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Copy number variation of *EIF4A2* loci related to phenotypic traits in Chinese cattle

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Abstract

Background: Generally, copy number variation (CNV) is a large-scale structural variation between 50 bp and 1 kb of the genome. It can affect gene expression and is an important reason for genetic diversity and phenotypic trait diversity. Studies have shown that the eukaryotic translation initiation factor 4A2 (*EIF4A2*) gene plays an essential role in muscle development in both humans and pigs. However, the influence of bovine *EIF4A2*'s copy number change on phenotypic traits has not been reported.

Objectives: To detect the tissue expression profile of the *EIF4A2* gene in adult cattle and individuals' CNV type of variation. Then, we explored the correlation between *EIF4A2*-CNV and growth traits in Chinese cattle breeds.

Methods: Real-time fluorescent quantitative reverse transcription PCR (qRT-qPCR) was used to determine the expression profile of the *EIF4A2* gene. Real-time fluorescent quantitative PCR (qPCR) was used to detect the CNV type of bovine populations. Then, SPSS 26.0 was used for association analysis.

Results: In this study, a total of 513 individuals in four cattle breeds (Qinchuan cattle [QC], Yunling cattle [YL], Pinan cattle [PN] and Jiaxian cattle [JX]) were detected for *EIF4A2* gene's CNV. The results showed that *EIF4A2*-CNV has an essential impact on hip width (HW) and rump length (RL) in QC, heart girth (HG), chest depth (CD) and RL in YL and HW in PN. However, it had no significant effect on JX.

Conclusions: The above results suggest that *EIF4A2* gene's CNV can be used as a molecular marker for cattle breeding, which is helpful to accelerate the breeding of superior beef cattle breeds.

KEYWORDS
association analysis, Chinese cattle, CNV, EIF4A2, growth traits

Zijing Zhang, Mengyang Peng and Yifan Wen contributed equally to this work.

1 | INTRODUCTION

In many parts of the world, cattle are providing people with various nutritional products (Barendse et al., 1997). With the cattle genome assembly release and functional elements annotation (Elsik et al., 2009), genetic variations have been used to amend marbling grades and meat yield in livestock breeding (Casas et al., 2005). Copy number variation (CNV) is analogous to single nucleotide polymorphism (SNP) and indels, a kind of genetic variation, and is a widespread structural variation in the genome in which the composition is diversified, including amplification, deletion, insertion and inversion of DNA fragments (Feuk et al., 2006). CNV often involves unbalanced rearrangement of increasing or decreasing DNA content (Levy et al., 2007; Redon et al., 2006). The size of CNV is generally between 50 bp and 1 kb. Determination of CNV is recognised as an increasingly important factor for gene function, animal evolution and growth traits (Peng et al., 2020). In a small-scale insertion and deletion study of DNA fragments, Li et al. (2019) found that the type of mutation in the *CDKN3* gene of chickens is significantly related to individual growth traits. In a study of dairy goats, it was found that there was a significant correlation between the CNVs of *ADAMTS20* and *PAPPA2* genes and two milk yield traits (Kang et al., 2020). In a chicken study, it was found that the mRNA level of *RHACD8* was weakly negatively correlated with the copy number of DNA (X. Wang et al., 2010). The *CADM2* gene of sheep found that the withers height (WH) and body length (BL) of individuals with a lack of CNV are significantly better than those of other types, so it is a potential selection site for sheep molecular marker breeding (Xu et al., 2020).

Eukaryotic translation initiation factor 4A (EIF4A) is a typical member of the dead-box protein family, which has nine characteristic motifs that form the conservative helicase core (Andreou & Klostermeier, 2013). There are three kinds of EIF4A: EIF4A1, EIF4A2 and EIF4A3, which have similar structures and different functions (Nielsen & Trachsel, 1988). In a study of plants, Bush et al. (2009) found that *EIF4A2* inhibits mRNA at translation initiation through interaction with the CCR4-NOT complex. Similarly, Wilczynska et al. (2019) also found that *EIF4A2* acts as an inhibitor directly to upstream of the AUG start codon. In addition, the high expression of the *EIF4A2* gene is observed in cancer (Chen et al., 2019; Quanico et al., 2017). These diseases can be alleviated by downregulating the expression of the gene (Liu et al., 2019; Ndzinu et al., 2018).

Qinchuan cattle (QC), Yunling cattle (YL), Pinan cattle (PN) and Jiaxian cattle (JX) are four representative local cattle breeds that are listed as the key national protection resources in China. They are good meat varieties with excellent production performance and good meat production characteristics. QC are the first of the five local cattle in China and have become an important source of increased income for farmers in many Chinese provinces. QC have a long history, which began in the eighth century BC, and they were mainly used for food and farming. With the development of the planting industry, the body size of QC has increased, and the working ability and meat quality have been improved. After more than 20 years of breeding, QC have formed a new breed of meat. The number of QC in Shaanxi Province is about 1.8 million now. YL were the first cattle bred by ternary cross breeding, including Brahman, Murray Grey and Yunnan native cattle. It is the fourth new breed of specialised beef cattle cultivated independently by China. In 2014, it was approved by the National Variety Resources Committee. It shows good reproductive ability and growth rate under high temperature and high humidity conditions. There are 7583 purebred YL in Yunnan Province, including 1380 bulls and 6203 cows. JX are an indigenous cattle breed in China, and they were draft cattle long ago that had a large body size. This breed has been widely bred for beef since 30 years ago and has evolved into a beef cattle breed with excellent meat quality, tough feeding resistance and high fertility (Xia et al., 2021). JX are mainly produced in Jiaxian County, Henan Province. At present, there are more than 60,000 cattle in the county. PN are a hybrid between Piedmont cattle and Nanyang cattle in China with excellent performance. Currently, there are 146,000 PN in Xinye County. Its cultivation began in 1987, and it has now become a beef cattle breed that produces high-grade beef and has much room for improvement. In brief, these cattle breeds play an irreplaceable role in Chinese animal husbandry industry and have great potential.

At present, most of the CNV studies are limited to the discovery of CNVs by various platforms, including comparative genomic hybridisation, SNP arrays and next-generation sequencing. Real-time fluorescent quantitative PCR (qPCR) and association analysis could assist in validating the findings of the above methods and exploring the influence on a certain trait. (Bickhart et al., 2012; Cicconardi et al., 2013; Hou et al., 2012; Jiang et al., 2012).

There are many studies on the *EIF4A2* gene in humans and mice, and most of them are related to disease. However, studies on this gene in cattle and its effects on livestock and poultry have not been reported. At present, there is also no research on the *EIF4A2* gene in cattle. In this study, the relationship between CNVs and growth traits was found by association analysis to provide a basis for further use of *EIF4A2* CNV as a molecular marker for bovine economic traits.

2 | MATERIALS AND METHODS

2.1 | Animals and samples

In the experiment, blood samples of QC ($N = 100$), YL ($N = 197$), PN ($N = 133$) and JX ($N = 83$) were collected from Shaanxi, Henan, Yunnan and Henan Provinces, respectively. Their ages ranged from 1 to 6 years. Several growth traits of 513 individuals were measured, including WH, height at hip cross (HHC), BL, heart girth (HG), chest width (CW), chest depth (CD), hip width (HW), hucklebone width (HBW), rump length (RL) and body weight (BW). Full names, abbreviations and descriptions of the above-related nouns are listed in Table S1. The heart, liver, kidney, lung, spleen and muscle (longissimus dorsi) tissues of three adult cattle were collected in the slaughterhouse.

2.2 | Acquisition of genomic DNA and cDNA

Genomic DNA was extracted from blood samples from the jugular vein by phenol–chloroform extraction according to the method of Ozsensoy

TABLE 1 Information on the primers used in this study

	Locus	Primer sequences(5′ to 3′)	Amplification length (bp)
DNA level	*EIF4A2*	F1:5′- ACCAAGGCTATCTTGGTTTCTG -3′ R1:5′-GGTGAAAAAGGAAGAATTGACCC-3′	156
	BTF3	F2:5′- AACCAGGAGAAACTCGCCAA -3′ R2:5′- TTCGGTGAAATGCCCTCTCG -3′	166
RNA level	*EIF4A2*	F1:5′- CAGCAGAGAGCTATTATTCCATGT -3′ R1:5′- TCTCCAAGTGCCAGAATTACCT -3′	200
	GAPDH	F2:5′- AAGTTCAACGGCACAGTCA -3′ R2:5′- GTCATAAGTCCCTCCACGAT -3′	365

and Kara (2019). The concentration and purity of DNA were detected by a Nanodrop 2000 Spectrophotometer. The samples were stored in a refrigerator at −80°C. The extracted DNA was dissolved to 10 ng/μl by double distilled waster (ddH_2O) when running PCR or qPCR. Total RNA was extracted from the heart, liver, kidney, lung, spleen and muscle tissues of adult cattle. The cDNA of different tissues was obtained by a reverse transcription kit (Evo M-MLV Mix Kit with gDNA Clean for qPCR, accurate biology).

2.3 | Primer design

According to whole genome resequencing (Huang et al., 2021), the CNV was found in the sequence from 81,347,201 to 81,351,200 on the *EIF4A2* gene (target gene) based on the Btau_5.0.1 assembly. Then, we regarded the bovine *EIF4A2* gene sequence (NC_007299.6) published by NCBI (National Center for Biotechnology information) as the reference sequence, and the primers included in this region were designed by Prime 5.0 software and compared in NCBI_BLAST. The primer sequences can be found in Table 1. Because Bickhart et al. (2012) found that the *BTF3* gene appeared stable with two copies, we decided to use the bovine *BTF3* gene as the reference gene and designed primers to amplify the specific fragment (166 bp). The primer sequences are shown in Table 1. The amplification system included BioEasy Master Mix (Probe, high ROX; BIOER) 5.0 μl, forward and revers primers (10 pmol/μl) 0.2 μl, DNA (10 ng/μl) 1 μl and ddH_2O 3.6 μl.

2.4 | Measurement of CNV and expression profiling

To detect the CNV type of each sample, we used the CFX 96TM real-time detection system (Bio-Rad, Hercules) and BioEasy Master Mix to estimate the CNV. The reaction system was as follows: (1) The reaction proceeded at 95°C for 1 min. Then, amplification was performed according to '(2)', (2) denaturation at 95°C for 15 s, annealing at 60°C for 1 min, for a total of 35 cycles.

EIF4A2 primers and *GAPDH* primers were used according to the same reaction system and procedure. The amplification of each sample was carried out three times for the parallel control. The experimental results are calculated using the $2^*2^{-\Delta\Delta CT}$ method, and Ct is the cycle threshold, which is the number of amplification cycles after the fluorescence signal of the amplified product reaches the set threshold. The CNV results are divided into CN = 0, CN = 1, CN = 2, CN = 3, CN = 4 and CN ≥ 5. Then, CN = 0 and CN = 1 were divided into deletion types. CN = 2 was regarded as the normal type. All the rest were considered to be the duplication type. The mean of CN is copy number.

2.5 | Association analysis

SPSS (26.0) was used for association analysis. In the data processing, according to the different factors affecting the traits, taking into account the environmental effects, age, sex, genetic effects and their interaction effects, a fixed model was used for analysis, while simplifying according to the actual situation. The complete model is as follows: $P_{ijk} = \mu + A_i + S_j + CNV_k + e_{ijk}$. P_{ijk} is the phenotypic traits record of the ijkth individual growth traits; μ is the overall mean of each trait. A_i is the effect of age. S_j is the effect due to sex. CNV_k is the influence of the copy numbers of the *EIF4A2*. E_{ijk} is the random error.

SPSS software was used to carry out simple correlation analysis to study the correlation between various traits of Chinese cattle. The simple correlation coefficient r is calculated according to the Pearson correlation.

3 | RESULTS

3.1 | Expression profile

The purpose of this experiment was to study the expression pattern of the *EIF4A2* gene in Chinese cattle. The results showed that the expression level of the *EIF4A2* gene was different in different tissues of adult cattle (Figure 1). The expression level of the *EIF4A2* gene in muscle tissue was significantly higher than that in other tissues. Subsequently, the middle expression level was in the liver and kidney. While its expression level is similar in the spleen, lung and kidney, its expression level is low as well.

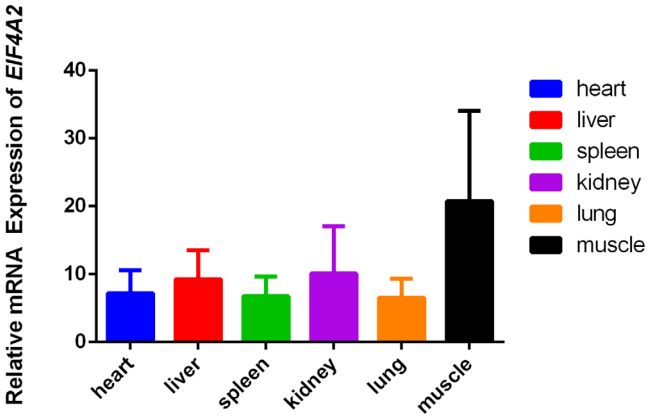

FIGURE 1 Expression pattern of the bovine eukaryotic translation initiation factor 4A2 (*EIF4A2*) gene in tissues

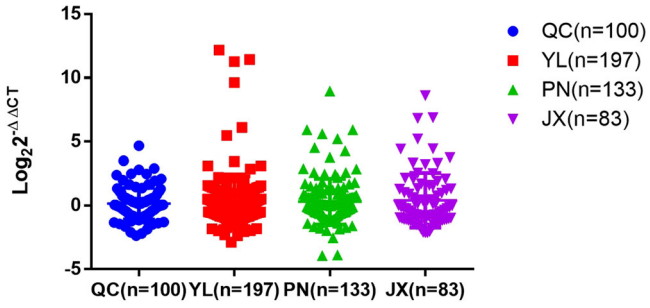

FIGURE 2 Distribution of different copy number variation (CNV) types in four cattle populations. JX, Jiaxian cattle; QC, Qinchuan cattle; PN, Pinan cattle; YL, Yunling cattle

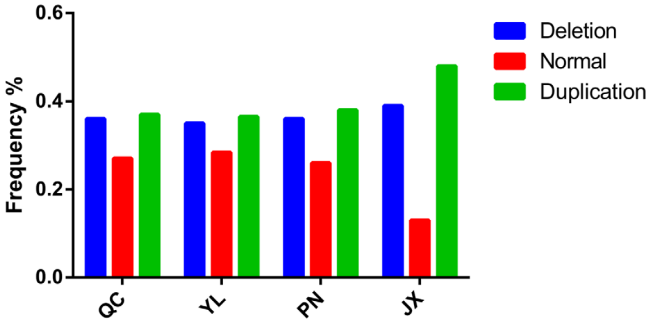

FIGURE 3 The frequency of different CNV types in four cattle populations. JX, Jiaxian cattle; QC, Qinchuan cattle; PN, Pinan cattle; YL, Yunling cattle

3.2 | CNV type distribution in different breeds

qPCR was used to detect the copy number of four cattle populations. According to Figure 2, the copy number distribution of the four cattle populations was concentrated, and the dispersion degree was similar. The tendency of CNV type distribution in the four breeds was consistent (Figure 3). The proportion of deletion type and duplication type was almost equal, while the normal type was the lowest.

TABLE 2 Frequency of different copy number variation (CNV) types in four cattle populations

	CNV types					
	Deletion		Normal	Duplication		
Breed	CN = 0	CN = 1	CN = 2	CN = 3	CN = 4	CN ≥ 5
QC	0.08	0.28	0.27	0.15	0.11	0.11
YL	0.071	0.279	0.284	0.208	0.076	0.081
PN	0.11	0.25	0.26	0.18	0.09	0.11
JX	0.19	0.20	0.13	0.13	0.17	0.18

Abbreviations: JX, Jiaxian cattle; PN, Pinan cattle; QC, Qinchuan cattle; YL, Yunling cattle.

Then, the frequency was calculated, and the results are listed in Table 2. The copy number distribution of the four breeds of cattle was relatively uniform. As a whole, in the deletion type, the bovine individual quantity with CN = 1 was more than CN = 0. The individual proportion with CN = 3 was dominant in the duplication type. Besides, the frequency of the normal type in JX was lower than that in other breeds. The reason could be the smaller population.

3.3 | Association analysis with growth traits

Association analysis is a method to determine the effect of sequence variation on economic traits (Heaton et al., 2002). The three types of copy numbers are designated deletion (CN = 0 and CN = 1), normal (CN = 2) and duplication (CN = 3, CN = 4 and CN ≥ 5). Then, we explored the association between these six CNV classifications and growth traits. The results of association analysis show that the CNV of *EIF4A2* has a significant association with one or several growth traits in QC, YL and PN ($p < 0.05$; Table 3, 4 and 5) but has no significant effect on JX (Table 6).

For QC, *EIF4A2*-CNV was significantly associated with HW and RL ($p < 0.05$). Among them, the individuals with CN = 0 had a better HW. The next was cattle with CN ≥ 5. The worst was CN = 4. Regarding RL, the situation was the same. The RL was remarkably wider in CN = 0 than in the other types except CN ≥ 5 ($p < 0.05$). For YL, *EIF4A2*-CNV obviously impacted HG ($p < 0.05$) and had a significant influence on CD and RL ($p < 0.01$). The duplication type (CN = 3, 4, ≥ 5) had better size in the above three growth traits, especially cattle with five or more copies. Then, compared to the duplication, the above indicators of cattle with CN = 0 were worse, but they had no significant differences. In addition, CNV could lead to harmful effects when CN = 1/2. For PN, HW was significantly affected by *EIF4A2*-CNV ($p < 0.05$). In this breed, the individuals with CN = 4 gained wider HW, and it was the most advantageous variation. Second, the animals belonging to CN ≥ 5 were the second-best group. The worst individuals usually belonged to the deletion type (CN = 0/1).

TABLE 3 Association analysis of eukaryotic translation initiation factor 4A2 (EIF4A2)-CNV and growth traits of Qinchuan cattle (QC)

		CNV types (mean ± SE)						
		Deletion		Normal	Duplication			
Breed	Growth traits	CN = 0 (*n* = 8)	CN = 1 (*n* = 28)	CN = 2 (*n* = 27)	CN = 3 (*n* = 15)	CN = 4 (*n* = 11)	CN ≥ 5 (*n* = 11)	*p*-value
QC	Withers height (WH; cm)	131.13 ± 2.17	130.86 ± 1.00	128.63 ± 1.24	130.07 ± 1.83	129.91 ± 2.78	129.55 ± 2.05	0.863
	Height at hip cross (HHC; cm)	127.88 ± 2.07	127.73 ± 1.11	126.72 ± 1.10	127.33 ± 1.93	127.86 ± 2.89	127.00 ± 2.45	0.993
	Body length (BL; cm)	149.50 ± 3.24	139.96 ± 1.99	138.11 ± 2.38	137.87 ± 3.21	134.09 ± 2.87	137.45 ± 3.86	0.101
	Heart girth (HG; cm)	193.63 ± 3.62	182.34 ± 3.03	181.59 ± 3.33	180.13 ± 3.96	176.55 ± 5.00	181.36 ± 3.30	0.306
	Chest width (CW; cm)	42.38 ± 1.05	38.70 ± 0.78	39.46 ± 1.10	38.87 ± 1.07	37.91 ± 1.66	39.73 ± 1.0	0.404
	Chest depth (CD; cm)	67.88 ± 0.74	65.50 ± 0.94	66.59 ± 1.13	65.33 ± 1.49	62.95 ± 1.80	64.45 ± 1.66	0.363
	Hip width (HW; cm)	48.00^{a} ± 2.03	$42.64^{a,b}$ ± 0.85	$44.04^{a,b}$ ± 1.03	41.20^{b} ± 1.25	41.18^{b} ± 2.09	$44.45^{a,b}$ ± 1.66	0.045
	Hucklebone width (HBW; cm)	24.44 ± 1.37	23.75 ± 0.81	24.50 ± 0.73	24.90 ± 1.09	21.91 ± 0.96	24.41 ± 0.84	0.449
	Rump length (RL; cm)	48.00^{a} ± 2.15	43.00^{b} ± 0.61	$43.85^{a,b}$ ± 0.73	42.53^{b} ± 1.16	42.41^{b} ± 1.43	$45.59^{a,b}$ ± 1.18	0.019
	Body weight (BW; kg)	469.34 ± 22.96	393.6 ± 16.68	387.34 ± 19.69	379.33 ± 22.04	354.24 ± 26.30	380.83 ± 23.24	0.143

^{a,b} indicate that $p < 0.01$ is significant.

TABLE 4 Association analysis of EIF4A2-CNV and growth traits of Yungling cattle (YL)

		CNV types (mean ± SE)						
		Deletion		Normal	Duplication			
Breed	Growth traits	CN = 0 (*n* = 14)	CN = 1 (*n* = 55)	CN = 2 (*n* = 56)	CN = 3 (*n* = 41)	CN = 4 (*n* = 15)	CN ≥ 5 (*n* = 16)	*p*-value
YL	WH (cm)	127.50 ± 1.21	127.77 ± 0.56	127.73 ± 0.69	128.93 ± 0.67	128.13 ± 1.03	129.94 ± 1.17	0.444
	HHC (cm)	131.57 ± 1.14	130.24 ± 2.33	131.21 ± 0.73	132.41 ± 0.69	131.67 ± 1.43	132.81 ± 1.05	0.913
	BL (cm)	154.21 ± 2.42	156.00 ± 1.04	155.54 ± 1.23	157.17 ± 1.43	157.80 ± 1.55	161.69 ± 2.64	0.158
	HG (cm)	$195.64^{a,b}$ ± 2.09	193.43^{b} ± 1.57	$195.46^{a,b}$ ± 1.48	$196.29^{a,b}$ ± 1.23	$198.73^{a,b}$ ± 1.86	202.81^{a} ± 2.77	0.040
	CW (cm)	49.86 ± 1.61	49.00 ± 0.52	48.48 ± 0.60	49.05 ± 0.75	50.60 ± 0.89	51.88 ± 1.26	0.115
	CD (cm)	$70.00^{a,b,c}$ ± 1.14	$66.95^{b,c}$ ± 0.69	$68.79^{b,c}$ ± 0.78	$70.78^{a,b}$ ± 1.03	73.67^{a} ± 1.46	$71.38^{a,b}$ ± 1.43	0.000
	HW (cm)	59.57 ± 1.34	57.94 ± 0.70	57.02 ± 0.68	57.15 ± 0.91	55.53 ± 1.81	56.06 ± 2.37	0.421
	HBW (cm)	21.64 ± 0.68	22.04 ± 0.25	22.52 ± 0.30	22.61 ± 0.30	22.40 ± 0.39	23.38 ± 0.66	0.185
	RL (cm)	50.29^{b} ± 0.87	49.25^{b} ± 0.41	49.63^{b} ± 0.47	$51.10^{a,b}$ ± 0.50	$51.33^{a,b}$ ± 0.36	53.31^{a} ± 0.60	0.000
	BW (kg)	549.92 ± 18.18	549.46 ± 8.29	556.33 ± 9.56	569.88 ± 9.57	567.92 ± 14.33	586.54 ± 21.02	0.357

^{a,b} indicate that $p < 0.01$ is significant.

3.4 | The simple correlation of growth traits in three cattle breeds

We selected three cattle breeds that could be affected by this variation for simple correlation analysis. Pearson correlations were used to determine associations between all traits affected by *EIF4A2*-CNV and other growth traits.

The results within the QC population showed that HW had a positive highly significant correlation with HG (r = 0.800**) and BW (0.800**), with a moderately significant correlation with WH

TABLE 5 Association analysis of EIF4A2-CNV and growth traits of Pinan cattle (PN)

		CNV types (mean ± SE)						
		Deletion		Normal	Duplication			
Breed	Growth traits	CN = 0 (*n* = 15)	CN = 1 (*n* = 33)	CN = 2 (*n* = 34)	CN = 3 (*n* = 24)	CN = 4 (*n* = 12)	CN ≥ 5 (*n* = 15)	*p*-value
PN	WH (cm)	124.73 ± 1.57	126.24 ± 0.84	125.56 ± 0.78	125.96 ± 1.18	126.75 ± 1.87	126.93 ± 1.88	0.888
	HHC (cm)	132.07 ± 1.51	133.36 ± 0.79	132.41 ± 0.80	133.75 ± 1.10	134.92 ± 1.64	133.60 ± 1.93	0.701
	BL (cm)	146.33 ± 2.75	148.21 ± 1.27	147.94 ± 1.60	150.38 ± 1.73	153.00 ± 3.03	151.47 ± 2.97	0.337
	HG (cm)	173.80 ± 3.21	174.24 ± 2.12	175.47 ± 1.68	173.92 ± 2.13	178.67 ± 3.71	177.40 ± 3.20	0.784
	HW (cm)	46.13^{b} ± 1.10	46.97^{b} ± 0.50	47.65^{a,b} ± 0.63	47.35^{a,b} ± 0.61	50.42^{a} ± 1.22	47.67^{a,b} ± 0.92	0.048
	RL (cm)	48.47 ± 0.73	48.58 ± 0.55	49.47 ± 0.56	48.75 ± 0.71	50.25 ± 1.07	49.27 ± 0.97	0.625

^{a,b} indicate that $p < 0.01$ is significant.

TABLE 6 Association analysis of EIF4A2-CNV and growth traits of Jiaxian cattle (JX)

		CNV types (mean ± SE)						
		Deletion		Normal	Duplication			
Breed	Growth traits	CN = 0 (*n* = 16)	CN = 1 (*n* = 17)	CN = 2 (*n* = 11)	CN = 3 (*n* = 11)	CN = 4 (*n* = 14)	CN ≥ 5 (*n* = 14)	*p*-value
JX	WH (cm)	124.69 ± 1.61	122.71 ± 1.49	124.50 ± 1.59	125.32 ± 1.82	123.93 ± 1.93	124.29 ± 2.02	0.929
	HHC (cm)	124.13 ± 1.54	123.59 ± 1.23	125.27 ± 0.98	125.77 ± 2.25	123.07 ± 1.79	119.18 ± 5.89	0.662
	BL (cm)	144.47 ± 2.54	143.82 ± 2.68	142.27 ± 3.07	145.09 ± 1.76	143.86 ± 3.81	142.14 ± 2.17	0.976
	HG (cm)	175.88 ± 2.94	173.94 ± 3.20	177.82 ± 3.75	176.27 ± 2.91	168.43 ± 6.20	173.93 ± 3.43	0.655
	CW (cm)	37.94 ± 1.13	38.21 ± 1.28	39.09 ± 1.25	38.86 ± 1.14	37.64 ± 0.89	38.07 ± 2.17	0.982
	CD (cm)	64.59 ± 1.30	65.44 ± 1.26	63.50 ± 1.39	64.50 ± 1.37	63.82 ± 1.94	66.61 ± 4.44	0.943
	HW (cm)	46.22 ± 1.15	45.53 ± 0.94	45.32 ± 0.78	44.55 ± 1.22	44.71 ± 1.63	44.81 ± 0.93	0.907
	HBW (cm)	23.22 ± 1.51	22.24 ± 1.22	22.41 ± 2.01	21.09 ± 1.74	22.86 ± 1.88	26.0 ± 42.22	0.508
	RL (cm)	46.59 ± 1.14	44.88 ± 0.98	45.91 ± 1.00	46.32 ± 0.70	46.21 ± 1.40	44.46 ± 1.95	0.802
	BW (kg)	418.23 ± 19.38	408.93 ± 21.22	420.57 ± 22.59	418.951 ± 5.91	391.96 ± 34.15	402.51 ± 20.19	0.950

^{a,b} indicate that $p < 0.01$ is significant.

($r = 0.501^{**}$), HHC ($r = 0.484^{**}$), BL ($r = 0.594^{**}$), CW ($r = 0.659^{**}$), CD ($r = 0.619^{**}$) and RL ($r = 0.752^{**}$), with a significant correlation with HBW ($r = 0.249^{*}$). RL has a moderately significant correlation with WH ($r = 0.520^{**}$), HHC ($r = 0.412^{**}$), BL ($r = 0.666^{**}$), HG ($r = 0.719^{**}$), CW ($r = 0.574^{**}$), CD ($r = 0.601^{**}$) and BW ($r = 0.754^{**}$). A significant correlation was observed between RL and HBW ($r = 0.256^{*}$; Table 7).

The findings in PN indicated that HW had a positive moderately significant correlation with WH ($r = 0.592^{**}$), HHC ($r = 0.580^{**}$), BL ($r = 0.637^{**}$), HG ($r = 0.663^{**}$) and RL ($r = 0.706^{**}$; Table 8).

In YL, HG had a positive moderately significant correlation with WH ($r = 0.518^{**}$) and BW ($r = 0.634^{**}$), with a significant correlation with HHC ($r = 0.234^{**}$), BL ($r = 0.349^{**}$), CW ($r = 0.321^{**}$), CD ($r = 0.271^{**}$), HBW ($r = 0.372^{**}$), RL ($r = 0.367^{**}$) and not significant with HW ($r = 0.107$). For CD, there was a positive moderately significant correlation between CD and RL ($r = 0.521^{**}$). CD had a significant correlation with WH ($r = 0.301^{**}$), HHC ($r = 0.182^{*}$), BL ($r = 0.446^{**}$), CW ($r = 0.299^{**}$) and BW ($r = 0.262^{**}$), with a negative highly significant correlation with HW ($r = -0.181^{*}$), and was not significant with HBW ($r = 0.127$). RL had a positive moderately significant correlation with BL ($r = 0.514^{**}$), with a significant correlation with WH ($r = 0.356^{**}$), CW ($r = 0.304^{**}$), HBW ($r = 0.242^{**}$) and BW ($r = 0.339^{**}$), with a negative significant correlation with HW ($r = -0.206^{**}$) and with no significance with HHC ($r = 0.134$; Table 9).

4 | DISCUSSION

The *EIF4A2* gene has been identified as a gene biomarker related to the upregulation of cell proliferation in rheumatoid arthritis (Lu et al., 2012). Some papers have shown that the abnormal expression of *EIF4A2* is significantly associated with several types of cancer, including non-small cell lung cancer (Shaoyan et al., 2013) and malignant peripheral neurilemmoma (Oblinger et al., 2016). The above report shows that the *EIF4A2* gene plays an important role in cell activity and human

TABLE 7 Correlation analysis of growth traits in QC

	WH	HHC	BL	HG	CW	CD	HW	HBW	RL	BW
WH		0.804**	0.567**	0.580**	0.386**	0.594**	0.501**	0.083	0.520**	0.621**
HHC	0.000		0.552**	0.544**	0.356**	0.451**	0.484**	0.042	0.412**	0.580**
BL	0.000	0.000		0.697**	0.477**	0.572**	0.594**	0.275**	0.666**	0.842**
HG	0.000	0.000	0.000		0.784**	0.757**	0.800**	0.437**	0.719**	0.967**
CW	0.000	0.000	0.000	0.000		0.670**	0.659**	0.513**	0.574**	0.740**
CD	0.000	0.000	0.000	0.000	0.000		0.619**	0.504**	0.601**	0.749**
HW	0.000	0.000	0.000	0.000	0.000	0.000		0.249*	0.752**	0.800**
HBW	0.411	0.681	0.006	0.000	0.000	0.000	0.012		0.256*	0.412**
RL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010		0.754**
BW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Note: The upper triangle is the correlation coefficient, and the lower triangle is the *p*-value.
*significant correlation ($p < 0.05$).
** indicates an extremely significant correlation ($p < 0.01$).

TABLE 8 Correlation analysis of growth traits in PN

	WH	HHC	BL	HG	HW	RL
WH		0.942**	0.688**	0.748**	0.592**	0.688**
HHC	0.000		0.677**	0.743**	0.580**	0.659**
BL	0.000	0.000		0.709**	0.637**	0.737**
HG	0.000	0.000	0.000		0.663**	0.728**
HW	0.000	0.000	0.000	0.000		0.706**
RL	0.000	0.000	0.000	0.000	0.000	

Note: The upper triangle is the correlation coefficient, and the lower triangle is the *p*-value.
*significant correlation ($p < 0.05$).
** indicates an extremely significant correlation ($p < 0.01$).

disease. Studies have also shown that the *EIF4A2* gene plays a vital role in muscle development. Human *EIF4A2* was thought to have a possible influence on muscle cells because it is expressed in a variety of normal tissues and is especially highly expressed in skeletal muscle (Sudo et al., 1995). H. Wang et al. showed that the *EIF4A2* gene is expressed in 11 tissues of pigs and is abundant in muscle. At the same time, the pattern of *EIF4A2* expression during embryonic muscle development and continuing through adulthood indicated *EIF4A2* plays a significant role in the formation and development of muscle tissue (H. Wang et al., 2007). In addition, the EIF4A family is highly conserved among mammals through evolution. Therefore, we explored the expression in bovine tissues. Our results showed that the bovine *EIF4A2* gene was widely expressed in the tissues, so it is speculated to play an important role in animal growth and development. Meanwhile, the expression of *EIF4A2* was highest and indicated that it plays an important role in muscle tissue. In addition, there is an association between muscle and bone. Skeletal muscle secretes bone inducible factors, which promote bone development and increase bone mass (Tagliaferri et al., 2015). In livestock, meat yield is also reflected in bovine BW. Based on these reports, we then explored the correlation between CNV of the *EIF4A2* gene and the growth traits of cattle.

According to Figure 3 and Table 2, the CNV distributions of the four cattle breeds are similar, which may be the result of long-term artificial and natural selection (Lehnert et al., 2007).

Beckmann et al. (2007) speculated that CNVs may represent the main genetic component of phenotypic variation and the source of genetic variation between individuals and human populations of different ethnic origins. Moreover, qPCR can be used to verify the CNV situation in the population, and at the same time, it can be associated with objective traits, which has the potential of molecular marker-assisted breeding. With the completion of bovine genome sequencing, bovine genomic CNVs have become a research hotspot. For example, Hou et al. (2011) found that the loss, median and gain types of *SCP2*, *ULBP* and *WC1.1* genes were related to lipid transport metabolism in cattle; Stothard et al. (2011) found that the change in copy number of the *PLA2G2D* gene was related to milk yield and meat quality. Bickhart et al. (2012) found that the involvement of the repetitive myosin heavy chain 1 gene in skeletal muscle development was related to bovine growth traits. Yang et al. (2020), through the association analysis of *MLLT10* gene CNVs and the growth traits of Chinese native cattle, found significant differences between the type of CNV and many growth traits. Shi et al. (2016) found that the expression of the *LEPR* gene decreased with the increase of copy numbers. In the Nanyang cattle population, the gain and median copy number types were better than the loss types in BW, body height and BL. Studies have shown that the CNVs of some genes are related to the expression of genes at the corresponding loci and various economic traits of cattle.

Our study indicated that *EIF4A2*-CNV has an essential impact on HW and RL in QC, HG, CD and RL in YL and HW in PN. On the basis of association analysis, QC with CN = 0/≥5, relative traits were promoted. However, YL with duplication or CN = 0, cattle perform better on certain growth traits. It is easy to find that this CNV had different effects on growth traits among these four cattle breeds, and the eminent genotypes were not completely consistent, which could be explained as being influenced by hereditary backgrounds (Lehnert et al., 2007) and sample size. JX cattle are a native breed in Henan

TABLE 9 Correlation analysis of growth traits in YL

	WH	HHC	BL	HG	CW	CD	HW	HBW	RL	BL
WH		0.419**	0.339**	0.518**	0.271**	0.301**	0.180*	0.362**	0.356**	0.627**
HHC	0.000		0.063	0.234**	0.087	0.182*	0.168*	0.066	0.134	0.333**
BL	0.000	0.393		0.349**	0.288**	0.446**	−0.047	.318**	0.514**	0.509**
HG	0.000	0.001	0.000		0.321**	0.271**	0.107	0.372**	0.367**	0.634**
CW	0.000	0.241	0.000	0.000		0.299**	0.071	0.087	0.304**	0.338**
CD	0.000	0.013	0.000	0.000	0.000		−0.181*	0.127	0.521**	0.262**
HW	0.015	0.023	0.530	0.150	0.339	0.014		0.156*	−0.206**	0.336**
HBW	0.000	0.371	0.000	0.000	0.238	0.086	0.035		0.242**	0.368**
RL	0.000	0.069	0.000	0.000	0.000	0.000	0.005	0.001		0.339**
BW	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Note: The upper triangle is the correlation coefficient, and the lower triangle is the *p*-value.
*significant correlation ($p < 0.05$).
** indicates an extremely significant correlation ($p < 0.01$).

Province, and their living environment is different from that of QC cattle. Meanwhile, its genetic background is different from that of YL and PN, which are bred by hybridisation. In addition, the number of JX cattle used for the test was smaller than that of other groups. The above two points may be responsible for the absence of significance in this group. In addition, although each growth trait involves a different part of the trunk, their growth may interact with each other and thus increase or decrease simultaneously (Zhou et al., 2016). Therefore, we conducted a simple correlation analysis on the growth traits of bovine breeds affected by CNV and focused on comparing the correlation of these four traits (HW, BL, HG, CD) with other traits. According to Tables 7 and 8, HW had a positive significant correlation with HG, WH, HHC, BL and RL. RL had a positive significant correlation with WH, HHC, BL, HG and HW. The correlation between these traits was moderate or high. Therefore, combined with the previous association analysis (Tables 3 and 5), although some of these traits are not statistically significant, they are most likely also affected by *EIF4A2*-CNV. Regarding YL, the situation was slightly different. HW had a negative significant correlation with CD and RL. This may be because its genetic background is quite different from that of other cattle breeds. This is why QC with zero and five copies had a better growth advantage in both HW and RL, while YL with four and five copies had a better development in HG, CD and RL.

In conclusion, this is the first detection and validation of CNV of the *EIF4A2* gene in four Chinese cattle breeds. The distribution of CNV among varieties was almost consistent. At the same time, *EIF4A2*-CNV can have a certain degree of useful influence on different growth traits of Chinese cattle. Studying the effects of *EIF4A2*-CNVs on growth traits is an important way to screen molecular markers for breeding. This study may provide a reference for the application of CNVs as a new molecular marker in cattle breeding.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

The protocols used in this study and for the animals were recognised by the Faculty Animal Policy and Welfare Committee of Northwest A&F University (FAPWC-NWAFU, Protocol number, NWAFAC1008).

AUTHOR CONTRIBUTIONS

Methodology: Mengyang Peng and Yifan Wen. *Conceptualisation*: Zijing Zhang, Mengyang Peng and Yifan Wen. F*ormal analysis*: Mengyang Peng and Yifan Wen. *Investigation*: Zijing Zhang, Yanan Chai and Xian Liu. *Resources and data curation*: Juntong Liang, Peng Yang. *Visualisation and supervision*: Jungang Li, Yajun Huang, Lijuan Li, Weihong Huang, Zengfang Qi, Guojie Yang, Fuying Chen, Qiaoting Shi and Zhiming Li. *Validation*: Baorui Ru, Chuzhao Lei, Eryao Wang and Yongzhen Huang. *Writing–original draft preparation*: Mengyang Peng and Yifan Wen. *Writing–review and editing*: Yifan Wen.

DATA AVAILABILITY STATEMENT

Research data are not shared.

REFERENCES

Andreou, A. Z., & Klostermeier, D. (2013). The DEAD-box helicase eIF4A: Paradigm or the odd one out? *RNA Biology*, *10*, 19–32.

Barendse, W., Vaiman, D., Kemp, S. J., Sugimoto, Y., Armitage, S. M., Williams, J. L., Sun, H. S., Eggen, A., Agaba, M., Aleyasin, S. A., Band, M., Bishop, M. D., Buitkamp, J., Byrne, K., Collins, F., Cooper, L., Coppettiers, W., Denys, B., Drinkwater, R. D., … Zaragoza, P. (1997). A medium-density genetic linkage map of the bovine genome. *Mammalian Genome*, *8*, 21–28.

Beckmann, J. S., Estivill, X., & Antonarakis, S. E. (2007). Copy number variants and genetic traits: Closer to the resolution of phenotypic to genotypic variability. *Nature Reviews Genetics*, *8*, 639–646.

Bickhart, D. M., Hou, Y., Schroeder, S. G., Alkan, C., Cardone, M. F., Matukumalli, L. K., Song, J., Schnabel, R. D., Ventura, M., Taylor, J. F., Garcia, J. F., Van Tassell, C. P., Sonstegard, T. S., Eichler, E. E., & Liu, G. E. (2012). Copy number variation of individual cattle genomes using next-generation sequencing. *Genome Research*, *22*, 778–790.

Bush, M. S., Hutchins, A. P., Jones, A. M. E., Naldrett, M. J., Jarmolowski, A., Lloyd, C. W., & Doonan, J. H. (2009). Selective recruitment of proteins to 5' cap complexes during the growth cycle in Arabidopsis. *Plant Journal*, *59*, 400–412.

Casas, E., White, S. N., Riley, D. G., Smith, T. P. L., Brenneman, R. A., Olson, T. A., Johnson, D. D., Coleman, S. W., Bennett, G. L., & Chase, C. C. (2005). Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in Bos indicus cattle. *Journal of Animal Science*, *83*, 13–19.

Chen, Z.-H., Qi, J.-J., Wu, Q.-N., Lu, J.-H., Liu, Z.-X., Wang, Y., Hu, P.-S., Li, T., Lin, J.-F., Wu, X.-Y., Miao, L., Zeng, Z.-L., Xie, D., Ju, H.-Q., Xu, R.-H., & Wang, F. (2019). Eukaryotic initiation factor 4A2 promotes experimental metastasis and oxaliplatin resistance in colorectal cancer. *Journal of Experimental & Clinical Cancer Research*, *38*, 196.

Cicconardi, F., Chillemi, G., Tramontano, A., Marchitelli, C., Valentini, A., Ajmone-Marsan, P., & Nardone, A. (2013). Massive screening of copy number population-scale variation in Bos taurus genome. *Bmc Genomics*, *14*, 124.

Elsik, C. G., Tellam, R. L., Worley, K. C., Gibbs, R. A., Muzny, D. M., & Weinstock, G. M., Adelson, D. L., Eichler, E. E., Elnitski, L., Guigó, R., Hamernik, D. L., Kappes, S. M., Lewin, H. A., Lynn, D. J., Nicholas, F. W., Reymond, A., Rijnkels, M., Skow, L. C., Zdobnov, E. M., … Zhao, F.-Q. (2009). The genome sequence of taurine cattle: A window to ruminant biology and evolution. *Science*, *324*, 522–528.

Feuk, L., Carson, A. R., & Scherer, S. W. (2006). Structural variation in the human genome. *Nature Reviews Genetics*, *7*, 85–97.

Heaton, M. P., Harhay, G. P., Bennett, G. L., Stone, R. T., Grosse, W. M., Casas, E., Keele, J. W., Smith, T. P. L., Chitko-Mckown, C. G., & Laegreid, W. W. (2002). Selection and use of SNP markers for animal identification and paternity analysis in the U.S. beef cattle. *Mammalian Genome*, *13*, 272–281.

Hou, Y., Bickhart, D. M., Hvinden, M. L., Li, C., Song, J., Boichard, D. A., Fritz, S., Eggen, A., DeNise, S., Wiggans, G. R., Sonstegard, T. S., Van Tassell, C. P., & Liu, G. E. (2012). Fine mapping of copy number variations on two cattle genome assemblies using high density SNP array. *BMC Genomics [Electronic Resource]*, *13*, 376.

Hou, Y., Liu, G. E., Bickhart, D. M., Cardone, M. F., Wang, K., Kim, E. S., Matukumalli, L. K., Ventura, M., Song, J., VanRaden, P. M., Sonstegard, T. S., & Van Tassell, C. P. (2011). Genomic characteristics of cattle copy number variations. *BMC Genomics [Electronic Resource]*, *12*, 127.

Huang, Y., Li, Y., Wang, X., Yu, J., Cai, Y., Zheng, Z., Li, R., Zhang, S., Chen, N., Nanaei, H. A., Hanif, Q., Chen, Q., Fu, W., Li, C., Cao, X., Zhou, G., Liu, S., He, S., Li, W., … Jiang, Y. (2021). An atlas of CNV maps in cattle, goat and sheep. *Science China Life Sciences*, *64*(10), 1747–1764.

Jiang, Li., Jiang, J., Wang, J., Ding, X., Liu, J., & Zhang, Q. (2012). Genome-wide identification of copy number variations in Chinese Holstein. *Plos One*, *7*, e48732.

Kang, X., Li, M., Liu, M., Liu, S., Pan, M. G., Wiggans, G. R., Rosen, B. D., & Liu, G. E. (2020). Copy number variation analysis reveals variants associated with milk production traits in dairy goats. *Genomics*, *112*, 4934–4937.

Lehnert, S. A., Reverter, A., Byrne, K. A., Wang, Y., Nattrass, G. S., Hudson, N. J., & Greenwood, P. L. (2007). Gene expression studies of developing bovine longissimus muscle from two different beef cattle breeds. *BMC Developmental Biology*, *7*, 95.

Levy, S., Sutton, G., Ng, P. C., Feuk, L., Halpern, A. L., Walenz, B. P., Axelrod, N., Huang, J., Kirkness, E. F., Denisov, G., Lin, Y., Macdonald, J. R., Pang, A. W. C., Shago, M., Stockwell, T. B., Tsiamouri, A., Bafna, V., Bansal, V., Kravitz, S. A., … Venter, J. C. (2007). The diploid genome sequence of an individual human. *PLoS Biology*, *5*, e254.

Li, W., Liu, D., Tang, S., Li, D., Han, R., Tian, Y., Li, H., Li, G., Li, W., Liu, X., Kang, X., & Li, Z. (2019). A multiallelic indel in the promoter region of the cyclin-dependent kinase inhibitor 3 gene is significantly associated with body weight and carcass traits in chickens. *Poultry Science*, *98*, 556–565.

Liu, M., Gong, C., Xu, R., Chen, Yu., & Wang, X. (2019). MicroRNA-5195-3p enhances the chemosensitivity of triple-negative breast cancer to paclitaxel by downregulating EIF4A2. *Cellular & Molecular Biology Letters*, *24*, 47.

Lu, C., Niu, X., Xiao, C., Chen, G., Zha, Q., Guo, H., Jiang, M., & Lu, A. (2012). Network-based gene expression biomarkers for cold and heat patterns of rheumatoid arthritis in traditional Chinese medicine. *Evidence-Based Complementary and Alternative Medicine*, *2012*, 203043.

Ndzinu, J. K., Takeuchi, H., Saito, H., Yoshida, T., & Yamaoka, S. (2018). eIF4A2 is a host factor required for efficient HIV-1 replication. *Microbes and Infection*, *20*, 346—352.

Nielsen, P. J., & Trachsel, H. (1988). The mouse protein synthesis initiation factor 4A gene family includes two related functional genes which are differentially expressed. *EMBO Journal*, *7*, 2097–2105.

Oblinger, J. L., Burns, S. S., Akhmametyeva, E. M., Huang, J., Pan, L., Ren, Y., Shen, R., Miles-Markley, B., Moberly, A. C., Kinghorn, A. D, Welling, D. B, & Chang, L.-S. (2016). Components of the eIF4F complex are potential therapeutic targets for malignant peripheral nerve sheath tumors and vestibular schwannomas. *Neuro-Oncology*, *18*, 1265–1277.

Özşensoy, Y., & Kara, H. (2019). Investigation of GH and GHR Alu I gene polymorphisms on meat yields in Anatolian water buffalo breed using PCR-RFLP method. *Turkish Journal of Zoology*, *43*, 560–565.

Peng, S.-J., Cao, X.-K., Dong, D., Liu, M., Hao, D., Shen, X.-M., Huang, Y.-Z., Lei, C.-Z., Ma, Y., Bai, Y.-Y., Hu, L.-Y., Qi, X.-L., Chaogetu, B., & Chen, H. (2020). Integrative analysis of APOL3 gene CNV for adult cattle stature. *Animal Biotechnology*, *31*, 440–446.

Quanico, J., Franck, J., Cardon, T., Leblanc, E., Wisztorski, M., Salzet, M., & Fournier, I. (2017). NanoLC-MS coupling of liquid microjunction microextraction for on-tissue proteomic analysis. *BBA-Proteins and Proteomics*, *1865*, 891–900.

Redon, R., Ishikawa, S., Fitch, K. R., Feuk, L., Perry, G. H., Andrews, T. D, Fiegler, H., Shapero, M. H., Carson, A. R., Chen, W., Cho, E. K., Dallaire, S., Freeman, J. L., González, J. R., Gratacòs, M., Huang, J., Kalaitzopoulos, D., Komura, D., Macdonald, J. R., … Hurles, M. E. (2006). Global variation in copy number in the human genome. *Nature*, *444*, 444–454.

Shaoyan, X., Juanjuan, Y., Yalan, T., Ping, H., Jianzhong, L., & Qinian, W. (2013). Downregulation of EIF4A2 in non-small cell lung cancer associates with poor prognosis. *Clinical Lung Cancer*, *14*, 658–665.

Shi, T., Xu, Y., Yang, M., Huang, Y., Lan, X., Lei, C., Qi, X., Yang, X., & Chen, H. (2016). Copy number variations at LEPR gene locus associated with gene expression and phenotypic traits in Chinese cattle. *Animal Science Journal*, *87*, 336–343.

Stothard, P., Choi, J. W., Basu, U., Sumner-Thomson, J. M., Meng, Y., Liao, X., & Moore, S. S. (2011). Whole genome resequencing of black Angus and Holstein cattle for SNP and CNV discovery. *BMC Genomics [Electronic Resource]*, *12*, 559.

Sudo, K., Takahashi, E., & Nakamura, Y. (1995). Isolation and mapping of the human EIF4A2 gene homologous to the murine protein synthesis initiation factor 4A-II gene Eif4a2. *Cytogenetics and Cell Genetics*, *71*, 385–388.

Tagliaferri, C., Wittrant, Y., Davicco, M.-J., Walrand, S., & Coxam, V. (2015). Muscle and bone, two interconnected tissues. *Ageing Research Reviews*, *21*, 55–70.

Wang, H., Wang, H., Zhu, Z., Yang, S., & Li, K. (2007). Molecular cloning, mapping, and expression analysis of the EIF4A2 gene in pig. *Biochemical Genetics*, *45*(1-2), 51–62.

Wang, X., Nahashon, S., Feaster, T. K., Bohannon-Stewart, A., & Adefope, N. (2010). An initial map of chromosomal segmental copy number variations in the chicken. *BMC Genomics [Electronic Resource]*, *11*, 351.

Wilczynska, A., Gillen, S L., Schmidt, T., Meijer, H. A., Jukes-Jones, R., Langlais, C., Kopra, K., Lu, W.-T., Godfrey, J. D., Hawley, B. R., Hodge, K., Zanivan, S., Cain, K., Le Quesne, J., & Bushell, M. (2019). eIF4A2 drives repression of translation at initiation by Ccr4-Not through purine-rich motifs in the 5′ UTR. *Genome Biology*, *20*, 262.

Xia, X., Zhang, S., Zhang, H., Zhang, Z., Chen, N., Li, Z., Sun, H., Liu, X., Lyu, S., Wang, X., Li, Z., Yang, P., Xu, J., Ding, X., Shi, Q., Wang, E., Ru, B., Xu, Z., Lei, C., … Huang, Y. (2021). Assessing genomic diversity and signatures of selection in Jiaxian Red cattle using whole-genome sequencing data. *BMC Genomics [Electronic Resource]*, *22*(1), 43.

Xu, Z., Wang, X., Zhang, Z., An, Q., Wen, Y., Wang, D., Liu, X., Li, Z., Lyu, S., Li, L., Wang, E., Ru, B., Xu, Z., & Huang, Y. (2020). Copy number variation of the CADM2 gene revealed its association with growth traits across Chinese Capra hircus (goat) populations. *Gene*, *741*, 144519.

Yang, P., Zhang, Z., Xu, J., Qu, K., Lyv, S., Wang, X., Cai, C., Li, Z., Wang, E., Xie, J., Ru, B., Xu, Z., Lei, C., Chen, H., Huang, B., & Huang, Y. (2020). The association of the copy number variation of the mllt10 gene with growth traits of Chinese cattle. *Animals (Basel)*, *10*(2), 250.

Zhou, Y., Utsunomiya, Y. T., Xu, L., Hay, E. H., Bickhart, D. M., Alexandre, P. A., Rosen, B. D., Schroeder, S. G., Carvalheiro, R., de Rezende Neves, H. H., Sonstegard, T. S., Tassell, C. P. V., Ferraz, J. B. S., Fukumasu, H., García, J. F., & Liu, G. E. (2016). Genome-wide CNV analysis reveals variants associated with growth traits in Bos indicus. *BMC Genomics [Electronic Resource]*, *17*, 419.

SUPPORTING INFORMATION

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