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Research Article

IGF-1 Gene Polymorphisms Influence Bovine Growth Traits in Chinese Qinchuan Cattle

Lin-sheng GUI [1,2] Zhi-you WANG [1,2] Jian-lei JIA [1,2]
Cheng-tu ZHANG [3] Yong-zhong CHEN [3] Sheng-zhen HOU [1,2]

[1] State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, Qinghai Province 810016, P.R. CHINA
[2] College of Agriculture and Animal Husbandry, Qinghai University, Xining, Qinghai Province 810016, P.R. CHINA
[3] Animal Husbandry and Veterinary Station of Xining, Qinghai Province 810016, P.R. CHINA

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Abstract

Insulin-like growth factor I (IGF-1) influences gonadotrophin-releasing hormone (GnRH) neurons during puberty, and plays an important role in muscle growth in farm animals. The objective of this study was to estimate the genotype frequencies of the polymorphisms of IGF-1 and to determine associations between these polymorphisms and growth traits in Chinese Qinchuan cattle breeds. Five single nucleotide polymorphisms (SNPs), including one in intron 2 (g.5172T>G) and four in intron 3 (g.56495C>A, g.56501C>T, g.56801C>T and g.71090T>G) were identified according to the sequencing results of 487 individual of a Qinchuan cattle population. The chi-square test showed g.5172T>G, g.56495C>A and g.56801C>T were in Hardy-Weinberg equilibrium in all the samples. Six different haplotypes were identified, of which two major haplotypes had a frequency of 48.2% (-TCTTT-) and 16.3% (-TCCTT-). The single marker association analysis showed that the three polymorphisms (g.5172T>G, g.56501C>T and g.71090T>G) were significantly associated with growth traits (at P<0.01 or P<0.05). In conclusion, our results suggest that some polymorphisms in IGF-1 may play a role in determining one of the important genetic factors that influence growth traits in Chinese Qinchuan cattle.

Keywords: *IGF-1 gene, Polymorphisms, Growth traits, Chinese Qinchuan cattle*

IGF-1 Gen Polimorfizmi Çin Qinchuan Sığırında Büyüme Özelliklerini Etkiler

Öz

İnsülin benzeri büyüme faktörü (IGF-1) çiftlik hayvanlarında gelişme döneminde gonadotropin salgılatıcı hormon nöronlarını etkiler ve kas gelişiminde önemli bir rol oynar. Bu çalışmanın amacı, Çin Qinchuan sığırında IGF-1 polimorfizminin genotip çeşitliliğini tespit ederek büyüme özellikleri üzerine etkisini belirlemektir. Qinchuan sığır popülasyonunda 487 sığırın sekans sonuçlarına göre, intron 2'de bir adet (g.5172T>G) ve intron 3'de dört adet (g.56495C>A, g.56501C>T, g.56801C>T and g.71090T>G) olmak üzere toplam beş adet tek nükleotid polimorfizmi tespit edildi. Ki kare testine göre, g.5172T>G, g.56495C>A ve g.56801C>T tüm örneklerde Hardy-Weinberg eşitliğindeydi. Altı farklı haplotip tespit edildi ve bunlardan iki ana haplotip %48.2 (-TCTTT-) ve %16.3 (-TCCTT-) sıklıkta gözlemlendi. Tek marker asosiasyon analizi, üç polimorfizmin (g.5172T>G, g.56501C>T ve g.71090T>G) anlamlı derecede olmak üzere büyüme özellikleri ile ilişkili olduğunu gösterdi (P<0.01 veya P<0.05). Sonuç olarak, IGF-1'deki bazı polimorfizmler Çin Qinchuan sığırında büyüme özelliklerini etkileyen önemli genetik faktörleri belirlemede etkili olabilir.

Anahtar sözcükler: *IGF-1 geni, Polimorfizm, Büyüme özellikleri, Çin Qinchuan sığırı*

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) is predominantly synthesized and secreted by the liver as an endocrine hormone as well as in target tissues via paracrine/autocrine [1], which has similar molecular structure to insulin and plays a pivotal role in growth, development, and metabolism in mammals [2], particularly during puberty [3]. IGF-1 has a broad tissue distribution detected by quantitative real-time PCR (qPCR), such as muscle, liver, kidney, heart, brain and intestine [4,5]. It means that IGF-1 may participate in modulation of the cellular development

İletişim (Correspondence)
+86 971 5315935 Fax: +86 971 5315935
qhdxdkfz@163.com

and proliferation in various tissues like muscle, cartilage and bones [6]. In Calu-1 cells, IGF-1 phosphorylates M2-pyruvate kinase (PKM2) and alters its activity, resulting in increased expression of hypoxia inducible factor-1α (HIF1α), hexokinase 2 (HK2), and glucose transporter 1 (GLUT1), thereby regulates glycolysis rate [7]. Additionally, Mice with an IGF-1 deletion were caused a number of abnormalities, including intrauterine growth retardation, developmental defects, and perinatal mortality [8,9]. In turn, overexpression or administration of IGF-1 peptide remarkably increased in body weight and carcass weight in rat [10]. Genetic deletion of IGF-1 receptor in preosteoblastic cells showed lower bone mass and mineral deposition rates in mice [11]. All the results support the hypothesis that IGF-1 is a potential target for the selection of growth-related traits in livestock.

Qinchuan cattle were famous for their excellent adaptability, meat quality, and physical features [12]. Compared with imported commercial beef cattle breeds, such as Angus and Charolais, Qinchuan cattle have several drawbacks including underdeveloped hind hip and slower growth rate [13]. The bovine IGF-1 gene localizes on chromosome 5, which harbors quantitative trait locus (QTLs) for growth traits in cattle [14]. However, there is limited information with regard to the influence of IGF-1 gene on growth traits in Qinchuan cattle. This study aimed to identify single nucleotide polymorphisms (SNPs) in the bovine IGF-1 gene and to carry out haplotypes construction and association analysis so as to contribute to the understanding of the role of IGF-1 gene in the variation of growth traits in cattle.

MATERIAL and METHODS

Experimental Population

A total of 487 female Qinchuan cattle aged from 18 to 24 months were randomly collected from the experimental farm of National Beef Cattle Improvement Center (Yangling Shaanxi, China). All heifers were raised in similar management and rearing environment. Growth traits, including body length, withers height, chest depth, chest circumference, and pin bone width, were measured using the standard method [15]. Genomic DNA of 487 cattle was extracted from blood samples collected from the jugular vein and all DNA samples were stored at -20°C for subsequent analysis.

Variant Detection and Genotyping

Primers used to amplify the bovine IGF-1 gene were designed according to the GenBank accession numbers (AC_000162.1). The total volume of the PCR reaction was 20 μL, which contained 50 ng of genomic DNA, 10 pM of each upstream and downstream primer, 0.20 mM dNTPs, 2.5 mM $MgCl_2$ and 0.5 U Taq polymerase (TaKaRa, Dalian, China). The PCR conditions were: 95°C, for 5 min (preliminary denaturation) followed by 35 cycles at 94°C for 30 s (denaturation), 30 s of annealing (at a temperature specific for a single analyzed fragment), 72°C for 40 s (extension). The final extension was performed at 72°C for 10 min.

Aliquots of 10 μL PCR products were digested with 10 U restriction enzymes for 8 h at 37°C, respectively. The digested products were detected by electrophoresis on a 3.5% agarose gel stained with ethidium bromide.

Data Analysis

Genotypic frequencies, gene heterozygosity (He) and polymorphism information content (PIC) were directly calculated. The Hardy-Weinberg equilibrium (HWE) was estimated through a χ2 test performed by the PopGene software (Version 3.2). The formulas were as follows:

$$He = 1 - \sum_{i=1}^{m} p_i^2 \qquad PIC = 1 - \sum_{i=1}^{m} - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2p_i^2 p_j^2$$

Where P_i, P_j are the frequency of the i and j allele, m is the number of allele.

$$P_A = {(2 * \mathrm{AA} + \mathrm{AB})}/{(2 * \mathrm{N})} \qquad P_B = 1 - P_A$$

Where P_A is the frequency of allele A, P_A is the frequency of allele B, N is the numbers of individuals, AA is the number of genotype AA, BB is the number of genotype BB

$$\chi^2 = \sum_{i=1}^{n} \frac{\left(|O_i - E_i| - {1}/{2}\right)^2}{E_i}$$

Where E_i is the theoretical value, O_i is the actual value, n is the numbers of allele.

The Linkage disequilibrium (LD) as measured by D' and r^2 were performed with the HAPLOVIEW software (Version 3.32) [16]. Haplotypes were obtained for each animal using the PHASE computer program (Version 2.1) [17]. The association analyses between SNP marker genotypes and growth traits were performed by the least squares method as applied in the general linear model (GLM) procedure of SPSS 16.0 software (IBM Company, NY, USA). The model applied was: $Y_i = \mu + G_i + A_i + E_i$, where Y_i is the trait value for each individual, μ is the overall population mean, G_i is the fixed effect associated with the ith genotype, A_i is the fixed effect of the ith age and E_i is the random error.

RESULTS

SNP Detection and Genetic Diversity Analyses

As shown in *Fig. 1* and *Fig. 2,* a total of five variations were revealed, including five SNPs in the introns: T→C at position g.5172T>G, C→A at position g.56495C>A, C→T at position g.56501C>T, C→T at position g.56801C>T and T→G at position g.71090T>G. According to the sequence

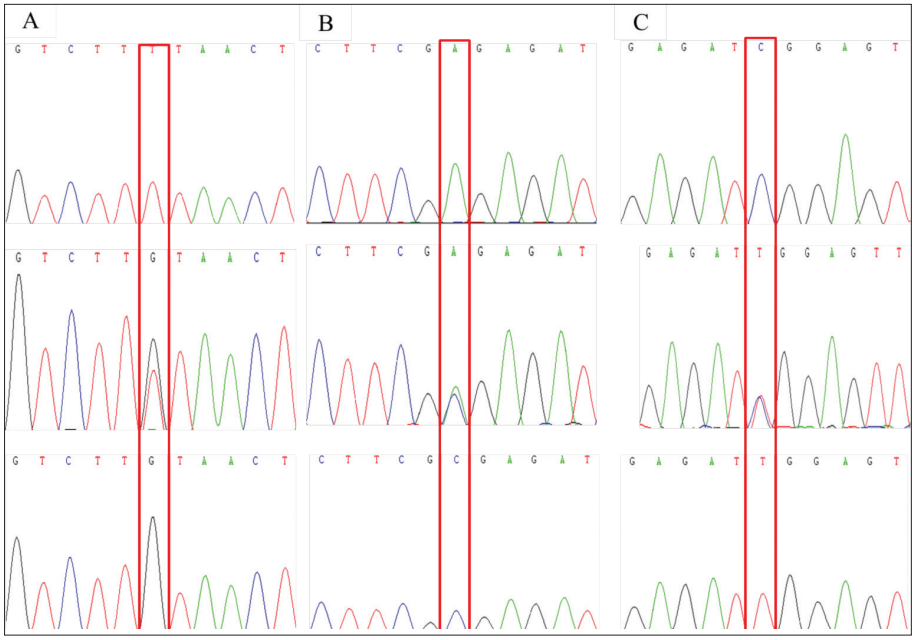

Fig 1. The sequencing map of the g.5172T>G (A), g.56495C>A (B) and g.56501C>T (C)

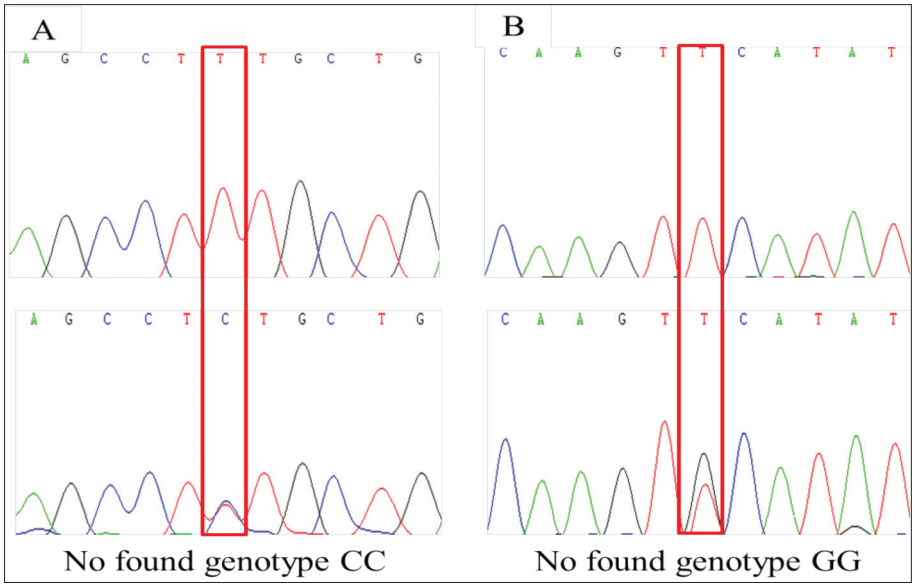

Fig 2. The sequencing map of the g.56801C>T (A) and g.71090T>G (B)

information of variations, the PCR products could be digested with *MaeIII, Taq I, Mbo I, Bfm I* and *HpyCH4V* restriction enzymes to genotype the individuals. To utilize those restriction enzymes better, five pairs of primers were redesigned using primer premier 5 [18]. The detailed information about oligonucleotide primers, location, annealing temperatures, and different pattern sizes is shown in *Table 1*.

Table 2 displays the allele and genotypic frequencies, He, PIC and HWE. The major allele frequencies ranged from 0.6982 to 0.9035 for each locus. The chi-square test showed that both g.56501C>T and g.71090T>G were severely out of the HWE, whereas the individual frequencies of the genotypes agreed with the HWE for the other three SNPs. The PIC values for SNPs ranged from 0.1592 to 0.3327. According to the convention for classification of PIC, g.5172T>G and g.5172T>G exhibited intermediate genetic diversity (0.2500<P<0.5000), while three others possessed a low genetic diversity (P<0.2500).

Linkage Disequilibrium and Haplotype Analysis

The linkage disequilibrium among the four SNPs was measured by D' and r^2 through HAPLOVIEW. The values of D' varied from 0.053 to 0.847 and the r^2 values were from 0.001 to 0.531 *(Table 3)*. Our results indicated strong linkage between g.56501C>T and g.56801C>T.

Additionally, the haplotype structure analysis was performed using PHASE software. As shown in *Table 4,* the five SNPs showed six different haplotypes in population studied (haplotypes with frequencies lower than 5% were not included). In detail, the Hap4 (-TCTTT-) had the highest frequencies (48.20%), followed by Hap6 (-TCCTT-) and Hap1 (-GATTT-), 16.3% and 13.2%, respectively.

Association Analysis

Table 5 summarizes the effects of the five SNPs on growth traits in Qinchuan cattle. At the g.5172T>G locus, animals with genotype GG had higher values of body length (P=0.031) and chest circumference (P=0.042) compared to animals with genotype TT. At the g.56501C>T locus, significant differences in body length, withers height, chest depth, chest circumference and pin bone width were observed between CC and TT; animals with genotype CC had increased with body length (P=0.000), chest depth (P=0.006), chest circumference (P=0.000) and pin bone width (P=0.001) compared to those with genotype TT. In addition, the withers height values were also higher in genotype CC carriers than genotype TT (P=0.012). At the g.71090T>G locus, the withers height (P=0.032) and chest circumference values of animals with genotype TG were higher than those of individuals

Table 1. *Primers used for PCR amplification of the IGF1 in Qinchuan cattle*

Locus	Primer Sequence (5′ to 3′)	Tm (°C)	Restriction Enzymes	Length	Genotype Pattern (bp)
g.5172T>G	GACCCAGGAGGAAGATGA	61.7	MaeIII (^GTNAC)	432 bp	GG: 39 and 393 TG: 39, 393, and 432 TT: 432
	TTTTTTGACCACGCCCTTTA				
g.56495C>A	AGGAATCGTGGATGAGTG	60.0	TaqI(T^CGA)	403 bp	AA: 175 and 228 CA: 175, 228 and 403 CC: 403
	CAAAGCCCAGGTTTAGTC				
g.56501C>T	CTGAGGAGGCTGGAGATG	63.8	MboI(^GATC)	225 bp	CC: 82 and 143 CT: 82, 143, and 225 TT: 225
	CCAGAAGTCTATGAGGGTATG				
g.56801C>T	ACTTCTGGTTCATAGGGTCA	57.5	BfmI(C^TRYAG)	239 bp	TT: 10 and 229 CT: 10, 229, and 239
	TCATCAGCAGAGGCTTGG				
g.71090T>G	GTGTTCACGGTGTTCCAG	60.0	HpyCH4V (TG^CA)	686 bp	TT: 686 TG: 138, 548, and 686
	ATGTATCCAACCCATCTC				

Table 2. *Genotype frequencies (%) of the IGF1 gene for the SNPs in Qinchuan cattle populations*

SNPs	Genotypic Frequencies			N	Allelic Frequencies		χ^2 (HWE)	PIC	He
g.5172T>G	TT	TG	GG	487	T	G			
	0.4908	0.4148	0.0944		0.6982	0.3018	0.1226	0.3327	0.4215
g.56495C>A	CC	CA	AA	487	C	A			
	0.5975	0.3285	0.0740		0.7618	0.2382	4.3685	0.2971	0.3629
g.56501C>T	CC	CT	TT	487	C	T			
	0.0513	0.2320	0.7167		0.1674	0.8326	13.6494*	0.2399	0.2787
g.56801C>T	TT	CT	CC	487	T	C			
	0.8070	0.1930	0.0000		0.9035	0.0965	5.5567	0.1592	0.1744
g.71090T>G	TT	TG	GG	487	T	G			
	0.6961	0.3039	0.000		0.8480	0.1520	15.6348*	0.2245	0.2577

χ^2 (HWE): *Hardy-Weinberg equilibrium χ^2 value, * Hardy-Weinberg disequilibrium (χ^2 >5.991) ,* ***PIC:*** *polymorphism information content,* ***He:*** *gene heterozygosity*

Table 3. *Estimated values of linkage disequilibrium for SNPs bovine IGF1 Qinchuan* cattle

SNPs (r^2/D)	g.56495C>A	g.56501C>T	g.56801C>T	g.71090T>G
g.5172T>G	0.274/0.616	0.005/0.104	0.001/0.053	0.002/0.072
g.56495C>A		0.002/0.171	0.001/0.059	0.002/0.201
g.56501C>T			0.531/0.847	0.184/0.454
g.56801C>T				0.198/0.577

with genotype TT (P =0.029).

The effects of genetic variations of a gene could be more reliable by integrating the haplotype combinations and the single analysis with traits [19]. In the present study, the association analysis suggested that no significant differences were detected between the combined genotypes of the five SNPs and growth traits in Qinchuan cattle *(Table 6)*.

DISCUSSION

Nowadays, growing studies suggested that IGF-1 participates in the modulation of a variety of physiological activities, including in cellular development, differentiation and proliferation [20]. For its positive role in improving muscle growth, association between IGF-1 polymorphisms and growth traits in livestock was extensively studied. Among five pig breeds, two SNPs (ss539003291 and rs81385751) were found to be associated with the body size and average daily gain [21]. In Mexican beef cattle, a novel SNP in the promoter region (IGF-1/SnaBI) were detected by Reyna et al.[22], which was significantly associated with weaning weight. Similarly, this SNP has a significant effected on growth curves of Angus bull calves at ages between 66 and 291 days [23]. Furthermore, two SNPs (g.4700T>C and g.5524C>T) detected in hircine IGF-1 were candidate markers for body weight at different ages in Indian goat breeds [24]. Taken together, these findings lend credence to the hypothesis that the IGF-1 is an excellent candidate gene for growth-related traits in livestock.

In this study, five SNPs were identified by PCR-RFLP. Among those, both g.56801C>T and g.71090T>G had 2 genotypes, and the genotype CC and GG were not observed in the sampled animals. The absence of that genotype in this population might mean that the size of the experimental population was too small to capture its full genetic variation. Association analyses showed that except for g.

Table 4. *Major haplotypes of IGF1 gene and their frequencies in Qinchuan cattle*

Haplotype	g.5172T>G	g.56495C>A	g.56501C>T	g.56801C>T	g.71090T>G	Frequency
Hap1	G	A	T	T	T	0.132
Hap2	G	C	T	T	T	0.091
Hap3	T	A	T	T	T	0.059
Hap4	T	C	T	T	T	0.482
Hap5	T	C	T	T	G	0.051
Hap6	T	C	C	T	T	0.163

Table 5. *Association of different genotypes of SNPs in IGF1 with growth traits and meat quality traits in Qinchuan cattle*

SNPs	Genotype (N)	Body Length (cm)	Withers Height (cm)	Chest Depth (cm)	Chest Circumference (cm)	Pin Bone Width (cm)
g.5172T>G	TT (239)	140.149±0.531^{b}	124.916±0.430	62.916±0.362	172.266±0.868^{b}	20.004±0.156
	TG (202)	143.735±0.578	126.775±0.467	63.972±0.394	174.455±0.944	19.797±0.170
	GG (46)	146.522±1.112^{a}	127.728±0.769	64.098±0.774	177.848±1.585^{a}	20.880±0.322
	P	0.031	0.380	0.533	0.042	0.369
g.56495C>A	CC (291)	143.046±0.495	126.373±0.393	63.316±0.329	174.591±0.790	20.195±0.141
	CA (160)	140.863±0.658	125.341±0.530	62.491±0.444	172.197±1.066	19.575±0.191
	AA (36)	141.819±0.955	125.167±1.117	61.000±0.736	173.194±1.246	20.319±0.402
	P	0.411	0.114	0.136	0.072	0.095
g.56501C>T	CC (25)	151.880±1.306^{Aa}	132.280±1.303^{a}	68.300±0.884^{Aa}	187.920±1.618^{A}	23.160±0.462^{A}
	CT (113)	144.721±0.755^{b}	127.022±0.613	63.942±0.513^{b}	174.235±1.231^{B}	20.053±0.217^{B}
	TT (349)	140.744±0.430^{B}	125.153±0.349^{b}	62.139±0.292^{B}	172.510±0.701^{B}	19.758±0.124^{B}
	P	0.000	0.012	0.006	0.000	0.001
g.56801C>T	TT (393)	141.989±0.428	125.922±0.339	62.739±0.285	173.975±0.681	19.122±0.122
	CT (94)	143.282±0.875	126.080±0.693	63.436±0.582	172.559±1.392	20.021±0.250
	P	0.725	0.652	0.743	0.269	0.448
g.71090T>G	TT (339)	140.583±0.441	123.919±0.327^{b}	61.903±0.296	171.369±0.708^{b}	19.605±0.128
	TG (148)	146.030±0.667	130.473±0.494^{a}	65.098±0.448	179.044±1.072^{a}	20.909±0.193
	P	0.053	0.032	0.074	0.029	0.585

^{a,b} Means with different superscripts are significantly different (P<0.05); ^{A,B} Means with different superscripts are significantly different (P<0.01)

Table 6. *Associations of haplotypes with growth traits and meat quality traits in Qinchuan cattle*

Hap (N)	Body Length (cm)	Withers Height (cm)	Chest Depth (cm)	Chest Circumference (cm)	Pin Bone Width (cm)
Hap4/1 (65)	139.515±0.885	123.238±0.733	61.023±0.637	161.168±1.623	18.600±0.287
Hap4/2 (33)	141.288±1.241	124.530±1.028	63.470±0.895	172.030±1.778	18.879±0.403
Hap4/3 (29)	135.466±1.324	122.690±1.097	61.345±0.877	169.069±1.125	19.103±0.758
Hap4/4 (120)	140.467±0.651	123.942±0.539	61.525±0.469	171.608±1.194	19.933±0.211
P	0.055	0.307	0.106	0.186	0.331

56495C>A and g.56801C>T, all the other detected SNPs were associated with growth traits in Qinchuan cattle.

Interestedly, we note that although the g.5172T>G, g. 56501C>T and g. 71090T>G located in introns and do not alter amino acid sequence. However, these polymorphisms are significantly influence phenotypic differences manifested in the growth traits in Qinchuan cattle. Nowadays, growing observations indicate that SNPs in intron can affect protein expression as well by altering the stability of mRNA. Huang et al. reported that the variation (T4127G) in intron2 of CYP3A4 exists in the human liver, which could significantly decreases the hepatic microsomal testosterone 6-hydroxylase activity of cytochrome P450, family 3, subfamily A (CYP3A) [25]. Seo et al.[26] demonstrated that intronic SNP rs13438494 alters splicing efficient creating or disrupting a splicing motif, which may ultimately result in bipolar disorder in affected people. In Holsteins, Wang et al.[27] detected a SNP (c. 1033 + 2184 C>T) in intron 8 of CD46 molecule (CD46), which results in CD46-transcript

variant aberrant splice variant, and clearly associated with mastitis. Additionally, research of Gao et al.[28] showed that a point variation in intron1 (A3481C) of fatty acid binding protein 4, adipocyte (A-FABP) had strong effect on intramuscular fat content in Junmu No. 1 white swine.

In summary, five polymorphisms in the IGF-1 were identified in Qinchuan cattle. The association analysis of single markers (g. 5172T>G, g. 56501C>T and g. 71090T>G) revealed prominent effects on growth traits. The present report provides evidence that the three SNPs could be used as a molecular marker for eliminating or selecting preferred individuals in MAS.

Conflicts of Interest

The authors declare no conflict of interest.

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