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Identification of a novel 24 bp insertion–deletion (indel) of the androgen receptor gene and its association with growth traits in four indigenous cattle breeds

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Abstract. During the past decades, insertions and deletions (indels) have become increasingly popular in animal breeding for understanding the relationship between genotypes and phenotypes. The androgen receptor (AR) plays the vital role of a bridge on the function of the androgen and has sexual size dimorphism. For this reason, the objective of this study was to explore the novel indel variants within the cattle AR gene and to detect their effects on growth traits in four breeds of Chinese yellow cattle. Herein, we first confirmed a novel 24 bp indel (AC_000187.1g.4187270-4187293delAATTTATTGGGAGATTATTGAATT) within the intron of the cattle AR gene. This is consistent with the results predicted from the NCBI SNP database. The distribution of the indel genotypes of four Chinese yellow cattle were significantly different from each other ($P < 0.01$). After significant correlation analysis, many remarkable phenotypic differences among the three genotypes were found ($P < 0.05$). In conclusion, a novel 24 bp indel within the AR gene significantly affected growth traits, suggesting that this indel may be a useful DNA marker for the elimination or selection of excellent individuals for cattle breeding.

1 Introduction

Chinese yellow cattle are transitioning from their previous role as draft cows to beef cattle (Li et al., 2014; Liang et al., 2016). The muscling of their forequarters, which is the key characteristic of draft cows, has been disappearing, and they have taken on many traits characteristic of beef cattle. The important breeds, Qinchuan (QC) cattle, Nanyang (NY) cattle, Luxi (LC) cattle, and Jiaxian red (JX) cattle, have developed a few differences in genotype and phenotype (Liu et al., 2016; Han et al., 2017). It is worth mentioning that QC cattle have developed into a new beef line of QC cattle after nearly 30 years of systematic breeding (Liang et al., 2014; Liu et al., 2015).

Animal growth and development is a complicated process, and the molecular mechanisms of traditional breeding are poorly understood and merit further study. During the past few decades, insertion and deletions (indels) have become increasingly popular in the study of animal breeding for understanding the relationship between genotypes and phenotypes. They are an important form of marker-assisted selection (MAS; Xu et al., 2016; Yang et al., 2016; Zhou et al., 2016).

Androgens are key factors in differences in the growth traits of cows, bulls, and bullocks. They perform a great number of functions by combining with the androgen receptor (AR; O'Reilly et al., 2014). As a member of the nuclear receptor superfamily, the AR could regulate downstream target genes and thereby alter various cell functions. The AR plays the vital role of a bridge for the function of androgens (Myung et al., 2017; Ryan et al., 2017). The AR is a transcription factor that is activated upon binding to testosterone (T), and it is implicated in regulation of the expression of development-related and reproduction-related genes (Grigorova et al., 2017). Some studies have indicated that the AR mediated the expression of androgen-associated somatic traits, such as muscle mass and strength (Ryan et al., 2017).

The objective of this study was to explore the novel indel variants within the cattle AR gene and to evaluate their effects on growth traits in four breeds of Chinese yellow cattle,

Table 1. PCR primer sequences of the cattle AR gene.

	Primer sequences (5′–3′)	T_m (°C)	Product size (bp)	Notes
AR-P1	F: GCGTACTCAATAAAGCAGAA R: CAATCCCAAAGAAAGCAAT	TD-PCR	252	Pool DNA sequencing
AR-P2	F: GGCTGCACTCACCCTTG R: TGCCAGGAGAAATATCAATAAC	TD-PCR	249	Pool DNA sequencing
AR-P3	F: CACATCCTAGTTCCCAGTTT R: CTCAGCACAGGGCTTGCA	TD-PCR	320	Pool DNA sequencing
AR-P4	F: TGACTACAAAGGCTCACTG R: TTCCTGTTCTTGCCACCA	TD-PCR	262	Pool DNA sequencing, indel classification
AR-P5	F: TGGATGTGGCTGAGATGGG R: GGGCGGAAGGTCAGAAAC	TD-PCR	203	Pool DNA sequencing
AR-P6	F: CAGGAGACAGGCAAGGTG R: CCAAGTGAGGCTTCAACAG	TD-PCR	247	Pool DNA sequencing
AR-P7	F: ATCATGTCATCAGATCCCTAT R: CGGACAAAACTGAGCAAC	TD-PCR	243	Pool DNA sequencing

TD-PCR: touchdown polymerase chain reaction; AR: androgen receptor F: forward primer; R: reverse primer; T_m: melting temperature; P1–P7: first pair–seventh pair.

which would not only extend the spectrum of genetic variations in the cattle AR gene but also contribute to implementing MAS in genetics and breeding in Chinese yellow cattle.

2 Material and methods

All experiments performed in this study were approved by the International Animal Care and Use Committee of the Northwest A&F University (IACUC-NWAFU). Furthermore, the care and use of animals was fully compliant with local animal welfare laws, guidelines, and policies.

2.1 DNA samples and data collection

A total of 614 Chinese yellow cattle (2–5 years old) from four breeds were measured: Luxi cattle (LX, $n = 113$, Shandong Province), Qinchuan cattle (QC, $n = 227$, Shaanxi Province), Nanyang cattle (NY, $n = 134$, Henan Province), and Jiaxian red cattle (JX, $n = 140$, Henan Province). Growth traits for all healthy and unrelated individuals, specifically body weight (BW), body height (BH), body length (BL), chest circumference (ChC), chest depth (ChD), chest width (ChW), hucklebone width (HuW), hip width (HW), and cannon circumference (CaC), were measured by the same person using the same standards; consequently, body length index (BLI), chest circumference index (ChCI), chest width index (ChWI), cannon circumference index (CaCI), hucklebone width index (HuWI), and trunk index (TI) were also calculated on the basis of related reported descriptions (Lan et al., 2007; Jin et al., 2016).

2.2 DNA isolation and genomic DNA pool construction

DNA samples were extracted from the leukocytes of the blood by using the phenol–chloroform method (S. H. Zhang et al., 2015; X. Y. Zhang et al., 2015). The quality of DNA samples was assayed by using a NanoDrop 1000 (Thermo Scientific, Waltham, MA, US), and all the data of $OD_{268/280}$ remained within a range of 1.8 to 2.0. Every DNA sample was homogenized, and its concentration was diluted to 50 ng μL^{-1}. Considering workloads, every 25 samples were used to construct a genomic DNA pool for polymerase chain reaction (PCR) and sequencing to find a potential indel locus in the cattle AR gene (Chen et al., 2016).

2.3 Primer design and PCR amplification

Based on the SNP database from NCBI (https://www.ncbi.nlm.nih.gov/snp), the loci of seven possible indels were found on the cattle AR intron. The seven pairs of primers were designed by Primer Premier software 5.0 (Premier Biosoft International, USA) based on the cattle AR gene sequence (GenBank NC_ 019460.1; Table 1). The PCR amplification system adopts a 20 µL volume system: 10 µL 2 × PCR mix, 0.5 µM of forward and reverse primers, 50 ng genomic DNA, and 8 µL ddH_2O. The protocol for PCR was touchdown PCR, with steps listed as follows: denatured at 95 °C for 5 min, followed by 18 cycles of 95 °C for 30 s, 68 °C declining to 50 °C, decreasing one degree per cycle for 30 s and 72 °C for 20 s, transition from 68 °C to 53 °C for 30 s over the course of 20 cycles, followed by final extension at 72 °C for 10 min. The products were detected by electrophoresis of 2.5 % agarose gel stained with GelRed (Solar-

bio Life Science, China), and the products were sequenced only when a given pair of primers had different genotypes (S. H. Zhang et al., 2015; Yang et al., 2016).

2.4 Statistical analyses

The sequence was contrasted and analyzed with BioEdit Software (UK) using the website (http://www.msrcall.com/) to calculate and analyze the genetic data for Hardy–Weinberg equilibrium (HWE), homozygosity (Ho), heterozygosity (He), effective allele numbers (Ne), and polymorphism information content (PIC; Chen et al., 2016). The chi-square test between varieties and ANOVA in varieties was conducted using SPSS software (version 18.0; International Business Machines, US) The results were subjected to statistical testing ($P < 0.05$; Pan et al., 2013; Zhang et al., 2016).

3 Results

Through the pooled DNA sequencing and indel classification, only one polymorphism was found in the cattle AR gene (Table 1). After 50 min of agarose gel (2 %) electrophoresis, three genotypes showed different bands (Fig. 1). The II (insertion–insertion) type showed one band (262 bp), the DD (deletion–deletion) type displayed one band (238 bp), and the ID (insertion–deletion) type showed two bands (262 bp, 238 bp). Based on the sequencing results (Fig. 2), genotype II showed two replica and the DD genotypes only had one replica; the del portion is AATTTATTGGGAGATTATTGAATT, the same as the information in the NCBI database (AC_000187.1g.4187270:4187293).

As shown in Table 2, the genotype frequencies, allelic frequencies, and population parameters of four cattle breeds (LX, QC, NY, and JX) were calculated. For the current locus, only JX showed Hardy–Weinberg equilibrium (HWE; $P > 0.05$). The effective allele numbers (Ne) of LX, JX, and NY were greater than 1.900. The NY cattle were highly polymorphic, and the other breeds had intermediate polymorphism information contents. The four breeds had a different superior genotype; ID was the greater genotype in NY and JX cattle, while DD was the dominant genotype in QC and LX cattle. With the exception of the allelic frequency between LX and NY, the frequency and allelic frequency distribution of all genotypes differed significantly among all four breeds on the χ^2 test ($P < 0.01$; Table 3).

The associations between the 24 bp indel and the cattle growth traits were investigated. Significant differences were found in the body height, chest circumference, chest depth, waist height, hip cross height, body length, body weight, and croup height index of female QC cattle among different genotypes ($P < 0.05$). Significant differences were observed in body height, body length, chest circumference, hip width, body weight, and croup height index of female LX cattle across three genotypes ($P < 0.05$). Significant differences were also found in body height, chest circumference, hip width, hip cross height, and body weight of male LX cattle among three genotypes ($P < 0.05$). Additionally, there is significant difference in the hip width ($P = 0.04$) of female NY cattle among different genotypes. Moreover, hip height ($P = 0.05$) and abdominal circumference ($P = 0.05$) differed significantly among three genotypes in female JX red cattle (Table 4). No significant relationship was observed among growth traits.

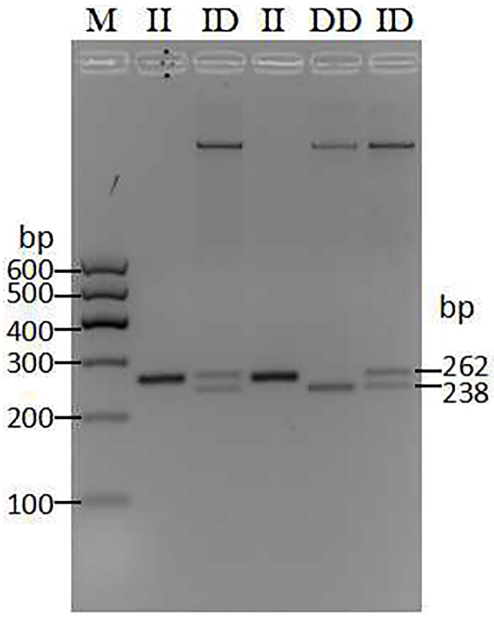

Figure 1. The agarose gel electrophoresis patterns of the 24 bp indel within the cattle AR gene. PCR products showed two genotypes at this locus where the insertion–insertion type (II genotype) consisted of 262 bp, deletion–deletion types (DD genotype) consisted of 238 bp, and the heterozygote type (ID genotype) showed 262 and 238 bp, which were detected by 2.5 % agarose gel electrophoresis. M: maker I (600, 500, 400, 300, 200, and 100 bp).

4 Discussion

The process of animal growth and development is regulated by an extremely complicated network, which contains numerous signaling pathways and genes, including the AR. Recently, many studies have confirmed that the AR is a key hub in the process of animal growth and development, not just in males but also in females (Wu et al., 2017). It is a master regulator that plays a vital role in the regulation of growth and the protection of endangered breeds. Current studies pay more attention to the function of lipometabolism and cancer, placing almost no focus on animal growth and development (Cameron et al., 2016; Ponce-González et al., 2016; Hu et al., 2017; Recouvreux et al., 2017). Herein, we first confirmed the existence of a novel 24 bp indel (AC_000187.1g.4187270-4187293del AATTTATTGGGAGATTATTGAATT) within the intron of

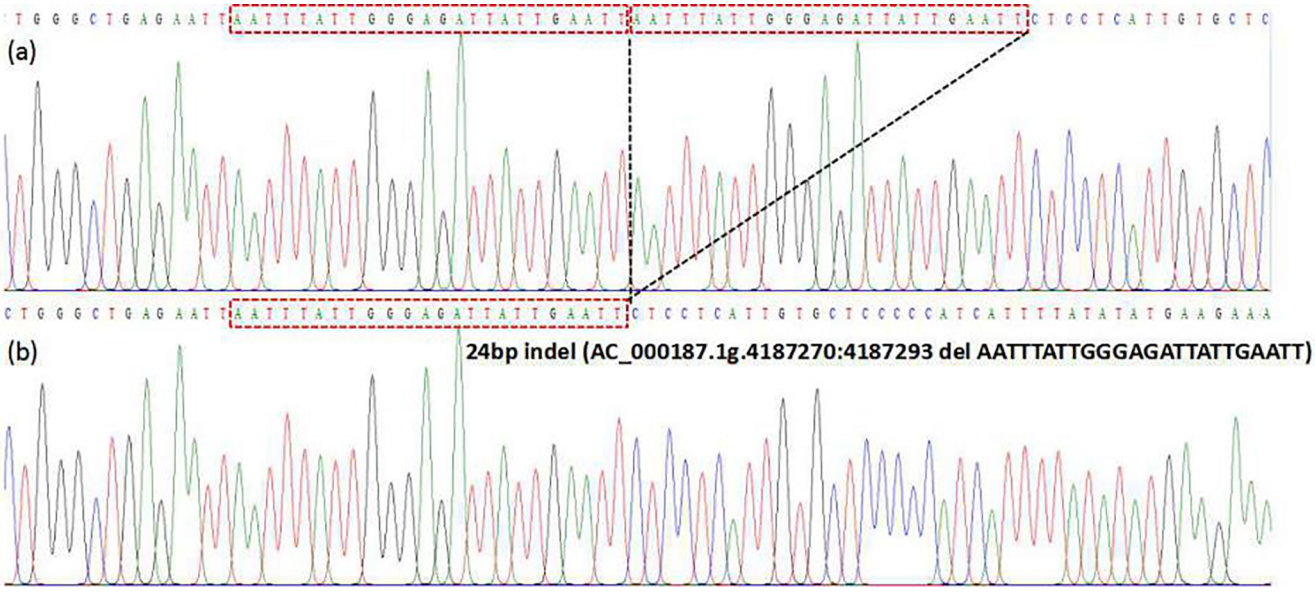

Figure 2. Sequencing maps for the 24 bp indel in the cattle AR gene. Panel **(a)**: homozygotic insertion type (II); panel **(b)**: homozygotic deletion type (DD). The sequence with the red border is the 24 bp deletion.

Table 2. Genotypes, alleles, He, Ne, and PIC for the novel indel of the cattle AR gene.

Breeds	Sizes	Genotypic frequencies			Allelic frequencies		HWE	Population parameters			
	N	II	ID	DD	I	D	*P* values	Ho	He	Ne	PIC
LX	113	29	40	44	0.434	0.564	$P<0.05$	0.509	0.491	1.965	0.371
QC	227	133	68	26	0.736	0.264	$P<0.05$	0.611	0.388	1.636	0.313
NY	134	13	79	42	0.489	0.511	$P<0.05$	0.523	0.477	1.911	0.670
JX	140	49	71	20	0.604	0.396	$P>0.05$	0.521	0.479	1.918	0.364

N: number; HWE: Hardy–Weinberg equilibrium; Ho: homozygosity; He: heterozygosity; Ne: effective allele numbers; PIC: polymorphism information content; LX: Luxi cattle; QC: Qinchuan cattle; NY: Nanyang cattle: JX: Jiaxian red cattle; II: insertion–insertion; ID: insertion–deletion; DD: deletion–deletion.

cattle AR genes in four breeds of indigenous cattle. This is consistent with that predicted from the NCBI SNP database. Androgens target many organs, including those of the female reproductive pathways (ovaries), male reproductive pathways, bone, the cardiovascular system, the immune system, skin, the kidney, the lungs, fat tissue, and the brain (Usui et al., 2014). Androgens combine with androgen receptors, causing animals to accumulate fat and indirectly regulating growth (O'Reilly et al., 2014). Most importantly, androgens control sexual dimorphism (Pollock et al., 2017).

As shown in the analysis of population genetics data, the number of effective alleles was close to 2 in the LC, NY, and JX cattle. The results showed that this locus had a uniform distribution in the three cattle breeds. According to the standards of polymorphism information content, these four Chinese yellow cattle breeds had intermediate polymorphism information. That is to say, the genetic variation in this site is abundant, which could have a pronounced effect on selective breeding (Li et al., 2016; Ahmad et al., 2017). The genotypic distribution of QC cattle, LX cattle, and JX cattle was not in Hardy–Weinberg equilibrium (HWE) ($P>0.05$). This was because these cattle live in a relatively stable environment and had reached a dynamic balance after long-term atresia breeding. However, the other three breeds of cattle were not at HWE ($P>0.05$). This could be because of excessive human intervention in the management of livestock farms. The indel genotype distributions of four Chinese yellow cattle breeds were significantly different from each other ($P<0.01$), which illustrated the transformation of Chinese cattle from draft cows to beef cattle (François et al., 2017; Mouresan et al., 2017).

After significant correlation analysis, many remarkable phenotypic differences were found. The locus was located in introns, and there is no denying that this could affect animal growth in some way. First, the distance from 5′UTR to exon 1 was more than 100 000 bp, covering a functional area of an unknown gene according to the powerful effect on growth traits (Smith et al., 1977; Lèbre and Gascuel, 2017;

Table 3. The χ^2 test of different breeds on a novel indel of the cattle AR gene.

Types	Breeds	LX	QC	NY	JX
Genotypic frequencies	LX	–	$\chi^2 = 45.55$	$\chi^2 = 17.26$	$\chi^2 = 20.13$
	QC	$p < 0.01$	–	$\chi^2 = 84.89$	$\chi^2 = 20.12$
	NY	$p < 0.01$	$p < 0.01$	–	$\chi^2 = 29.02$
	JX	$p < 0.01$	$p < 0.01$	$p < 0.01$	–
Allelic frequencies	LX	–	$\chi^2 = 29.71$	$\chi^2 = 0.75$	$\chi^2 = 14.49$
	QC	$p < 0.01$	–	$\chi^2 = 22.43$	$\chi^2 = 7.00$
	NY	$p > 0.05$	$p < 0.01$	–	$\chi^2 = 24.57$
	JX	$p < 0.01$	$p < 0.01$	$p < 0.01$	–

AR: androgen receptor; LX: Luxi cattle; QC: Qinchuan cattle; NY: Nanyang cattle: JX: Jiaxian cattle.

Table 4. Relationship between the novel 24 bp indel of the cattle AR gene and growth traits in four cattle breeds.

Breeds	Sex	Growth traits	Observed genotypes (LSM$^a \pm$ SE)			
			II	ID	DD	*P* values
QC	Female	Body height	$126.71 \pm 0.65^b (n = 83)$	$127.37 \pm 0.71^b (n = 46)$	$135.55 \pm 1.17^a (n = 19)$	0.00
		Chest circumference	$187.65 \pm 1.60^{ab} (n = 79)$	$178.92 \pm 5.00^b (n = 45)$	$197.53 \pm 3.33^a (n = 19)$	0.01
		Chest depth	$64.33 \pm 0.71^b (n = 80)$	$63.39 \pm 1.47^b (n = 46)$	$69.66 \pm 1.09^a (n = 19)$	0.01
		Waist height	$122.14 \pm 0.57^b (n = 83)$	$123.61 \pm 0.71^b (n = 46)$	$129.84 \pm 1.16^a (n = 19)$	0.00
		Hip cross height	$130.43 \pm 0.55^b (n = 83)$	$130.90 \pm 0.76^b (n = 46)$	$136.30 \pm 1.23^a (n = 19)$	0.00
		Body length	$148.10 \pm 1.06^b (n = 81)$	$149.08 \pm 1.61^b (n = 45)$	$156.27 \pm 2.03^a (n = 19)$	0.01
		Body weight	$489.60 \pm 10.50^b (n = 79)$	$459.25 \pm 19.98^b (n = 45)$	$570.17 \pm 25.51^a (n = 19)$	0.00
		Croup height index	$103.02 \pm 0.35^a (n = 83)$	$102.82 \pm 0.49^a (n = 46)$	$100.60 \pm 0.49^b (n = 19)$	0.01
LX	Female	Body height	$130.00 \pm 1.29^c (n = 22)$	$133.34 \pm 0.79^b (n = 32)$	$136.64 \pm 1.27^a (n = 21)$	0.00
		Body length	$144.14 \pm 1.48^b (n = 22)$	$147.50 \pm 1.33^{ab} (n = 32)$	$150.90 \pm 1.60^a (n = 21)$	0.01
		Chest circumference	$171.18 \pm 2.35^b (n = 22)$	$176.56 \pm 1.21^a (n = 32)$	$178.38 \pm 2.05^a (n = 21)$	0.03
		Hip width	$44.55 \pm 0.85^b (n = 22)$	$46.84 \pm 0.59^a (n = 32)$	$47.62 \pm 1.03^a (n = 21)$	0.03
		Body weight	$367.32 \pm 13.76^b (n = 22)$	$398.75 \pm 8.85^{ab} (n = 32)$	$419.67 \pm 14.36^a (n = 21)$	0.02
		Croup height index	$102.05 \pm 1.47^a (n = 22)$	$99.43 \pm 0.39^b (n = 32)$	$98.87 \pm 0.45^b (n = 21)$	0.03
LX	Male	Body height	$131.43 \pm 3.13^b (n = 7)$	$131.00 \pm 4.65^b (n = 5)$	$140.00 \pm 1.55^a (n = 17)$	0.02
		Chest circumference	$169.43 \pm 3.12^b (n = 7)$	$168.40 \pm 6.90^b (n = 5)$	$184.11 \pm 3.12^a (n = 17)$	0.01
		Hip width	$40.00 \pm 1.31^b (n = 7)$	$40.40 \pm 2.66^b (n = 5)$	$45.71 \pm 0.95^a (n = 17)$	0.01
		Hip cross height	$130.43 \pm 4.17^b (n = 7)$	$132.20 \pm 2.71^{ab} (n = 5)$	$138.35 \pm 1.48^a (n = 17)$	0.05
		Body weight	$356.28 \pm 17.71^b (n = 7)$	$324.20 \pm 24.11^b (n = 5)$	$441.29 \pm 22.64^a (n = 17)$	0.01
NY	Female	Hip width	$128.14 \pm 3.25^{ab} (n = 7)$	$131.42 \pm 0.98^a (n = 24)$	$124.44 \pm 9.83^b (n = 9)$	0.04
JX	Female	Hip height	$126.65 \pm 0.94^{ab} (n = 33)$	$129.33 \pm 0.67^a (n = 52)$	$127.90 \pm 1.53^b (n = 10)$	0.05
		Abdominal circumference	$196.97 \pm 2.82^{ab} (n = 33)$	$203.67 \pm 1.80^a (n = 52)$	$195.70 \pm 2.11^b (n = 10)$	0.05

AR: androgen receptor; LSM: least squares technique; SE: standard error; LX: Luxi cattle; QC: Qinchuan cattle; NY: Nanyang cattle: JX: Jiaxian red cattle; II: insertion–insertion; ID: insertion–deletion; DD: deletion–deletion. $^{a, b, c} = P < 0.05$.

Li and Lan, 2015). Second, some studies have confirmed that introns can also affect the expression of genes (Parenteau et al., 2008, 2011; Chorev and Carmel, 2013; Ramke et al., 2017). Third, this locus could affect the expression of the AR gene by regulating alternative splicing (Yang et al., 2017). As this research progresses, it could provide evidence that the combined effects of all of the genomic differences regulate the complex process of animal growth and development. As shown in Table 4, the optimal genotypes were not the same for each breed. The DD genotype was the best genotype in LC and QC cattle, but the ID genotype was the best in NY and JX cattle. This could be due to underpopulation.

In conclusion, we performed a comprehensive study of molecular genetic markers. Ideally, the use of indels will allow us to uncover every gene associated with growth. It could then be combined with the traditional methods of single-strand conformation polymorphism (SSCP), restriction fragment length polymorphism (RFLP), and amplification-created restriction site (ACRS), as well as new methods such as whole-genome resequencing (WGR) and genome-wide

association study (GWAS; Tian et al., 2008; Niu et al., 2017; Hibicke et al., 2017; Y. Zhang et al., 2015; Choi et al., 2015; Jiang et al., 2016). Comparatively analyzing the value of molecular markers should be an important choice. Briefly, a novel 24 bp indel within the AR gene significantly affected growth traits, suggesting that this indel may be a potentially useful DNA marker for eliminating or selecting excellent individuals for MAS breeding in cattle.

5 Conclusion

Our results confirmed the existence of a 24 bp indel within the introns of AR genes in four breeds of Chinese yellow cattle, and we verified their association with growth traits.

Data availability. Data are available upon request.

Author contributions. XS and RD designed the experiments; HZ, MW, SW, XY, and ZL collected DNA samples; HZ and MW carried out experiments; HZ and MW analyzed the experimental data; and XS and HZ wrote the paper.

Competing interests. The authors declare that they have no conflict of interest.

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