DOI: 10.1002/vms3.371

ORIGINAL ARTICLE

WILEY

A novel $(ATC)_n$ microsatellite locus is associated with litter size in an indigenous Chinese pig

Guofang Wu[1,2] | Wenjuan Shen[1] | Xingxing Xue[1] | Lei Wang[1,2] | Yuhong Ma[1] | Jiping Zhou[1]

[1]Academy of Animal Science and Veterinary Medicine, Qinghai University, Xining, China

[2]State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, China

Correspondence
Lei Wang, Academy of Animal Science and Veterinary Medicine, Qinghai University, Xining, Qinghai 810016, China.
Email: qhdxwanglei@126.com

Funding information
This work was funded by Achievement Transformation Project of Science and Technology of Qinghai Province (2019-NK-102).

Abstract

Simple sequence repeats (SSRs) are an important part of the genome and have become powerful auxiliary DNA markers in animal breeding using marker-assisted selection (MAS). Based on previous sequencing data of Qinghai Bamei pigs, a total of three novel candidate SSR loci were analysed in this study. Time-of-flight mass spectrometry (TOF-MS) was used for SSR genotyping, and association analyses between SSRs and the litter size of Qinghai Bamei sows was also performed. The results of genotyping showed that the $(ATC)_n$-P1, $(AC)_n$-P2 and $(AC)_n$-P3 loci had 2, 3 and 18 genotypes, respectively; 2, 3 and 8 alleles were also identified at these loci. Except for the $(AC)_n$-P2 locus, the polymorphism information content (*PIC*) values of other loci were greater than 0.25. Association analyses indicated that only the $(ATC)_n$-P1 locus was significantly associated with the litter size of Qinghai Bamei sows ($p = .047$). Compared to 189-/189- genotype, individuals with the 189-/195- genotype had the senior litter size, which was 9.04 ± 0.21. Our results enrich the data on SSRs in Qinghai Bamei pigs and indicate that $(ATC)_n$-P1 is a candidate locus for MAS in the pig industry.

KEYWORDS

litter size, pig, simple sequence repeat (SSR), time-of-flight mass spectrometry (TOF-MS)

1 | INTRODUCTION

Microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STR), are repeat sequences of 2–6 nt length that play important roles in genomes (Liu et al., 2017). They have become powerful DNA markers in animal breeding using marker-assisted selection (MAS; Beuzen et al., 2000). They can be used in the identification of economically important traits and diseases, individuals and infer parent–child relationships. In humans, SSRs are associated with a variety of diseases, such as cancer and Kennedy's disease (Breza & Koutsis, 2019; Fujimoto et al., 2020; Vieira et al., 2016). In cattle and goats, they significantly affect growth traits, milk quality, and virus resistance (Avondo et al., 2019; Dux et al., 2018; Karim et al., 2011). However, few studies have

Abbreviations: *BMPR-1B*, bone morphogenetic protein receptor type 1B; *FSHR*, follicle stimulating hormone subunit beta; *He*, heterozygosity; *Ho*, homozygosity; HWE, Hardy–Weinberg equilibrium; indel, insertions/deletion; MAS, marker-assisted selection; *Ne*, effective allele numbers; PCR, polymerase chain reaction; *PIC*, polymorphism information content; *PRL*, prolactin; *SLA-11*, swine leukocyte antigen 11; SNP, nucleotide polymorphisms; SSR, simple sequence repeat; STR, short tandem repeats; TOF-MS, time-of-flight mass spectrometry.

Guofang Wu and Wenjuan Shen have contributed equally to this work.

considered the associations between SSRs and economic traits in indigenous Chinese pigs.

There are two traditional methods for detecting SSRs, namely, sequencing and gel-based approaches. However, these two methods are time-consuming and provide very little information about quantitative differences (Vogel et al., 2009). Fortunately, in recent years, SSR detection technology has been reformed. Time-of-flight mass spectrometry (TOF-MS), a powerful tool for qualitative research and relative quantitative determination of variation (Seichter et al., 2004), can greatly improve the accuracy and efficiency of SSR detection (Bonk et al., 2003). It measures the molecular mass of the target and essentially minimizes inaccuracies (Ahlstrom et al., 2014). In a single experiment, it can reveal variability in several repeat lengths (Vogel et al., 2009). Therefore, screening of SSRs with TOF-MS can provide a theoretical basis for MAS of pigs and technical support for the breeding of superior traits.

The litter size of females has an important influence on the reproductive success of livestock. Litter size can be affected by genetics, nutrition, the environment and disease. Genetic factors have far-reaching effects. For example, the bone morphogenetic protein receptor type 1B (*BMPR-1B*, also known as *FecB*) gene in sheep may have major effect on their reproduction (Qi et al., 2020). When A746G mutation occurred in *FecB* gene sequence, BB genetype would show more litter size (Chen et al., 2015). The equivalent gene in pigs is follicle stimulating hormone subunit beta (*FSHR*; Bernard & Tran, 2013). However, like most economically important traits, litter size is a complex quantitative trait that might be controlled by a major gene along with several minor ones. Apart from the *FSHR* gene, mutations in *Cytochrome B*, prolactin (*PRL*), swine leukocyte antigen 11 (*SLA-11*) and other genes can also significantly affect the litter size of sows (Korwin-Kossakowska et al., 2009; Pradhan et al., 2018; Zhang et al., 2019). Therefore, to improve the litter size and thus production efficiency of females, it is important to select dominant stable genotypes using an advanced technology such as MAS.

Qinghai Bamei pigs are strongly adaptive and resistant to stress, have high fat deposition, resist of crude feed and feature stable genetics (Zhang et al., 2018), which are one of the important economic breeds in Qinghai. However, its female fertility is lower than that of commercial pigs in China. On the one hand, Qinghai Bamei sows reach sexual maturity at 120 days and can be bred at 300 days after birth. On the other hand, Qinghai Bamei sows have fewer litter size with only about eight live litter size (China National Commission of Animal Genetic Resources, 2011). Enhancing its fecundity, increasing the number of litter size and expanding the population size are the problems that need to be solved urgently (Wu et al., 2019). To this end, it is of great value to study the association between SSRs and litter size in this breed.

In this study, three specific SSR loci were selected using both transcriptome and reduced-representation sequencing. TOF-MS was used to genotype large samples. Then, the associations between these SSR loci and litter size of Qinghai Bamei sows were analysed. We identified dominant and stable genotypes, providing a scientific basis and theoretical guidance for the subsequent improvement of litter size in these pigs.

2 | MATERIALS AND METHODS

All experimental procedures and animal experimentation were performed in agreement with the guidelines of our ethics committee. The study was approved by the Institutional Animal Care and Use Committee of the school.

2.1 | Samples and phenotypic data collection

Ear samples of 256 adult Qinghai Bamei sows were randomly selected from the breeding farms (Qinghai, China). Some of these sows had records of litter size. All tissues were immediately frozen in liquid nitrogen, brought back to our lab and stored at −80°C.

2.2 | DNA extraction

DNA was extracted using a high salt-extraction method (Aljanabi & Martinez, 1997), and then diluted to a standard concentration (10 ng/μl) and stored at −20°C for the detection of genetic variation (Hui et al., 2020; Wang, et al., 2020). The Nanodrop 2000 was used to detect the purity (A_{260}/A_{280} ratio) and quality of DNA.

2.3 | Primer design

Based on preliminary sequencing results (data not shown), three SSR loci were selected for further analysis. $(ATC)_n$-P1, $(AC)_n$-P2 and $(AC)_n$-P3 represented $(ATC)_n$, $(AC)_n$ and $(AC)_n$, respectively. Primer 5.0 and the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) primer designing tool were used to design the primers (Zhang et al., 2020). Primer information is shown in Table 1.

2.4 | Detection of SSR variants using TOF-MS

The specific primers and genome DNA of Qinghai Bamei sows were used to perform polymerase chain reaction (PCR; Smołucha et al., 2019). The products were sent to Saisike Biotechnology Co., Ltd. for TOF-MS analyses. The specific experimental methods followed Seichter et al. (2004) and Rau et al. (2019).

2.5 | Statistical analyses

The methods of Nei and Roychoudhury (1974) and Botstein et al. (1980) were used to detect homozygosity (*Ho*), heterozygosity (*He*), effective allele numbers (*Ne*) and polymorphism information content (*PIC*) using PopGene version 1.3.1 (Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, AB, Canada). The SHEsis program (http://analysis.bio-x.cn) was used to calculate Hardy–Weinberg equilibrium (HWE; Wang, et al., 2020).

TABLE 1 The primers used for SSR analysis

Primer names	Primer sequences (5′–3′)	Sizes (bp)	Repeat motif	Location
$(ATC)_n$-P1	F: TTCTCCTTCCCCATAATGCTT R: GGTTGCAGATGCAGTTCAGA	190	$(ATC)_n$	Chr17:13600112-13600301 (*LOC100155289* gene)
$(AC)_n$-P2	F: AACTTCAGGTGCACAGCAAA R: ATAGATGGGTAACGGGGACC	139	$(AC)_n$	Chr13:145368763-145368901 (*ZBTB20* gene)
$(AC)_n$-P3	F: CTGATCCTTTCAAGATGAGTGAA R: GCATTTTGCAATCTGGGAGT	134	$(AC)_n$	Chr15:2003259-203396 (intergenic region)

A Student's *t* test (T-test) with a paired two-tailed distribution and ANOVA were used to analyse the associations between SSR variation and litter size.

3 | RESULTS

3.1 | SSR Identification

As shown in Table 2, TOF-MS identified two alleles (189- and 195-) and two genotypes (189-/189- and 189-/195-) at the $(ATC)_n$-P1 locus (Figure 1). The frequency of the 189-/195- (0.930) genotype was higher than that of 189-/189- (0.070). Three alleles (126-, 128- and 136-) and three genotypes (126-/136-, 128-/136- and 136-/136-) were found at the $(AC)_n$-P2 locus (Figure 2). In addition, the 136-/136- genotype showed the highest frequency (0.929). Similarly, there were various alleles (8 types) and genotypes (18 types) at the (AC)n-P3 locus (Figure 3).

3.2 | Genetic parameters

Next, the genotypes, allele frequencies and genetic parameters, including *Ho*, *He*, *Ne* and *PIC*, were calculated and analysed (Table 2). The values of *Ho*, *He*, *Ne* and *PIC* were 0.502, 0.498, 1.992 and 0.374 at the $(ATC)_n$-P1 locus; 0.932, 0.068, 1.073 and 0.067 at the $(AC)_n$-P2 locus; and 0.253, 0.747, 3.958 and 0.709 at the $(AC)_n$-P3

TABLE 2 Allelic frequency, genotypic frequency and genetic diversity of SSR in pigs

Loci	Sample size	Genotypes	Genotypic frequencies	Allele	Allelic frequencies	Population parameters				*P* (HWE) *p* value
						Ho	*He*	*Ne*	*PIC*	
$(ATC)_n$-P1	$n = 256$	189-/189-	0.070	189-	0.535	0.502	0.498	1.992	0.374	$p < .05$
		189-/195-	0.930	195-	0.465					
$(AC)_n$-P2	$n = 255$	126-/136-	0.055	126-	0.027	0.932	0.068	1.073	0.067	$p > .05$
		128-/136-	0.016	128-	0.008					
		136-/136-	0.929	136-	0.965					
$(AC)_n$-P3	$n = 242$	128-/128-	0.116	128-	0.353	0.253	0.747	3.958	0.709	$p < .05$
		128-/130-	0.198	130-	0.308					
		128-/134-	0.041	134-	0.081					
		128-/136-	0.161	136-	0.151					
		128-/140-	0.012	138-	0.002					
		128-/142-	0.041	140-	0.027					
		128-/146-	0.021	142-	0.041					
		130-/130-	0.112	146-	0.037					
		130-/134-	0.029	-	-					
		130-/136-	0.058	-	-					
		130-/138-	0.004	-	-					
		130-/140-	0.041	-	-					
		130-/142-	0.025	-	-					
		130-/146-	0.037	-	-					
		134-/134-	0.045	-	-					
		136-/136-	0.025	-	-					
		136-/142-	0.017	-	-					
		136-/146-	0.017	-	-					

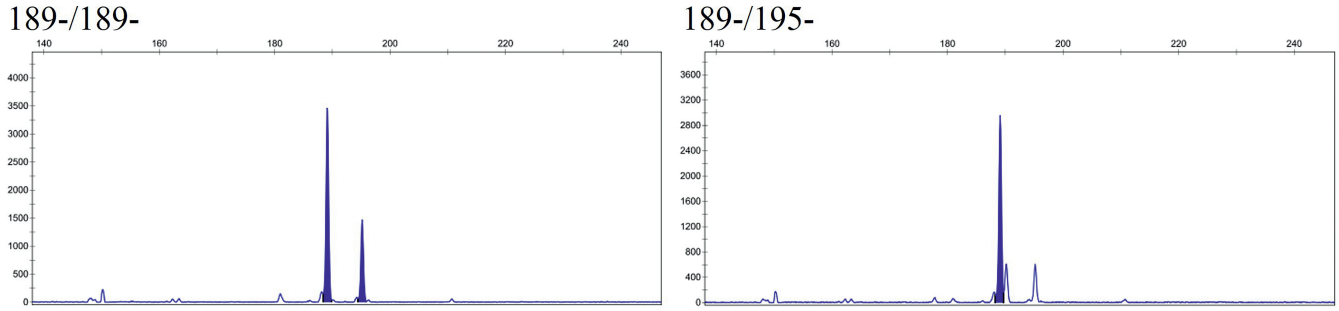

FIGURE 1 The TOF-MS of $(ATC)_n$-P1 locus in pigs

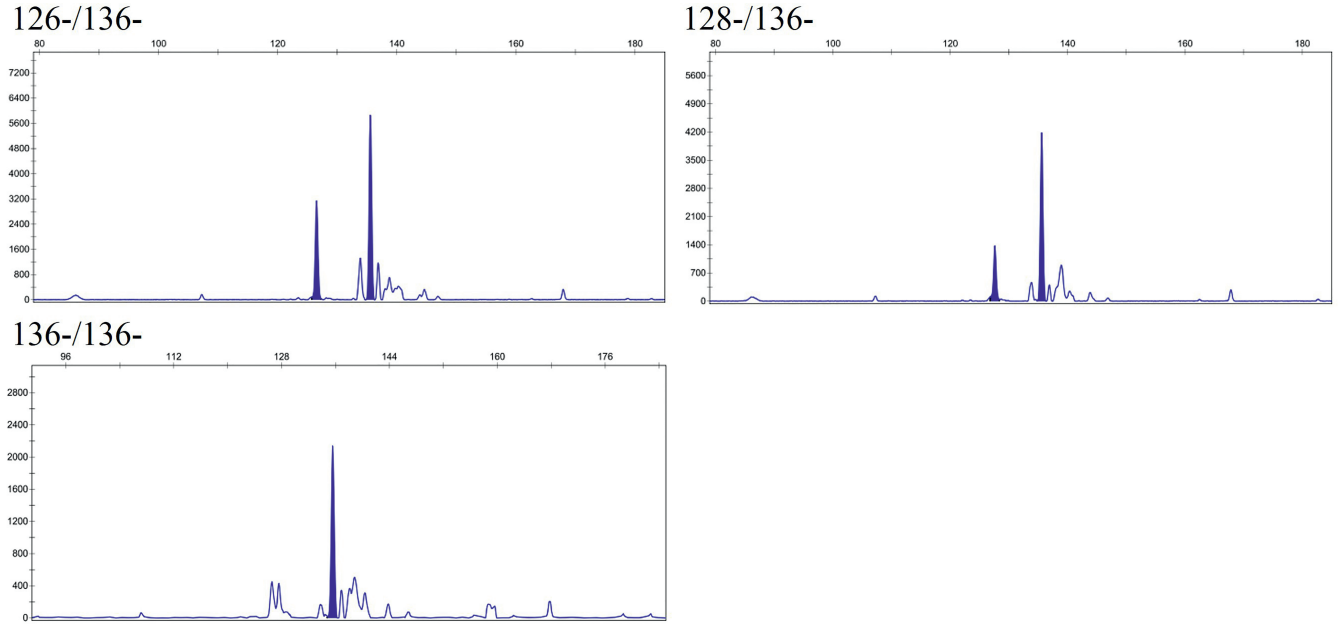

FIGURE 2 The TOF-MS of $(AC)_n$-P2 locus in pigs

locus, respectively. According to their respective *p* values, only the $(AC)_n$-P2 locus was at HWE.

3.3 | Association analysis of mutations

The T-test and ANOVA results indicated that among the three SSR loci, only $(ATC)_n$-P1 was associated with litter size (Table 3). In particular, 189-/195- individuals had larger litters (9.04 ± 0.21), followed by 189-/189- sows ($p = .047$). There were no significant differences in litter size at the other two loci, with *p* values of .328 and .220, respectively.

4 | DISCUSSION

As DNA markers, SSRs were highly emphasized two or three decades ago, but have received less attention in the last decade. The main reasons for this are their low accuracy of detection and the low degree of automation in the techniques used to identify them. However, it is worth noting that SSRs are widespread in the animal genome (Zhao et al., 2018). Moreover, compared to the more commonly used single nucleotide polymorphisms (SNPs) and insertions/deletions (indels), the mutation rates of SSRs are higher (Fotsing et al., 2019). In addition, some SSRs have important physiological functions in animals. Karim and his colleagues found that SSRs in PLAG1-CHCHD7 intergenic region can affect promoter activity and influence the binding of nuclear factor (Karim et al., 2011). Not only that, the SSRs of *IGF2R* and *LEP* genes in cattle may influence the milk traits, feed intake, milk fatty acid composition and metabolic state (Avondo et al., 2019; Dux et al., 2018). Therefore, they are important targets to inform breeding practices. Fortunately, the emergence of TOF-MS and other methods makes the screening of SSRs more accurate and convenient than what can be attained using the traditional methods (Beuzen et al., 2000).

In previous work, we identified some potential microsatellites in the genome of Qinghai Bamei pigs (data not shown). The $(ATC)_n$-P1 locus was located on Chr17: 13600112–13600301, which belonged to an uncharacterized *LOC100155289* gene (Table 1). Similarly, the $(AC)_n$-P2 locus was located in the zinc finger and BTB domain containing 20 (*ZBTB20*) gene belonging to chromosome 13. The $(AC)_n$-P3 locus was found between the uncharacterized *LOC106506168* gene

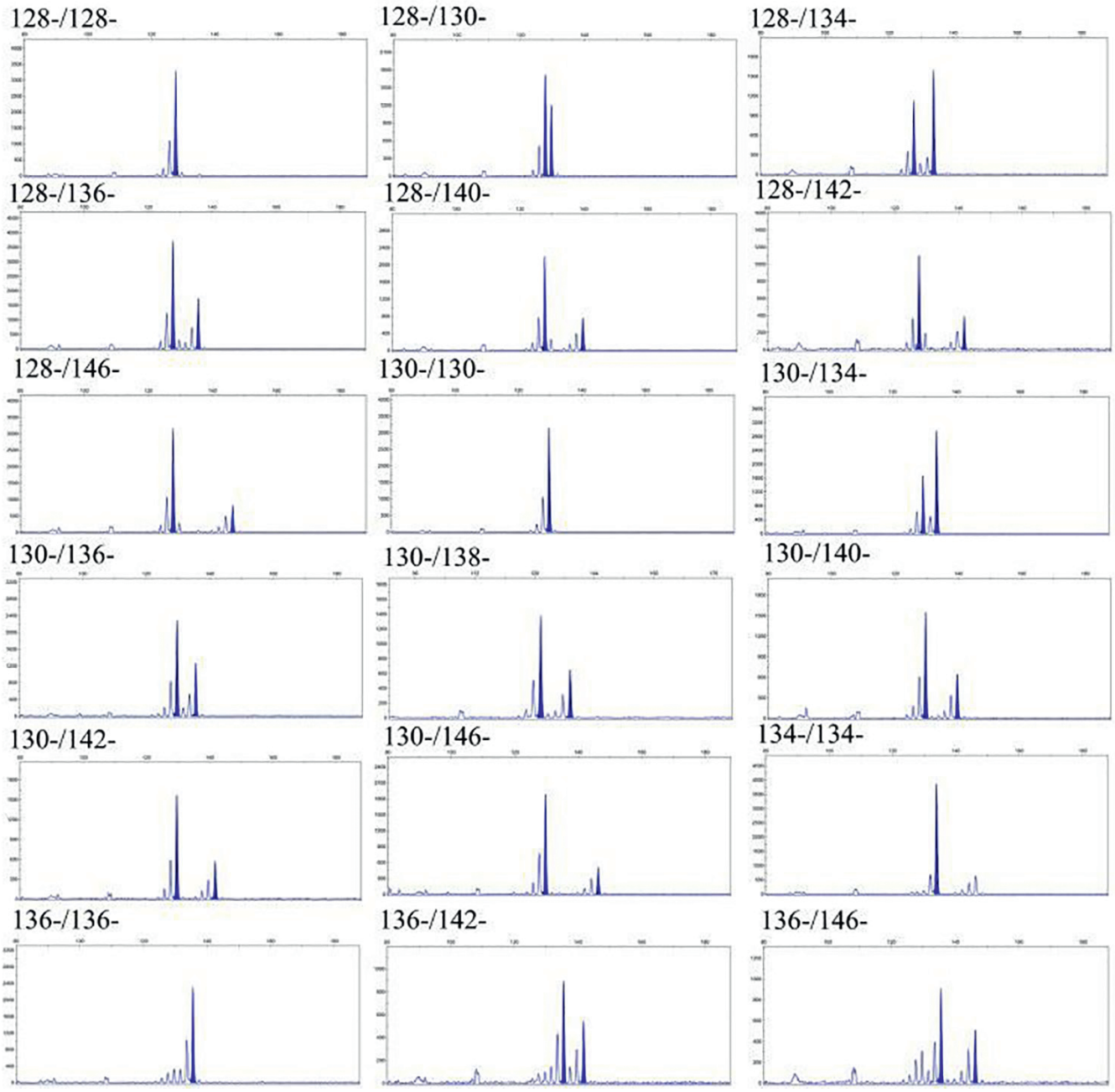

FIGURE 3 The TOF-MS of $(AC)_n$-P3 locus in pigs

and the uncharacterized *LOC102159607* gene in the intergenic region on chromosome 15.

After genotyping the three novel SSR loci using TOF-MS, we analysed the allelic frequency, genotypic frequency and genetic diversity of each locus following the method of Botstein et al. (1980). In genetic breeding, markers with *PIC* values greater than 0.5 are considered very informative and values between 0.25 and 0.5 are considered somewhat informative (Serrote et al., 2020). Our results indicated that the $(ATC)_n$-P1 and $(AC)_n$-P3 loci could be used as potential markers for selecting sows that would produce large litters (*PIC* values of 0.374 and 0.709, respectively).

Genetic parameter analyses indicated that these loci were not at HWE except for $(AC)_n$-P2 locus. The direct reason for this result was that there are more heterozygotes in the population of Qinghai Bamei sows (Graffelman et al., 2017). In addition, purification selection, inbreeding and population substructure in the breeding process were all potential causes for HWE-departure (Chen et al., 2017; Lee et al., 2008; Wang & Shete, 2012). These results were consistent with the genetic background of the Qinghai Bamei pigs.

Finally, association analyses indicated that only the $(ATC)_n$-P1 locus was significantly associated with litter size (p = .047). Individuals with the 189-/195- genotype had larger litter size compared to the other genotypes. Although the $(ATC)_n$-P1 locus is located on the intron of the *LOC100155289* gene, it may affect litter size in various ways. For instance, SSR mutations could directly regulate ontology gene expression (Meloni et al., 1998) or could

TABLE 3 The association analysis between different genotypes of different loci and the multiparity litter size in pigs

Loci	Genotypes	Mean ± *SEM* (number)	*p* values
$(ATC)_n$-P1	189-/195-	[a]9.04 ± 0.21 (*n* = 79)	*p* = .047
	189-/189-	[b]7.85 ± 0.43 (*n* = 11)	
$(AC)_n$-P2	126-/136-	8.00 ± 0.00 (*n* = 2)	*p* = .328
	128-/136-	10.84 ± 0.84 (*n* = 2)	
	136-/136-	8.98 ± 0.21 (*n* = 88)	
$(AC)_n$-P3	128-/128-	8.00 ± 0.50 (*n* = 9)	*p* = .220
	128-/130-	8.47 ± 0.35 (*n* = 25)	
	128-/136-	9.40 ± 0.56 (*n* = 15)	
	130-/130-	9.20 ± 0.43 (*n* = 15)	
	130-/136-	9.43 ± 1.09 (*n* = 7)	
	130-/140-	10.31 ± 1.07 (*n* = 4)	
	130-/146-	10.22 ± 1.13 (*n* = 3)	

Note: Values with different letters (a, b) differ significantly at $p < .05$.

co-regulate gene expression with SSRs in the 5′-UTR region (Akai et al., 1999). In addition, the locus could increase abnormal splicing of gene (Sirand-Pugnet et al., 1995) and even lead to gene silencing (Saveliev et al., 2003). However, the specific mechanism of $(ATC)_n$-P1 affecting litter size of Qinghai Bamei sows needs to be studied further. In addition, the *LOC100155289* gene is uncharacterized. According to our experimental results, the *LOC100155289* gene and the $(ATC)_n$-P1 locus have the potential to be important candidates for MAS to improve the litter size of Qinghai Bamei pigs.

In the future, further experiments will be carried out to explore how mutations in the introns of *LOC100155289* affect the litter size of Qinghai Bamei sows.

5 | CONCLUSIONS

Three SSRs of Qinghai Bamei sows were identified through TOF-MS. Among them, the $(ATC)_n$-P1 locus was significantly associated with the litter size of Qinghai Bamei sows. Meanwhile, 189-/195- was the dominant genotype. These findings provide a scientific basis and theoretical guidance for the subsequent improvement of litter size in Qinghai Bamei pigs.

ACKNOWLEDGEMENTS

We greatly thank the staffs of Qinghai Bamei Pig Original Breeding Farm, Qinghai Huzhu Bamei Pig Breeding Farm and Huangzhong County Breeding Farm, Qinghai province, P.R. China for collecting samples.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Guofang Wu: Conceptualization; Formal analysis; Funding acquisition; Project administration; Resources; Visualization; Writing-original draft; Writing-review & editing. **Wenjuan Shen:** Conceptualization; Investigation; Software; Visualization; Writing-original draft; Writing-review & editing. **Xingxing Xue:** Investigation; Methodology; Software. **Lei Wang:** Conceptualization; Formal analysis; Funding acquisition; Investigation; Project administration; Resources; Supervision; Validation; Writing-review & editing. **Yuhong Ma:** Investigation; Methodology; Resources. **Jiping Zhou:** Methodology; Resources.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.371.

ORCID

Guofang Wu https://orcid.org/0000-0002-5397-8390

REFERENCES

Ahlstrom, C., Barkema, H. W., & De Buck, J. (2014). Improved short-sequence-repeat genotyping of *Mycobacterium avium* subsp. *paratuberculosis* by using matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Applied and Environmental Microbiology*, *80*, 534–539.

Akai, J., Kimura, A., & Hata, R. I. (1999). Transcriptional regulation of the human type I collagen alpha2 (COL1A2) gene by the combination of two dinucleotide repeats. *Gene*, *239*, 65–73.

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, *25*, 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Avondo, M., Di Trana, A., Valenti, B., Criscione, A., Bordonaro, S., De Angelis, A., Giorgio, D., & Di Gregorio, P. (2019). Leptin gene polymorphism in goats fed with diet at different energy level: Effects on feed intake, milk traits, milk fatty acids composition, and metabolic state. *Animals (Basel)*, *9*, E424.

Bernard, D. J., & Tran, S. (2013). Mechanisms of activin-stimulated FSH synthesis: The story of a pig and a FOX. *Biology of Reproduction*, *88*, 78.

Beuzen, N. D., Stear, M. J., & Chang, K. C. (2000). Molecular markers and their use in animal breeding. *Veterinary Journal*, *160*, 42–52. https://doi.org/10.1053/tvjl.2000.0468

Bonk, T., Humeny, A., Gebert, J., Sutter, C., von Knebel Doeberitz, M., & Becker, C. M. (2003). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based detection of microsatellite instabilities in coding DNA sequences: A novel approach to identify DNA-mismatch repair-deficient cancer cells. *Clinical Chemistry*, *49*, 552–561. https://doi.org/10.1373/49.4.552

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, *32*, 314–331.

Breza, M., & Koutsis, G. (2019). Kennedy's disease (spinal and bulbar muscular atrophy): A clinically oriented review of a rare disease. *Journal of Neurology*, *266*(3), 565–573. https://doi.org/10.1007/s00415-018-8968-7

Chen, B., Cole, J. W., & Grond-Ginsbach, C. (2017). Departure from Hardy Weinberg equilibrium and genotyping error. *Frontiers in Genetics*, *8*, 167. https://doi.org/10.3389/fgene.2017.00167

Chen, X., Sun, H., Tian, S., Xiang, H., Zhou, L., Dun, W., & Zhao, X. (2015). Increasing litter size in a sheep breed by marker-assisted selection of BMPR1B A746G mutation. *Journal of Genetics*, *94*, 139–142. https://doi.org/10.1007/s12041-015-0470-8

China National Commission of Animal Genetic Resources. Animal genetic resources in China: Pigs. 2011. China Agriculture Press.

Dux, M., Muranowicz, M., Siadkowska, E., Robakowska-Hyzorek, D., Flisikowski, K., Bagnicka, E., & Zwierzchowski, L. (2018). Association of SNP and STR polymorphisms of insulin-like growth factor 2 receptor (IGF2R) gene with milk traits in Holstein-Friesian cows. *Journal of Dairy Research*, *85*, 138–141.

Fotsing, S. F., Margoliash, J., Wang, C., Saini, S., Yanicky, R., Shleizer-Burko, S., Goren, A., & Gymrek, M. (2019). The impact of short tandem repeat variation on gene expression. *Nature Genetics*, *51*, 1652–1659. https://doi.org/10.1038/s41588-019-0521-9

Fujimoto, A., Fujita, M., Hasegawa, T., Wong, J. H., Maejima, K., Oku-Sasaki, A., Nakano, K., Shiraishi, Y., Miyano, S., Yamamoto, G. O., Akagi, K., Imoto, S., & Nakagawa, H. (2020). Comprehensive analysis of indels in whole-genome microsatellite regions and microsatellite instability across 21 cancer types. *Genome Research*, *30*(3), 334–346. https://doi.org/10.1101/gr.255026.119

Graffelman, J., Jain, D., & Weir, B. (2017). A genome-wide study of Hardy-Weinberg equilibrium with next generation sequence data. *Human Genetics*, *136*, 727–741. https://doi.org/10.1007/s00439-017-1786-7

Hui, Y., Zhang, Y., Wang, K., Pan, C., Chen, H., Qu, L., Song, X., & Lan, X. (2020). Goat DNMT3B: An indel mutation detection, association analysis with litter size and mRNA expression in gonads. *Theriogenology*, *147*, 108–115. https://doi.org/10.1016/j.theriogenology.2020.02.025

Karim, L., Takeda, H., Lin, L. I., Druet, T., Arias, J. A. C., Baurain, D., Cambisano, N., Davis, S. R., Farnir, F., Grisart, B., Harris, B. L., Keehan, M. D., Littlejohn, M. D., Spelman, R. J., Georges, M., & Coppieters, W. (2011). Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. *Nature Genetics*, *43*, 405–413. https://doi.org/10.1038/ng.814

Korwin-Kossakowska, A., Wyszynska-Koko, J., Sender, G., Gajewska, A., Pierzchala, M., Kamyczek, M., & Kochman, K. (2009). Effect of the polymorphism in 5′ UTR region of pig prolactin gene on prolactin gene expression and reproduction performance in the female pig. *Neuro Endocrinology Letters*, *30*(2), 221–226.

Lee, S., Kasif, S., Weng, Z., & Cantor, C. R. (2008). Quantitative analysis of single nucleotide polymorphisms within copy number variation. *PLoS One*, *3*, e3906. https://doi.org/10.1371/journal.pone.0003906

Liu, C., Liu, Y., Zhang, X., Xu, X., & Zhao, S. (2017). Characterization of porcine simple sequence repeat variation on a population scale with genome resequencing data. *Scientific Reports*, *7*, 2376. https://doi.org/10.1038/s41598-017-02600-8

Meloni, R., Albanese, V., Ravassard, P., Treilhou, F., & Mallet, J. (1998). A tetranucleotide polymorphic microsatellite, located in the first intron of the tyrosine hydroxylase gene, acts as a transcription regulatory element in vitro. *Human Molecular Genetics*, *7*, 423–428. https://doi.org/10.1093/hmg/7.3.423

Nei, M., & Roychoudhury, A. K. (1974). Sampling variances of heterozygosity and genetic distance. *Genetics*, *76*, 379–390.

Pradhan, M., Pal, A., Samanta, A. K., Banerjee, S., & Samanta, R. (2018). Mutations in cytochrome B gene effects female reproduction of Ghungroo pig. *Theriogenology*, *119*, 121–130. https://doi.org/10.1016/j.theriogenology.2018.05.015

Qi, M. Y., Xu, L. Q., Zhang, J. N., Li, M. O., Lu, M. H., & Yao, Y. C. (2020). Effect of the Booroola fecundity (*FecB*) gene on the reproductive performance of ewes under assisted reproduction. *Theriogenology*, *142*, 246–250. https://doi.org/10.1016/j.theriogenology.2019.10.038

Rau, J., Eisenberg, T., Peters, M., Berger, A., Kutzer, P., Lassnig, H., Hotzel, H., Sing, A., Sting, R., & Contzen, M. (2019). Reliable differentiation of a non-toxigenic tox gene-bearing *Corynebacterium ulcerans* variant frequently isolated from game animals using MALDI-TOF MS. *Veterinary Microbiology*, *237*, 108399. https://doi.org/10.1016/j.vetmic.2019.108399

Saveliev, A., Everett, C., Sharpe, T., Webster, Z., & Festenstein, R. (2003). DNA triplet repeats mediate heterochromatin-protein-1-sensitive variegated gene silencing. *Nature*, *422*, 909–913. https://doi.org/10.1038/nature01596

Seichter, D., Krebs, S., & Forster, M. (2004). Rapid and accurate characterisation of short tandem repeats by MALDI-TOF analysis of endonuclease cleaved RNA transcripts. *Nucleic Acids Research*, *32*, e16. https://doi.org/10.1093/nar/gnh017

Serrote, C. M. L., Reiniger, L. R. S., Silva, K. B., Rabaiolli, S., & Stefanel, C. M. (2020). Determining the Polymorphism Information Content of a molecular marker. *Gene*, *726*, 144175. https://doi.org/10.1016/j.gene.2019.144175

Sirand-Pugnet, P., Durosay, P., Brody, E., & Marie, J. (1995). An intronic (A/U)GGG repeat enhances the splicing of an alternative intron of the chicken beta-tropomyosin pre-mRNA. *Nucleic Acids Research*, *23*, 3501–3507.

Smołucha, G., Kozubska-Sobocińska, A., Koseniuk, A., Żukowski, K., Lisowski, M., & Grajewski, B.(2019). Polymorphism of the myostatin (*MSTN*) gene in Landes and Kielecka geese breeds. *Animals (Basel)*, *10*, E10.

Vieira, M. L., Santini, L., Diniz, A. L., & Munhoz, C. (2016). Microsatellite markers: What they mean and why they are so useful. *Genetics and Molecular Biology*, *39*(3), 312–328. https://doi.org/10.1590/1678-4685-GMB-2016-0027

Vogel, N., Schiebel, K., & Humeny, A. (2009). Technologies in the whole-genome age: MALDI-TOF-based genotyping. *Transfusion Medicine and Hemotherapy*, *36*, 253–262. https://doi.org/10.1159/000225089

Wang, J., & Shete, S. (2012). Testing departure from Hardy-Weinberg proportions. *Methods in Molecular Biology*, *850*, 77–102.

Wang, K., Kang, Z., Jiang, E., Yan, H., Zhu, H., Liu, J., Qu, L., Lan, X., & Pan, C. (2020). Genetic effects of *DSCAML1* identified in genome-wide association study revealing strong associations with litter size and semen quality in goat (*Capra hircus*). *Theriogenology*, *146*, 20–25. https://doi.org/10.1016/j.theriogenology.2020.01.079

Wang, Z., Zhang, X., Jiang, E., Yan, H., Zhu, H., Chen, H., Liu, J., Qu, L., Pan, C., & Lan, X. (2020). InDels within caprine IGF2BP1 intron 2 and the 3′-untranslated regions are associated with goat growth traits. *Animal Genetics*, *51*, 117–121.

Wu, G. F., Wang, L., Zhou, J. P., Ma, Y. H., Han, Q. C., Xu, F. F., Mo, H. S., Ma, C. M., Zhan, S. J., Ma, L. M., Ye, Z. S., Li, Y. L., & Hu, C. Y. (2019). Relationships between different backfat thickness and reproductive performance of Qinghai Bamei sow. *Journal of Qinghai University*, *37*, 47–50.[Article in Chinese].

Zhang, J., Yang, B., Wen, X., & Sun, G. (2018). Genetic variation and relationships in the mitochondrial DNA D-loop region of Qinghai indigenous and commercial pig breeds. *Cellular and Molecular Biology Letters*, *23*, 31. https://doi.org/10.1186/s11658-018-0097-x

Zhang, S., Yang, J., Wang, L., Li, Z., Pang, P., & Li, F. (2019). SLA-11 mutations are associated with litter size traits in Large White and Chinese DIV pigs. *Animal Biotechnology*, *30*(3), 212–218.

Zhang, X., Cui, W., Wang, K. E., Chen, R., Chen, M., Lan, K., Wei, Y., Pan, C., & Lan, X. (2020). Chlorpyrifos inhibits sperm maturation and induces a decrease in mouse male fertility. *Environmental Research*, *188*, 109785. https://doi.org/10.1016/j.envres.2020.109785

Zhao, J., Li, T., Zhu, C., Jiang, X., Zhao, Y., Xu, Z., Yang, S., & Chen, A. (2018). Selection and use of microsatellite markers for individual identification and meat traceability of six swine breeds in the Chinese market. *Food Science and Technology International*, *24*, 292–300. https://doi.org/10.1177/1082013217748457

How to cite this article: Wu G, Shen W, Xue X, Wang L, Ma Y, Zhou J. A novel $(ATC)_n$ microsatellite locus is associated with litter size in an indigenous Chinese pig. *Vet Med Sci.* 2021;7:1332–1338. https://doi.org/10.1002/vms3.371