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

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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 (Elisk 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 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 Ozesensoy.
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 (ddH2O) 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 reverse primers (10 pmol/µl) 0.2 µl, DNA (10 ng/µl) 1 µl and ddH2O 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^\text{ΔΔCT} 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: $P_{ijk} = \mu + A_i + S_j + CN_V_k + e_{ijk}$. $P_{ijk}$ is the phenotypic traits record of the $i$th individual growth traits; $\mu$ is the overall mean of each trait. $A_i$ is the effect of age. $S_j$ is the effect due to sex. $CN_V_k$ 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.

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| Locus     | Primer sequences(5’ to 3’)                  | Amplification length (bp) |
|-----------|---------------------------------------------|---------------------------|
| EIF4A2    | F1:5’- ACCAGCCATCTCAGCTGACC-3’             | 156                       |
|           | R1:5’- GTGAAAAAGGAAATTGACCC-3’             |                           |
| BTF3      | F2:5’- AACCGAGAGATCGCCAA-3’                | 166                       |
|           | R2:5’- TCGGGAATGGCCTCCTG-3’                |                           |
| EIF4A2    | F1:5’- CACAGAGAGCTATTATGCATG-3’            | 200                       |
|           | R1:5’- TCTCAAGTGCCAGAATTACC-3’             |                           |
| GAPDH     | F2:5’- AAGTTCAACGGCAGCTACA-3’              | 365                       |
|           | R2:5’- GTCAATACTCCCTCCAGAT-3’              |                           |

#### TABLE 1 Information on the primers used in this study

| DNA level | RNA level |
|-----------|-----------|
| EIF4A2    | EIF4A2    |
| BTF3      | GAPDH     |
|           |           |
### 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.

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.

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

| Breed  | CNV types | Deletion | Normal | Duplication |
|--------|-----------|----------|--------|-------------|
| QC     | CN = 0    | 0.08     | 0.28   | 0.27        | 0.15        | 0.11        | 0.11        |
| YL     | CN = 1    | 0.071    | 0.279  | 0.284       | 0.208       | 0.076       | 0.081       |
| PN     | CN = 2    | 0.11     | 0.25   | 0.26        | 0.18        | 0.09        | 0.11        |
| JX     | CN = 3    | 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.

### 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-CN V 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-CN V 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-CN V (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)

| Breed  | Growth traits      | CNV types (mean ± SE) |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
|--------|--------------------|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|        |                    | Deletion              | Normal            | Duplication       |                  |                  |                  |                  |                  |                  |                  |                  |
|        |                    | 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 ± 2.03         | 42.64 ± 0.85      | 44.04 ± 1.03      | 41.20 ± 1.25     | 41.18 ± 2.09     | 44.45 ± 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 ± 2.15         | 43.00 ± 0.61      | 43.85 ± 0.73      | 42.53 ± 1.16     | 42.41 ± 1.43     | 45.59 ± 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             |                  |                  |                  |                  |

ab indicate that p < 0.01 is significant.

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

| Breed  | Growth traits      | CNV types (mean ± SE) |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
|--------|--------------------|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|        |                    | Deletion              | Normal            | Duplication       |                  |                  |                  |                  |                  |                  |                  |                  |
|        |                    | 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 ± 2.09         | 193.43 ± 1.57     | 195.46 ± 1.48     | 196.29 ± 1.23    | 198.73 ± 1.86    | 202.81 ± 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 ± 1.14          | 66.95 ± 0.69      | 68.79 ± 0.78      | 70.78 ± 1.03     | 73.67 ± 1.46     | 71.38 ± 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 ± 0.87          | 49.25 ± 0.41      | 49.63 ± 0.47      | 51.10 ± 0.50     | 51.33 ± 0.36     | 53.31 ± 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             |                  |                  |                  |                  |

ab 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-CNVS 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)

| Breed | Growth traits | CNV types (mean ± SE) |
|-------|---------------|-----------------------|
|       |               | Deletion    | Normal     | Duplication |
|       |               | 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 ± 1.10   | 46.97 ± 0.50   | 47.65 ± 0.63   | 47.35 ± 0.61   | 50.42 ± 1.22   | 47.67 ± 0.92   | 0.048 |
|       | RL (cm)       | 48.47 ± 0.73   | 48.58 ± 0.55   | 49.47 ± 0.56   | 48.75 ± 0.71   | 50.23 ± 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)

| Breed | Growth traits | CNV types (mean ± SE) |
|-------|---------------|-----------------------|
|       |               | Deletion    | Normal     | Duplication |
|       |               | 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 ± 1.13   | 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 | 418.95 ± 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**), HGC (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**), HGC (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


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 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 inductive 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.

### 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).
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. Formal 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: 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.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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