene and their association with reproductive traits in Landrace, Large Yorkshire and Jinhua sows. A total of 316 sows of three different breeds were used to examine the genotypes, and DNA sequencing method was employed to detect the potential SNPs in this study. There were two SNP loci found in these genes totally. One mutation (G→A) was detected in exon 1 of *MDR1* gene, which caused a synonymous mutation of amino acid (Leu→Leu), the other one (T→A) in exon 7 of *OPN* gene, resulting in a non-synonymous mutation (His→Gln). The association analysis indicated that the GG genotype of *MDR1* gene and the TT genotype of *OPN* gene had the highest values for the total number of piglets born (TNB), the number of piglets born alive (NBA), piglet weight at birth (PWB) and litter weight at birth (LWB) in both primiparous and multiparous sows of the three breeds. These results suggested that the SNP loci of *MDR1* and *OPN* genes can be used in Marker-Assisted Selection (MAS) programs for rapid improvement of the reproductive traits in sows.

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Authors' Contribution

JL and FW conceived and designed the experiments. YFN and JFC performed the experiments. JQH and JPF analyzed the data. JL and JFC contributed reagents/materials/analysis tools. YFN and FW contributed to the writing of the manuscript. JZZ revised the manuscript.

Key words

MDR1, *OPN*, Single-nucleotide Polymorphism, Reproductive traits, Sows

INTRODUCTION

Reproductive traits, especially litter traits, are one of the most economically important parameters in pig production (Linville *et al.*, 2001). As the same time, reproductive traits are highly complicated, however, with rather low heritability which is only about 0.1, among various characteristics (Johnson *et al.*, 1999; Campbell *et al.*, 2003). It is not obviously effective to apply conventional breeding methods to improve these traits. With the development of molecular biology, it has become possible to find genetic markers and to carry out marker-assisted selection (MAS).

The human *Multiple drug resistance gene 1* (*MDR1*) is located on the long arm of chromosome 7 and contains 28 introns (Pauli-Magnus *et al.*, 2004), while the pig *MDR1* is located on chromosome 9. The length of the *MDR1* is 4.7 kb, less than 5% of the entire genome, encoding P-glycoprotein (*P-gp*) weighed 40 ku which consisting of 1280 amino acids in total. The *P-gp* is an ATP-dependent transmembrane outward transporter protein which can actively pump out chemical substances and drugs that are passively diffused into cells, leading to multiple drug resistance

(Brambila-Tapia, 2013). As early as 1989, *P-gp* was reported to exist in tissues known to have blood-tissue barriers, such as the placenta (protecting the fetus), ovaries and testes, which were associated with reproduction (Sugawara *et al.*, 1988; Cordon-Cardo *et al.*, 1989). Later, Lankas *et al.* (1998) had found the importance of placental *P-gp* in protecting the fetus from potential teratogens, and the results showed that the fetus was more sensitive to teratogenic substances in pregnant mice lacking the *MDR1* transporter in the placenta. There was confirmed that multiple drug resistant gene1 was closely related to treatments of many human diseases (Obata *et al.*, 2006; Pauli-Magnus *et al.*, 2004). Pappas *et al.* (2014) had also found that *MDR1* gene expressed in placenta of human and guinea pig. However, the information on the association between polymorphism of *MDR1* gene and reproductive traits of sows is poorly unknown. Based on the relationship between the *MDR1* gene and the reproductive system diseases such as ovarian cancer (Obata *et al.*, 2006) discussed above, we can speculate that the *MDR1* gene may be closely related to animal reproductive organs and the polymorphism may be related to animal reproductive performance.

There was found that the *porcine osteopontin* gene (*OPN*) could increase the mRNA and protein expression of *P-gp* in a concentration- and time-dependent manner, and their results indicated that *OPN* was a potential therapeutic

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target for cancer therapy to reduce drug resistance in sensitive tumors (Hsieh *et al.*, 2013). The osteopontin is a secreted glycosylation protein isolated from bone matrix by Bernards *et al.* (2008) which is rich in arginine-glycine-aspartate (Arg-Gly-Asp, RGD) structures. It plays an important role in mediating the connection between bone matrix and bone tissue cells and that's why it's called osteopontin. The binding of osteopontin and CD44 receptor plays a vital role in mediating the interaction between cell matrix and cell signal transduction (Stier *et al.*, 2005). Meanwhile, osteopontin was also found to be involved in regulating embryonic growth and development, initiating and maintaining pregnancy (Garlow *et al.*, 2002; Goncalves *et al.*, 2007; Monaco *et al.*, 2009). There are also an increasing number of studies showing that the *Porcine Osteopontin* (*OPN* gene) is expressed in humans and other mammals. It has been found that osteopontin expressed in various tissues such as bone, liver, testis, ovary and placenta (Pines *et al.*, 1995). *OPN* gene, expressed abundantly in uterine epithelium and immune cells that were key contributors in pig embryo attachment and placentation, may interact with receptors and uterus to improve conceptus development and be the signaling between these tissues (Garlow *et al.*, 2002). *OPN* mRNA expression in the ovine uterus was induced by progesterone and led to the secretion of *OPN* gene into the uterine lumen by endometrial glandular epithelium (Johnson *et al.*, 2000). Given that *OPN* gene was detected in the uterus of humans, as well as pigs, Johnson *et al.* (2003) believed that the *OPN* gene had the potential to profoundly impact pregnancy. It has also been reported that the *OPN* gene expressed in both mature and immature oocytes and follicular cells (Monaco *et al.*, 2008). Moreover, Kapelański *et al.* (2013) found the marker genotypes characterized by *OPN* gene had an important influence on the weight of the uterus, the weight and volume of the ovary and other reproductive system indicators in both breeds (Polish Large White and Polish Landrace gilts). However, while the studies on *OPN* gene polymorphism and porcine fertility is available in artificial insemination boars (Lin *et al.*, 2006), Tibet pigs (Niu *et al.*, 2008), Polish Landrace and Polish Large White sows (Korwin-Kossakowska *et al.*, 2013) and other pig breeds, the literatures concerning *OPN* gene and prolificacy of Landrace, Yorkshire and Jinhua sows were limited. And we guess that the SNP of the *OPN* gene may be closely associated with reproductive traits of these sows through the above summary.

In this study, three sow breeds including Landrace, Large Yorkshire and Jinhua sows were selected as the research objects. Direct sequencing of DNA was applied to detect SNPs in exon 1 of *MDR1* gene and exon 7 of *OPN* gene, we analyzed the associations between the SNP

sites of *MDR1* and *OPN* genes, and productive traits such as total litter size, alive litter size and litter weight at birth in both primiparous and multiparous sows. The goal of the study was to provide useful information for marker-assisted breeding to improve reproductive performance of sows.

MATERIALS AND METHODS

Ethics statement

All procedures involving animals were conducted in accordance with Chinese guidelines for animal welfare and approved by the Laboratory Animal Center of Zhejiang University (Hangzhou, China).

Animals and DNA extraction

In the present experiment, ear tissue samples were collected from 316 sows, including 70 Landrace (18 primiparous sows and 52 multiparous sows), 140 Large Yorkshire (21 primiparous sows and 119 multiparous sows) and 106 Jinhua (17 primiparous sows and 89 multiparous sows), all from Zhejiang Jiahua Breeding Company Limited. All of these sows were provided same feed and water and kept under natural temperature conditions. The reproductive performance records of corresponding sows from 2011 to 2015 were analyzed and sorted out, including the total number of piglets born (TNB), the number of piglets born alive (NBA), piglet weight at birth (PWB) and litter weight at birth (LWB). 150 mg of pig ear tissue was cut into an EP tube, and DNA was extracted according to the instructions of the Tiangen Genomic DNA Extraction Kit. The extracted DNA was detected by 1.0% electrophoresis agarose gels, the DNA concentration was measured by Nano 2000 and diluted to 50ng L⁻¹, and then stored them at 20°C for subsequent experiments.

Primer design and PCR amplification

In this experiment, primer sequences based on the pig *MDR1* (GenBank NO. NC_010451.4) and *OPN* gene (GenBank NO. NC_010450.4) were designed by Primer 5.0 and synthesized by Invitrogen (Shanghai) Trading Company Limited. Taking the polymerase chain reaction (PCR) procedure into account, the primer sequences for *MDR1* were as followings: forward 5'-GCGGTCTGGCTGATTGGC-3' and reverse 5'-CCTCGGGCTTTCCCTCTG-3'; and the following were those for *OPN* gene: forward 5'-TGGATGCCACAGAGGAAG-3' and reverse 5'-CATTCGAGATATTTTATTCACA-3'. The PCR was performed in a reaction volume of 25 µL containing 12.5 µL 2×Taq Master Mix, 1µL of forward and reverse primer respectively, 2 µL DNA template and 8.5 µL ddH₂O. PCR amplification was performed using the following

conditions: initial denaturation at 94°C for 5 min, 35 amplification cycles including denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 4 min. The PCR amplification products were examined by electrophoresis on 1.0% agarose gel.

PCR product purification and sequencing

The PCR products were sent to Shanghai Shenggong Biotechnology Company Limited for purification and sequencing. Sequencing peak maps and sequences were analyzed using BioEdit and DNA star software.

Statistics analysis

Allele frequency, genotype frequency, χ^2 Hardy-Weinberg equilibrium estimations, polymorphism information content (PIC), population heterozygosity (He) and effective allele number (Ne) were calculated using POPGENE1.31. According to fixed effect model, General Linear Model (GLM) procedure in SAS 8.2 software was used to compare the reproductive performance of the four sow breeds among different genotypes of the 2 genes respectively. The following was the statistical models used in the analysis: $Y_{ijk} = \mu + G_i + P_j + S_k + e_{ijk}$. Where Y_{ijk} is observation of reproductive traits; μ is the population mean; G_i is effect of gene; P_j is effect of parity ($j=1, 2, 3 \dots 8$); S_k is environmental effect ($k=1, 2, 3, 4$); and e_{ijk} is random error.

RESULTS

PCR amplification of *MDR1* and *OPN* genes

Taking genomic DNA of different pig breeds as templates, the gene fragments of exon 1 of *MDR1* gene and exon 7 of *OPN* gene were amplified, and the amplified products were detected by electrophoresis on 1.0% agarose gel. The amplified fragments were 280 bp and 780 bp, respectively (Figs. 1, 2), which can be used for subsequent studies because of the consistence with the expected fragment sizes, better amplification effects, better banding specificity, and higher brightness. The positions of amplified fragments on chromosome 9 of *MDR1* and chromosome 8 of the *OPN* gene were from 93050045 to 93050281 and from 131077789 to 131078590 in accordance with Sscrofa11.1, respectively.

SNPs identification of *MDR1* and *OPN* genes and its genotyping

The peak maps and sequences obtained after purification and sequencing of the PCR products were compared and analyzed by software such as BioEdit and DNA star (Figs. 3, 4). In the examined population of the

three sow breeds, exon 1 of *MDR1* gene was mutated at 154 bp (G→A), thus, leading to appearance of the three genotypes, namely, GG, AA and AG. There was no amino acid change at the location, which caused a synonymous mutation (Leu→Leu) (Fig. 3). For *OPN* gene, one mutation was detected in exon 7 of which at position 288, and the two alleles (T and A) with three genotypes (TT, AA, and AT) were determined (Fig. 4). Mutations at this site was a nonsynonymous mutation (His→Gln).

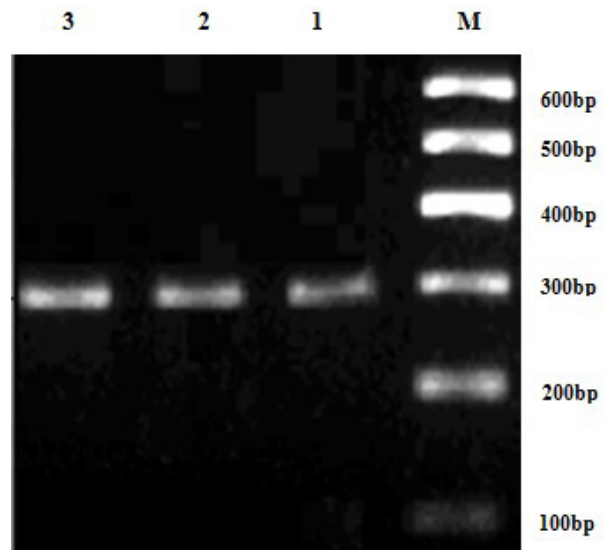


Fig. 1. The PCR results of *MDR1* gene.

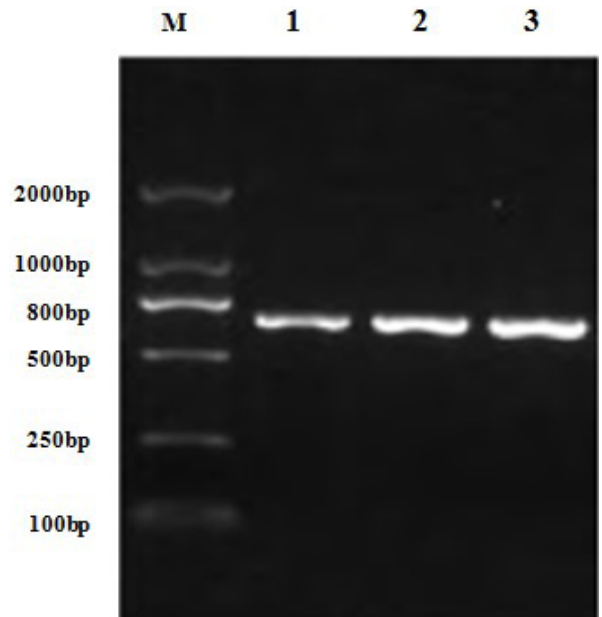


Fig. 2. The PCR results of *OPN* gene.

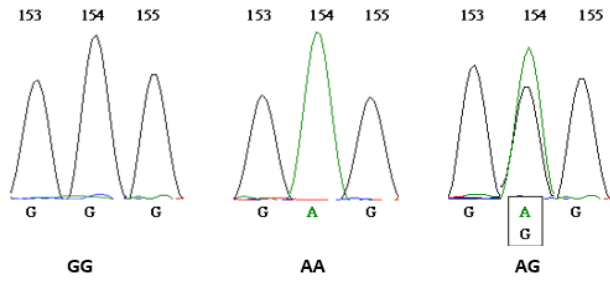


Fig. 3. The sequencing results of *MDR1* gene.

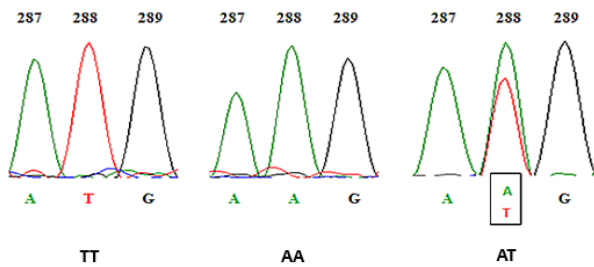


Fig. 4. The sequencing results of *OPN* gene.

Genotypes and allele frequency of MDR1 and OPN genes and sample genetic characteristics

In Landrace, Large Yorkshire, and Jinhua sows, the major alleles of *MDR1* and *OPN* genes were G and T, respectively. The allelic frequencies of *MDR1* and *OPN* genes of the three population were shown in the Tables I and II. It could be seen from Chi-square test that *MDR1* and *OPN* genes reached the Hardy-Weinberg equilibrium

in all four populations ($P>0.05$). By analyzing the PIC, He and Ne of *MDR1* and *OPN* genes in the three populations, it could be found that the PIC of *MDR1* and *OPN* genes were more than 0.25 and less than 0.5 in the populations, indicating that *MDR1* and *OPN* genes were in a moderate polymorphism in the study.

Association analysis of genetic polymorphism with reproductive performance

According to the two gene mutations for individual genotype, the least squares method was used to analyze the effects of different genotypes on traits (TNB, NBA, PWB and LWB) of the primiparous and multiparous sows of the three breeds in this experiment. From Tables III and IV, both *MDR1* and *OPN* genes had significant effects on reproductive traits of the three sow breeds ($P<0.05$). Interestingly, in the primiparous and multiparous Landrace, Large Yorkshire and Jinhua sows, the reproductive traits (TNB, NBA, PWB and LWB) of the different genotypes of *MDR1* gene all showed a trend of AA>GG>AG. The primiparous and multiparous sows with GG genotype had higher TNB, NBA, PWB and LWB values than AA genotype ($P<0.05$), although the AG genotypes were not significantly different from the GG and AA genotypes ($P>0.05$). The *OPN* gene also showed a similar trend that the TNB, NBA, PBW and LWB values of the TT genotype individuals were significantly higher than those of the AA genotype individuals ($P<0.05$); the difference between AT genotype individuals and TT genotype individuals was not significant; and either of AT genotype individuals or AA genotype individuals ($P>0.05$).

Table I. Genotype and allele frequencies at each locus of *MDR1* gene.

| Breeds | No. | Genotype frequency | | | Allele frequency | | χ^2 | PIC | He | Ne |
|-----------------|-----|--------------------|------------|------------|------------------|--------|----------|--------|--------|--------|
| | | GG | AG | AA | G | A | | | | |
| Landrace | 70 | 0.3857(27) | 0.3571(25) | 0.2571(18) | 0.5643 | 0.4357 | 0.2760 | 0.4905 | 0.4917 | 1.9673 |
| Large Yorkshire | 140 | 0.4500(63) | 0.3643(51) | 0.1857(26) | 0.6321 | 0.3679 | 3.1450 | 0.3569 | 0.4651 | 1.8695 |
| Jinhua | 106 | 0.4057(43) | 0.2830(30) | 0.3113(33) | 0.5472 | 0.4528 | 1.2360 | 0.3727 | 0.4955 | 1.9821 |

$\chi^2_{0.05(2)}=5.99$. PIC, polymorphism information content; He, heterozygosity; Ne, effective number of alleles.

Table II. Genotype and allele frequencies at each locus of *OPN* gene.

| Breeds | No. | Genotype frequency | | | Allele frequency | | χ^2 | PIC | He | Ne |
|-----------------|-----|--------------------|------------|------------|------------------|--------|----------|--------|--------|--------|
| | | TT | AT | AA | T | A | | | | |
| Landrace | 70 | 0.4143(29) | 0.4571(32) | 0.1286(9) | 0.6429 | 0.3571 | 3.4760 | 0.3537 | 0.4592 | 1.8491 |
| Large yorkshire | 140 | 0.4500(63) | 0.2571(36) | 0.2929(41) | 0.5786 | 0.4214 | 3.8750 | 0.3687 | 0.4876 | 1.9516 |
| Jinhua | 106 | 0.3962(42) | 0.2547(27) | 0.3491(37) | 0.5236 | 0.4764 | 3.0550 | 0.3606 | 0.4893 | 1.9581 |

$\chi^2_{0.05(2)}=5.99$. PIC, polymorphism information content; He, heterozygosity; Ne, effective number of alleles.

Table III. Reproductive traits in relation to *MDR1* gene.

| Breeds | Geno- type | Primiparous sows | | | | Multiparous sows | | | |
|--------------------|---------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| | | TNB | NBA | PWB | LWB | TNB | NBA | PWB | LWB |
| Landrace | GG | 13.45±0.21 ^a | 12.51±0.77 ^a | 1.71±0.27 ^a | 21.41±0.23 ^a | 13.96±0.35 ^a | 13.21±0.88 ^a | 1.78±0.28 ^a | 23.52±0.87 ^a |
| | AG | 13.09±0.42 ^{ab} | 12.11±0.38 ^{ab} | 1.62±0.08 ^{ab} | 19.62±0.41 ^{ab} | 13.52±0.53 ^{ab} | 12.73±0.16 ^{ab} | 1.68±0.18 ^{ab} | 21.38±0.44 ^{ab} |
| | AA | 12.51±0.26 ^b | 11.37±0.24 ^b | 1.52±0.15 ^b | 17.28±0.56 ^b | 13.05±0.25 ^b | 11.99±0.27 ^b | 1.58±0.16 ^b | 18.93±0.37 ^b |
| Large Yorkshire | GG | 11.51±0.21 ^a | 10.53±0.23 ^a | 1.47±0.28 ^a | 15.48±0.24 ^a | 12.13±0.14 ^a | 11.25±0.26 ^a | 1.51±0.43 ^a | 16.97±0.18 ^a |
| | AG | 10.94±0.23 ^{ab} | 10.07±0.24 ^{ab} | 1.35±0.18 ^{ab} | 13.61±0.25 ^{ab} | 11.58±0.33 ^{ab} | 10.74±0.31 ^{ab} | 1.41±0.28 ^{ab} | 15.15±0.18 ^{ab} |
| | AA | 10.31±0.34 ^b | 9.42±0.33 ^b | 1.29±0.13 ^b | 12.17±0.37 ^b | 10.89±0.19 ^b | 10.13±0.45 ^b | 1.31±0.12 ^b | 13.28±0.11 ^b |
| Jinhua | GG | 12.78±0.24 ^a | 12.01±0.31 ^a | 1.06±0.04 ^a | 12.74±0.34 ^a | 13.49±0.22 ^a | 12.68±0.34 ^a | 1.08±0.23 ^a | 13.82±0.14 ^a |
| | AG | 12.15±0.33 ^{ab} | 11.28±0.29 ^{ab} | 0.91±0.02 ^{ab} | 10.27±0.22 ^{ab} | 12.94±0.22 ^{ab} | 12.27±0.19 ^{ab} | 0.99±0.06 ^{ab} | 12.18±0.13 ^{ab} |
| | AA | 11.51±0.31 ^b | 10.79±0.36 ^b | 0.82±0.03 ^b | 8.85±0.34 ^b | 12.15±0.23 ^b | 11.71±0.66 ^b | 0.88±0.15 ^b | 10.31±0.24 ^b |

For the same trait among the same group of sows, values with different letter superscripts in the same line mean significant difference ($P < 0.05$); with the same or no letter superscripts in the same line mean no significant difference ($P > 0.05$). TNB, total number of piglets born; NBA, number of piglets born alive; PWB, pig weight at birth; LWB, litter weight at birth.

Table IV. Reproductive traits in relation to *OPN* gene.

| Breeds | Geno- type | Primiparous sows | | | | Multiparous sows | | | |
|--------------------|---------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| | | TNB | NBA | PWB | LWB | TNB | NBA | PWB | LWB |
| Landrace | TT | 13.54±0.23 ^a | 12.46±0.47 ^a | 1.70±0.17 ^a | 21.26±0.51 ^a | 14.11±0.35 ^a | 13.31±0.68 ^a | 1.75±0.18 ^a | 23.31±0.87 ^a |
| | AT | 13.06±0.32 ^{ab} | 12.09±0.36 ^{ab} | 1.61±0.07 ^{ab} | 19.51±0.85 ^{ab} | 13.62±0.23 ^{ab} | 12.77±0.19 ^{ab} | 1.65±0.17 ^{ab} | 21.06±0.44 ^{ab} |
| | AA | 12.61±0.26 ^b | 11.42±0.19 ^b | 1.51±0.17 ^b | 17.25±0.41 ^b | 13.09±0.24 ^b | 12.05±0.25 ^b | 1.56±0.15 ^b | 18.76±0.37 ^b |
| Large Yorkshire | TT | 11.29±0.22 ^a | 10.34±0.14 ^a | 1.48±0.48 ^a | 15.31±0.25 ^a | 11.89±0.24 ^a | 11.03±0.27 ^a | 1.53±0.49 ^a | 16.88±0.15 ^a |
| | AT | 10.98±0.24 ^{ab} | 10.03±0.09 ^{ab} | 1.36±0.28 ^{ab} | 13.65±0.26 ^{ab} | 11.49±0.33 ^{ab} | 10.72±0.33 ^{ab} | 1.41±0.26 ^{ab} | 15.11±0.11 ^{ab} |
| | AA | 10.54±0.33 ^b | 9.75±0.32 ^b | 1.31±0.01 ^b | 12.76±0.38 ^b | 11.12±0.21 ^b | 10.41±0.43 ^b | 1.33±0.11 ^b | 13.85±0.41 ^b |
| Jinhua | TT | 12.59±0.14 ^a | 11.81±0.11 ^a | 1.05±0.11 ^a | 12.42±0.14 ^a | 13.57±0.21 ^a | 12.81±0.31 ^a | 1.11±0.21 ^a | 14.23±0.14 ^a |
| | AT | 12.01±0.23 ^{ab} | 11.08±0.19 ^{ab} | 0.96±0.05 ^{ab} | 10.65±0.34 ^{ab} | 12.72±0.62 ^{ab} | 12.03±0.13 ^{ab} | 1.01±0.06 ^{ab} | 12.13±0.13 ^{ab} |
| | AA | 11.27±0.21 ^b | 10.69±0.33 ^b | 0.87±0.12 ^b | 9.42±0.31 ^b | 12.05±0.23 ^b | 11.31±0.46 ^b | 0.91±0.15 ^b | 10.33±0.24 ^b |

For the same trait among the same group of sows, values with different letter superscripts in the same line mean significant difference ($P < 0.05$); with the same or no letter superscripts in the same line mean no significant difference ($P > 0.05$). TNB, total number of piglets born; NBA, number of piglets born alive; PWB, pig weight at birth; LWB, litter weight at birth.

DISCUSSION

The reproductive traits of sows, including TNB, NBA, PWB and LWB, are important economic characteristics in modern pig production. The reproductive performance affects the production efficiency and economic benefits of farms directly. However, the heritability of reproductive traits is extremely low, and it is occurred at a low efficiency and rate about improvement of these traits (Bidanel *et al.*, 2008). Thus, it is meaningful to study the genes related to reproductive traits of pigs and reveal the genetic mechanism affecting the reproductive performance and the prolificacy of high-breeding pigs. At present, many reports on *MDR1* gene polymorphisms are related to its relationship with genital diseases. Compared to Landrace, Yorkshire, Duroc

and Jinhua sows, studies on *OPN* gene polymorphisms have mainly focused on ruminants, avian animals and other pigs. Therefore, this study analyzed *MDR1* and *OPN* genes of the three sow breeds to investigate the polymorphisms of the two genes in the pig populations. In the present study, we found that the polymorphisms of *MDR1* and *OPN* genes impacted reproductive traits of the sows and could be markers.

In the present study, the *MDR1* gene was used as a candidate gene to explore the reproductive performance of sows. The results showed that one mutation (G→A) occurred at 154 bp of exon 1 of *MDR1* gene; among the three genotypes presented (GG, AA, AG), the sows with GG genotype had higher values about reproductive traits. We found that the mutation in the *MDR1* gene had the

same effect on the reproductive performance of different sow populations selected in this study. Previous studies have shown that *MDR1* gene widely expressed in the fetal placenta and played pivotal roles in protecting the fetus from insults of drugs and xenobiotics, which might explain why *MDR1* gene could prevent drugs from reaching the litter through the placenta (Pappas *et al.*, 2014; Han *et al.*, 2018). In view of low immunity of litters, *MDR1* gene played an important role in protecting litters if the drug reached the body of the dam and might cause the death of litters. This may explain that the *MDR1* gene polymorphism has a significant effect on reproductive traits in sows. This is also consistent with our results, thus, these findings could suggest that *MDR1* gene may be a candidate gene associated with sow reproductive performance and that the mutation site detected in this gene may be a useful genetic marker for reproductive performance in pigs.

The studies on *OPN* gene have become increasingly popular these years, and many of them are the ones on the polymorphisms at present. In pregnant sows, *OPN* gene was induced by conceptual estrogen in the uterine luminal epithelium and was regulated in the glandular epithelium in a manner consistent with placental progesterone production (White *et al.*, 2005). Extracting the samples of the sow ovary and fallopian tube tissue, Goluch *et al.* (2009) detected two mutations (A→G) occurring at positions of 617 and 608, respectively. Nevertheless, it could be found that the major alleles and their frequencies of the *OPN* gene mutations varied from one study to the next. Lin *et al.* (2006) by investigating mutations in the *OPN* gene intron of the purebred Pitland boars, crossbred Hampshire Pietrain boars in northwestern Germany, found the frequencies of the B allele were 0.54 and 0.75, respectively. Niu *et al.* (2008) used the DNA mutation in the *OPN* gene to determine the associations between the genotype and litter size in Tibet pigs, and their data showed that B allele was dominant in Tibet pigs with a frequency of 0.804. Three different genotypes (AA, AB, BB) were found in the mutation of the intron 6 of the *OPN* gene in 71 sows (46 in the luteal and 25 in the follicular phase) in the Korwin-Kossakowska *et al.* (2013)'s experiment, and the frequency of major allele B was 0.61. However, Oztabak *et al.* (2008) found that the *OPN* gene had a mutation (T→G) and the frequency of T allele was higher. And in our study, the mutation (T→A) occurred at 288 bp of exon 7 of *OPN* gene, which caused the frequencies of major alleles in the four populations were 0.64, 0.58 and 0.52, respectively. Many studies have found that the *OPN* gene was closely related to the reproductive traits of pigs. Southwood *et al.* (1998) suggested that it was possible to use the microsatellite sequence in the *OPN* gene locus as a

marker of the reproductive traits TNB and NBA. They found 13 alleles, five of which were related to litter size in the synthetic line. And the polymorphism of *OPN* gene had a significant effect on boar fertility traits such as NBA (Lin *et al.*, 2006). Meanwhile, previous studies by Zhang *et al.* (2010) showed that the genotype of AA and AB of *OPN* gene could increase the LWB, but did not reach the significant level. The expression level of the *OPN* gene was significantly associated with LWB (Korwin-Kossakowska *et al.*, 2013). Among the mutation sites found in this study, three genotypes, TT, AA and AT, were detected, and TT was the dominant genotype. In the primiparous and multiparous sows of the 3 populations tested, the reproductive performance of the TT genotype was significantly better than the AA genotype ($P < 0.05$). However, considering that the reproductive performance of livestock is affected by many factors, and the genetic background of different varieties is very different, the effects of each genotype on reproductive performance can be different. Therefore, it is also necessary to expand the sample size to conduct further systematic studies on different breeds of pigs.

CONCLUSIONS

In conclusion, our results found that polymorphisms of the *MDR1* and *OPN* genes were associated with reproductive traits in Landrace, Large Yorkshire and Jinhua three sow breeds. The present study showed that sows with the GG genotype of *MDR1* gene or the TT genotype of *OPN* gene had the highest values for reproductive traits (TNB, NBA, PBW and LBW) in both primiparous and multiparous sows of the 3 breeds. We indicate that *MDR1* and *OPN* genes may be possible markers of reproduction traits. However, it is also necessary to conduct further systematic studies on other different breeds of pigs, as there may be different results for different breeds.

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Competing interests

All the authors declared no conflict of interests.

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