Polymorphisms of *CRELD1* and *DNAJC30* and their relationship with chicken carcass traits

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ABSTRACT Carcass traits play important roles in the broiler industry and single nucleotide polymorphism (**SNP**) can be efficient molecular markers for marker-assisted breeding of chicken carcass traits. Based on our previous RNA-seq data (accession number GSE58755), cysteine rich with epidermal growth factor like domains 1 (***CRELD1***) and DnaJ heat shock protein family member C30 (***DNAJC30***) are differentially expressed in breast muscle between white recessive rock chicken (**WRR**) and Xinghua chicken (**XH**). In this study, we further characterize the potential function and SNP mutation of *CRELD1* and *DNAJC30* in chicken for the first time. According to protein interaction network and enrichment analysis, *CRELD1* and *DNAJC30* may play some roles in chicken muscle development and fat deposition. In WRR and XH, the results of the relative tissue expression pattern demonstrated that *CRELD1* and *DNAJC30* are not only differentially expressed in breast muscle but also leg muscle and abdominal fat. Therefore, we identified 5 SNP sites of *CRELD1* and 7 SNP sites of *DNAJC30* and genotyped them in an F_2 chicken population. There are 4 sites of *CRELD1* and 3 sites of *DNAJC30* are associated with chicken carcass traits like breast muscle weight, body weight, dressed weight, leg weight percentage, eviscerated weight with giblet percentage, intermuscular adipose width, shank length, and girth. These results suggest that the SNP sites of *CRELD1* and *DNAJC30* can be potential molecular markers to improve the chicken carcass traits and lay the foundation for marker-assisted selection.

Key words: broiler, *CRELD1*, *DNAJC30*, carcass trait

INTRODUCTION

Domestic chicken (*Gallus gallus* domesticus) has become the most extensively distributed poultry in the world since their domestication (Scanes, 2007; Wang et al., 2020). Chicken also plays an irreplaceable role in the human diet nowadays and the production of chicken meat and eggs has been increasing quickly in recent years (Connerton et al., 2018; Cartoni Mancinelli et al., 2022). However, poultry is the terminal host of various diseases like avian influenza (Fusaro et al., 2019; Escalera-Zamudio et al., 2020). For this reason, the European Union has already placed a ban on live bird imports to prevent the spread of avian influenza, and the trade controls and import restrictions of live poultry can control the epidemic effectively (Reino et al., 2017; Chen et al., 2020).

Based on the above background, slaughtering poultry and storing it on ice for transportation to sale have gradually replaced the live poultry market, which makes carcass trait become one of the most important traits of poultry, especially in chicken. Carcass traits are one kind of quantitative trait and they can't be measured without slaughter, which becomes a major challenge to the further development of the poultry industry (Uemoto et al., 2009). Skeletal muscle development and fat deposition have a significant impact on poultry carcass traits (Zerehdaran et al., 2004; Lotfi et al., 2011; Li et al., 2021).

Xinhua chicken (**XH**) and white recessive rock chicken (**WRR**) are kinds of leaner and hypertrophic broiler respectively (Hu et al., 2013; Teng et al., 2019). WRR demonstrates significantly faster muscle growth and lower fat deposition compared to XH in chicken carcass traits due to the complex gene regulatory network (Qiu et al., 2006; Ouyang et al., 2015; Li et al., 2019). In our previous study, RNA sequencing was performed to construct a LncRNA-microRNA-gene interaction network between WRR and XH's breast muscle tissues (Li et al., 2019). Among all differentially expressed genes, *CRELD1* and DnaJ heat shock protein family member C30 (*DNAJC30*) are the top 50 significantly upregulated genes in WRR's breast muscle compared to XH, which may play some roles in chicken carcass traits.

Cysteine rich with epidermal growth factor like domains 1 (*CRELD1*) is a multifunctional gene that mostly plays important roles in myocardial development (Beckert et al., 2021) and immune system homeostasis (Bonaguro et al., 2020). Most human fetal and adult tissues express *CRELD1* and the gene expression in skeletal muscle and adult heart has a higher level (Rupp et al., 2002). Besides, *CRELD1* is identified as a key gene required for the synaptic expression of ionotropic acetylcholine receptors in C2C12 myoblasts (D'Alessandro et al., 2018). Studies revealed that *CRELD1* highly expresses and has an important biological function in skeletal muscle, but its relationship with chicken carcass traits are not yet investigated.

Heat shock proteins family (**HSP**) is one kind of molecular chaperone family that plays critical roles in protein folding and assembly (Gomez-Pastor et al., 2018). Many studies have shown that HSP play an important role in skeletal muscle development (Du SJ et al., 2008; Sin et al., 2019). *DNAJC30* is one of the HSP and it has been reported as an auxiliary component of ATP-synthase machinery in mitochondria (Tebbenkamp et al., 2018). In addition, *DNAJC30* is also the protein chaperone required for the exchange of damaged complex I subunits in mitochondria (Stenton et al., 2021). ATP-synthase and mitochondrial complex I subunits are highly correlated with the metabolism and development of muscle and fat, which suggested that *DNAJC30* may play some roles in skeletal muscle and fat metabolism (Hong et al., 2014; Sanchez-Gonzalez et al., 2022). However, there are no reports about their function in skeletal muscle development and their relationship with the chicken carcass.

Single nucleotide polymorphism (**SNP**) is an important genetic factor that is usually used for marker-assisted breeding of economic traits in agriculture (Sun et al., 2020). Studies have shown SNP in functional genes can be significantly associated with chicken carcass traits (Lei et al., 2005; Lu et al., 2012). In this study, we first examined the relative tissue expression pattern of *CRELD1* and *DNAJC30* and found that these 2 genes were significantly differentially expressed in WRR and XH's breast muscle,

leg muscle, and abdominal fat. After that, SNP sites in *CRELD1* and *DNAJC30* were detected and significantly associated with chicken carcass traits. Our study demonstrated that the SNP sites of *CRELD1* and *DNAJC30* were potential molecular markers in the breeding of carcass traits.

MATERIALS AND METHODS

Experimental Animals and Determination of Their Carcass Traits

The F_2 generation of Chinese indigenous chicken was the object of this study. All tissue samples and carcass trait data were collected from KwangFeng Industrial Co., Ltd (Guangzhou, Guangdong, China). The animal experiment performed in this study satisfied strict the requirements of the Institutional Animal Care and Use Committee at the South China Agricultural University (approval ID: 2021-f074).

Chinese indigenous spotted chicken line was crossed with Chinese indigenous yellow chicken to produce F_1 individuals. 15 pairs of F_1 individuals were used to construct full-sib families to produce the F_2 individual. The F_2 individuals were raised in floor pens and fed with commercial corn-soybean-based diets to 80 d of age. The 410 F_2 individuals were slaughtered according to standard procedures. Blood samples were collected and stored at $-20°C$ before slaughter. The measured carcass traits included subcutaneous fat thickness, intermuscular adipose width, eviscerated weight (**EW**), EW with giblet, abdominal visceral fat weight (**AVFW**), legs weigh (**LW**), breast muscle weight (**BMW**), body weight (**BW**), shank length (**SL**), shank girth (**SG**), dressed weight (**DW**), BMW percentage, LW percentage, AVFW percentage, EW with giblet percentage (**EWGP**), EW percentage and slaughter percentage.

Genomic DNA Extraction, PCR Amplification, and SNP Detection

The genomic DNA of the chicken blood sample was extracted using NRBC Blood DNA Kit (Omega, Georgia, CA) according to the manufacturer's protocol. The primers were designed using Primer 5. The genomic DNA of each chicken sample was subjected to PCR amplification using 2×Taq MasterMix (CWBIO, Jiangsu, China) according to the manufacturer's protocol. The annealing temperature of both genes was 58°C, and the cycle number was 32. The PCR products were sequenced using ABI-3730XL by Tianyi Biotech Co., Ltd (Guangzhou, China). The information on primers used for PCR amplification and Sanger sequencing was listed in Table S1. Names for SNP sites follow the nomenclature standards approved by the HGVS (Human Genome Variation Society).

RNA Extraction, cDNA Synthesis, and qPCR Assay

The total RNA of 12 tissues was extracted using RNAiso Plus (Takara, Kyoto, Japan) and the HiPure Universal RNA Mini Kit (Magen, Guangzhou, China) following the manufacturer's protocol. cDNA was synthesized using MonScript 5×RTIII All−in−One Mix Kits (Monad, Shanghai, China) for reverse transcription. Primers were designed in Primer 5 software. cDNA samples were subjected to ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) following the manufacturer's protocol. The $2^{-\Delta\Delta Ct}$ method and internal normalization were used to analyze quantification results. The information on primers used for qPCR amplification was listed in Table S1.

Protein Interaction Network, GO, and KEGG Analysis

STRING online database (https://cn.string-db.org/, accessed on 10 September 2021) was employed to analyze the protein interaction network between *CRELD1* and DNCJC30 (von Mering et al., 2005; Szklarczyk et al., 2017). Proteins enriched in the network were clustered into 3 clusters using the kmeans method. GO enrichment and KEGG pathway enrichment of these proteins were performed and visualized using the g: Profiler tool (https://biit.cs.ut.ee/gprofiler/gost, accessed on 10 September 2021) (Raudvere et al., 2019).

Statistical Analysis

SNP Genotyping was performed using DNASTAR software (DNASTAR, Inc. Madison, WI), Linkage disequilibrium (**LD**) and haplotype analysis were performed using SHEsis online tool (http://analysis.bio-x.cn/, accessed on 30 September 2021) (Shi and He, 2005; Li et al., 2009).

Microsoft Excel (Microsoft Corp., Redmond, WA) was used to calculate the genotype frequency allele frequency, and diversity parameters. Diversity parameters contained Hardy−Weinberg p-value (P_w), observed heterozygosity (**Ho**), expected heterozygosity (**He**), and the polymorphism information content (**PIC**).

Correlation among traits was considered by calculating the inter-trait Pearson's correlation for all pairwise combinations of traits within each class of traits. SPSS 25 software (SPSS Inc., Chicago, IL).

Association analysis of SNP sites and carcass traits was performed with a generalized linear model as the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Y_{ij} is the observed value of different carcass traits, μ is the overall population mean, G_i is the effect of each genotype, and e_{ij} is the random error. For each carcass trait, the analysis of variance was carried out using SAS 9.4 (SAS Institute Inc, Cary, NC), and the significance was determined by Duncan's multiple tests. Significance between SNP sites is represented by letters where different letters indicate a significant difference ($P < 0.05$).

RESULTS

The Interaction Network of CRELD1 and DNAJC30

STRING database was employed to construct the protein interaction network of these 2 genes (Figure 1A). Ninety genes were determined in the interaction network and Kmeans clustering was run to generate 3 clusters, including a muscle-related cluster (red cluster), an endocytosis-related cluster (green cluster), and an N-glycan-related cluster (blue cluster). *CRELD1* and *DNAJC30* were contained in the green cluster and blue cluster respectively, and these 2 clusters interacted through the muscle-related cluster. It indicated that *CRELD1* and *DNAJC30* probably play some role in muscle development. GO and KEGG enrichment analysis also suggested that the interaction network of *CRELD1* and *DNAJC30* enriched 23 muscle-related GO terms and KEGG pathway, which further confirmed the potential muscle-regulatory function of *CRELD1* and *DNAJC30* (Figure 1B). Besides, there are also 2 lipid-related GO terms, which indicated that *CRELD1* and *DNAJC30* may be also the candidate genes for lipid metabolism. Overall, these results showed that *CRELD1* and *DNAJC30* were associated with muscle and fat development and metabolism to some extent.

Relative mRNA Expression Level of CRELD1 and DNAJC30 in Tissue of WRR and XH

To further predict the potential function of *CRELD1* and *DNAJC30* in chicken, the relative mRNA expression level of these 2 genes was measured in XH and WRR using qPCR. The results showed that mRNA expression of *CRELD1* and *DNAJC30* between WRR and XH were significantly different in breast muscle, leg muscle, and abdominal fat ($P < 0.05$) but showed no significant differences in other 9 tissue (brain, cerebellum, hypothalamus, kidney, proventriculus, heart, liver, spleen, and lung) (Figure 2). In skeletal muscle tissues (breast and leg muscle), the mRNA expression of *CRELD1* in WRR was significantly higher than XH, with the expression level being the highest in the leg muscle ($P < 0.05$; Figure 2A). Interestingly, the expression level of *DNAJC30* in WRR was also significantly higher in skeletal muscle tissues but with the highest expression level in breast muscle (Figure 2B; $P < 0.05$). It is also interesting to note that both mRNA expression of *CRELD1* and *DNAJC30* in WRR was the significantly lowest expression in abdominal fat than
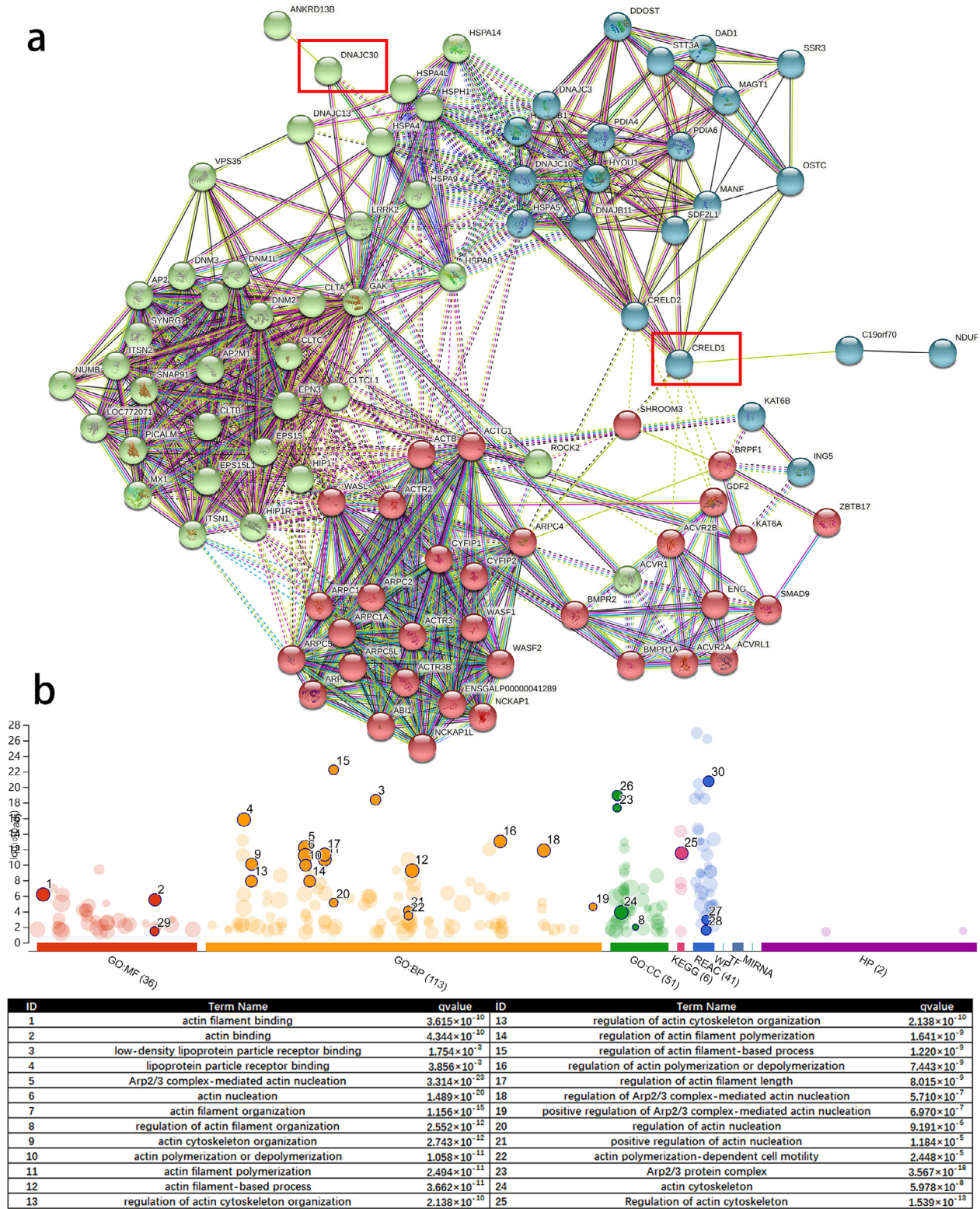
Figure 1. The protein interaction network and enrichment analysis. (A) 92 genes were determined in the interaction network and Kmeans clustering was run to generate 3 clusters (red, blue, and green). (B) Muscle and fat-related terms were enriched in the interaction network between *CRELD1* and *DNCJC30*. Abbreviations: *CRELD1*, cysteine rich with epidermal growth factor like domains 1; *DNAJC30*, DnaJ heat shock protein family member C30.

XH ($P < 0.05$; Figures 2A and 2B). The expression of *CRELD1* and *DNAJC30* in WRR were higher in skeletal muscle and lower in abdominal fat than XH, emphasizing their essential roles in the carcass trait of chicken.

The Polymorphism of CRELD1 *and* DNAJC30 *Genes*

The PCR-amplified DNA sequences of 410 individuals were confirmed by Sanger sequencing and genotyped by
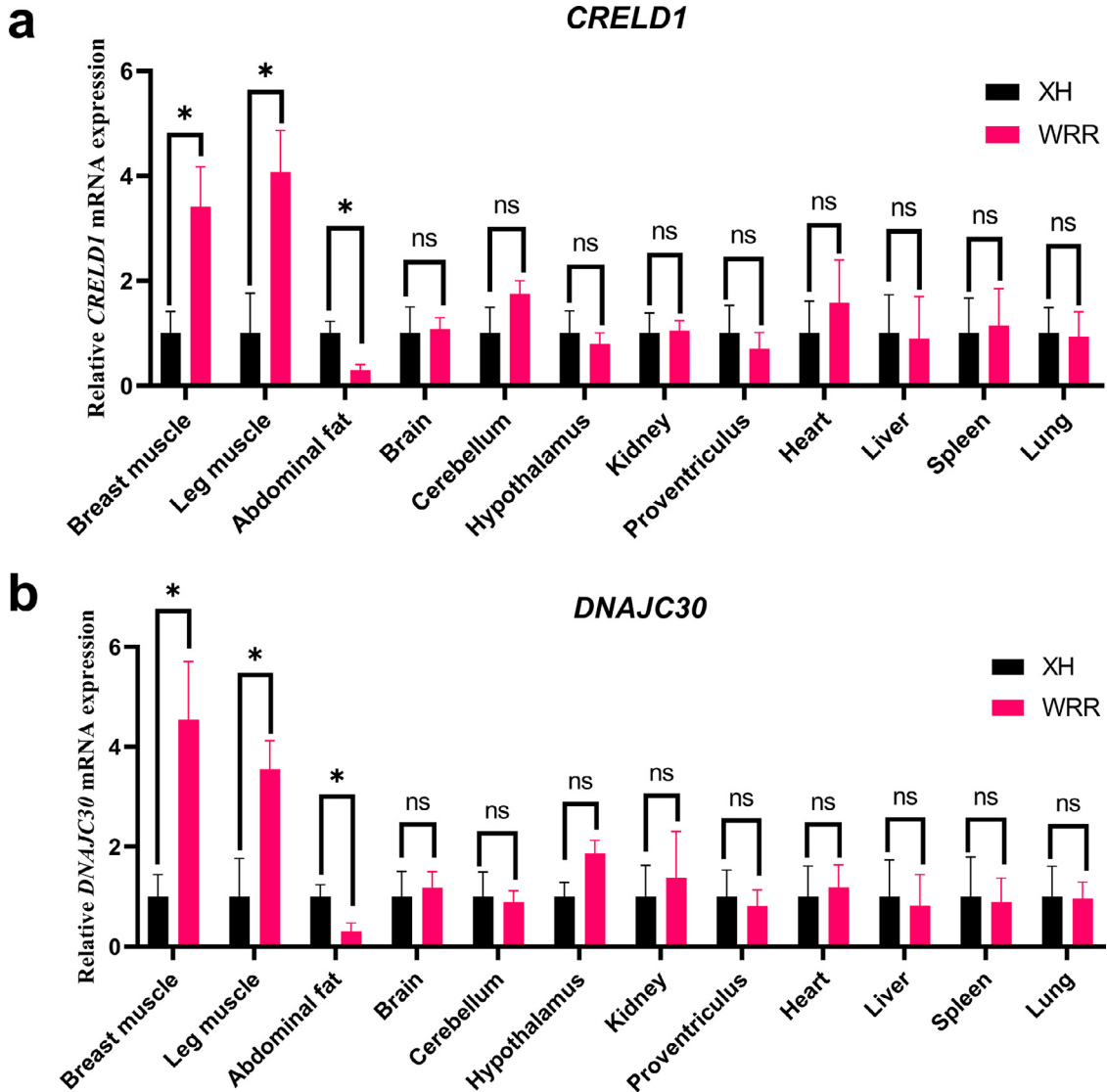
Figure 2. The relative tissue expression pattern of *CRELD1* and *DNAJC30* in Xinhua chicken (XH) and White Recessive Rock chicken (WRR). (A) Relative *CRELD1* expression of different tissues between WRR and XH. (B) Relative *DNAJC30* expression of different tissues between WRR and XH. Symbols "*" and "**" indicate a significant difference at $P < 0.05$ and $P < 0.01$, respectively. Abbreviations: *CRELD1*, cysteine rich with epidermal growth factor like domains 1; *DNAJC30*, DnaJ heat shock protein family member C30

DNASTAR. 5 SNP sites of *CRELD1* and 7 SNP sites of *DNAJC30* were obtained. In *CRELD1*, 2 SNP sites were located on exon and the other 3 sites were located on intron. These 2 sites in exon were synonymous mutations. Besides, In *DNAJC30*, 2 sites were in the exon and the other 5 sites were in the intron and the 2 exon-located sites were also synonymous mutations. The allele that was consistent with the NCBI reference genome (bGalGal1.mat.broiler. GRCg7b Primary Assembly) was defined as wild type. Otherwise, the allele was defined as mutant type (Table 1).

The 5 SNP sites of *CRELD1* detected in this study were NC_051543.1: g.11683326 (**C-SNP1**), NC_051543.1: g.11683378 (**C-SNP2**), NC_051543.1: g.11683497 (**C-SNP3**), NC_051543.1: g.11683588 (**C-SNP4**) and NC_051543.1: g.11683643 (**C-SNP5**) The 7 SNP sites of *DNAJC30* detected in this study were NC_052550.1: g.618125 (**D-SNP1**), NC_052550.1: g.618020 (**D-SNP2**), NC_052550.1: g.617962 (**D-SNP3**), NC_052550.1: g.617664 (**D-SNP4**), NC_052550.1: g.617659 (**D-SNP5**), NC_052550.1: g.617631 (**D-SNP6**), NC_052550.1: g.617467 (**D-SNP7**). The peak maps of all 3 genotypes of SNP sites mentioned above were shown in Figure 3.

After genotyping, the genotype frequency, allele frequency, and diversity parameters of SNP sites in *CRELD1* and *DNAJC30* were calculated and listed in Table 2. Interestingly, all 5 SNP sites in *CRELD1* were consistent with Hardy-Weinberg Equilibrium ($P_w > 0.05$) while all 7 SNP sites in *DNAJC30* deviated from Hardy-Weinberg Equilibrium ($P_w < 0.05$). These variations of *DNAJC30* may be due to a strong artificial selection. The PIC of SNP sites was calculated and revealed that all sites of *CRELD1* and *DNAJC30* were moderate polymorphism ($0.25 < PIC < 0.5$).

To further explore the relationship among the SNP sites in *CRELD1* and *DNAJC30* repetitively, LD and haplotype analyses were performed. LD analysis was

Table 1. The information on SNPs in *CRELD1* and *DNAJC30* in this study.

Gene	SNP ID	Abbreviation	Location	Amino change	Wild type	Heterozygote	Mutant type
CRELD1	NC_052543.1: g.11683326 G>A	C-SNP1	Intron 5	NO	GG (n = 115)	GA (n = 196)	AA (n = 98)
	NC_052543.1: g.11683378 G>A	C-SNP2	Exon 6	NO	GG (n = 206)	GA (n = 167)	AA (n = 36)
	NC_052543.1: g.11683497 T>C	C-SNP3	Intron 6	NO	TT (n = 38)	TC (n = 164)	CC (n = 207)
	NC_052543.1: g.11683588 A>G	C-SNP4	Exon 7	NO	AA (n = 36)	AG (n = 165)	GG (n = 208)
	NC_052543.1: g.11683643 G>C	C-SNP5	Intron 7	NO	GG (n = 231)	GC (n = 147)	CC (n = 31)
DNAJC30	NC_052550.1: g.618125 A>G	D-SNP1	Exon	NO	AA (n = 89)	AG (n = 104)	GG (n = 213)
	NC_052550.1: g.618020 T>C	D-SNP2	Exon	NO	TT (n = 118)	TC (n = 123)	CC (n = 165)
	NC_052550.1: g.617962 C>T	D-SNP3	Intron	NO	CC (n = 261)	CT (n = 88)	TT (n = 57)
	NC_052550.1: g.617664 T>C	D-SNP4	Intron	NO	TT (n = 33)	TC (n = 93)	CC (n = 280)
	NC_052550.1: g.617659 C>T	D-SNP5	Intron	NO	CC (n = 36)	CT (n = 88)	TT (n = 282)
	NC_052550.1: g.617631 G>T	D-SNP6	Intron	NO	GG (n = 294)	GT (n = 74)	TT (n = 38)
	NC_052550.1: g.617467 T>C	D-SNP7	Intron	NO	TT (n = 267)	TC (n = 70)	CC (n = 69)

Abbreviations: *CRELD1*, cysteine rich with epidermal growth factor like domains 1; *DNAJC30*, DnaJ heat shock protein family member C30; SNP, single nucleotide polymorphism.

performed by calculating D' and r^2 values and the results were shown in Figure 4. SNP sites in *CRELD1* and *DNAJC30* were at high LD level according to the D' and r^2 values (Table S2 and S3). Besides, 11 haplotypes (4 in *CRELD1* and 7 in *DNAJC30*) were found and the information on haplotypes was listed in Table S4 and Table S5.

Correlation Analysis of Chicken Carcass Traits

To further understand the detailed information of the 410 individual chicken population, the Pearson correlation coefficient was calculated among these 17 carcass traits. The values of pairwise correlation between chicken carcass traits were shown in Figure 5 and the descriptive statistics of 17 carcass traits in the study population were shown in Table S6. Interestingly, we found that SL and SG were significantly correlated with muscle development and fat deposition-related carcass traits AVFW, eviscerated weight with giblet percentage, LW, BW, BMW and so on ($P < 0.05$).

Association Analysis of CRELD1 and DNAJC30 SNPs With Chicken Carcass Traits

To explore the relationship between obtained SNP sites and chicken carcass traits, association analysis was performed and the association results were shown in Table 3. In *CRELD1*, all SNP sites were associated with chicken carcass traits except C-SNP5. C-SNP1 was significantly associated with 5 carcass traits like BMW, BW, DW, SL, and SG ($P < 0.05$). C-SNP2, C-

SNP3, and C-SNP4 were all associated with EWGP, and C-SNP4 was also associated with SG. It is interesting to note that the heterozygote of all obtained SNP sites in *CRELD1* was significantly higher than its wild type or mutant type in associated carcass traits. In *DNAJC30*, a total of 7 SNP sites were found but only 3 of them were associated with chicken carcass traits ($P < 0.05$). Both D-SNP1 and D-SNP2 were associated with EWGP, and D-SNP1 and D-SNP2 were also associated with SG and LW percentage, respectively ($P < 0.05$). D-SNP6 was associated with intermuscular adipose width and the mutant type was significantly higher than the heterozygote ($P < 0.05$). SL and SG were correlated with muscle development and fat deposition-related carcass traits according to correlation analysis results (Figure 5). Thus, the above results indicated that SNPs of *CRELD1* and *DNAJC30* can be potential molecular markers for marker-assist breeding of the chicken carcass traits.

DISCUSSION

Chicken consumption has become one of the most widespread meat in the world (Zhao et al., 2019). Carcass traits are one kind of the most important traits that have a great impact on the development of the domestic chicken industry (Santos et al., 2021). Promoting muscle development and reducing fat deposition are the top priority in chicken breeding (Guo et al., 2019). Therefore, many studies have been devoted to exploring the potential candidate genes, regulatory network, and molecular mechanism to improve chicken carcass traits (Li et al., 2015; Allais et al., 2019; Fu et al.,
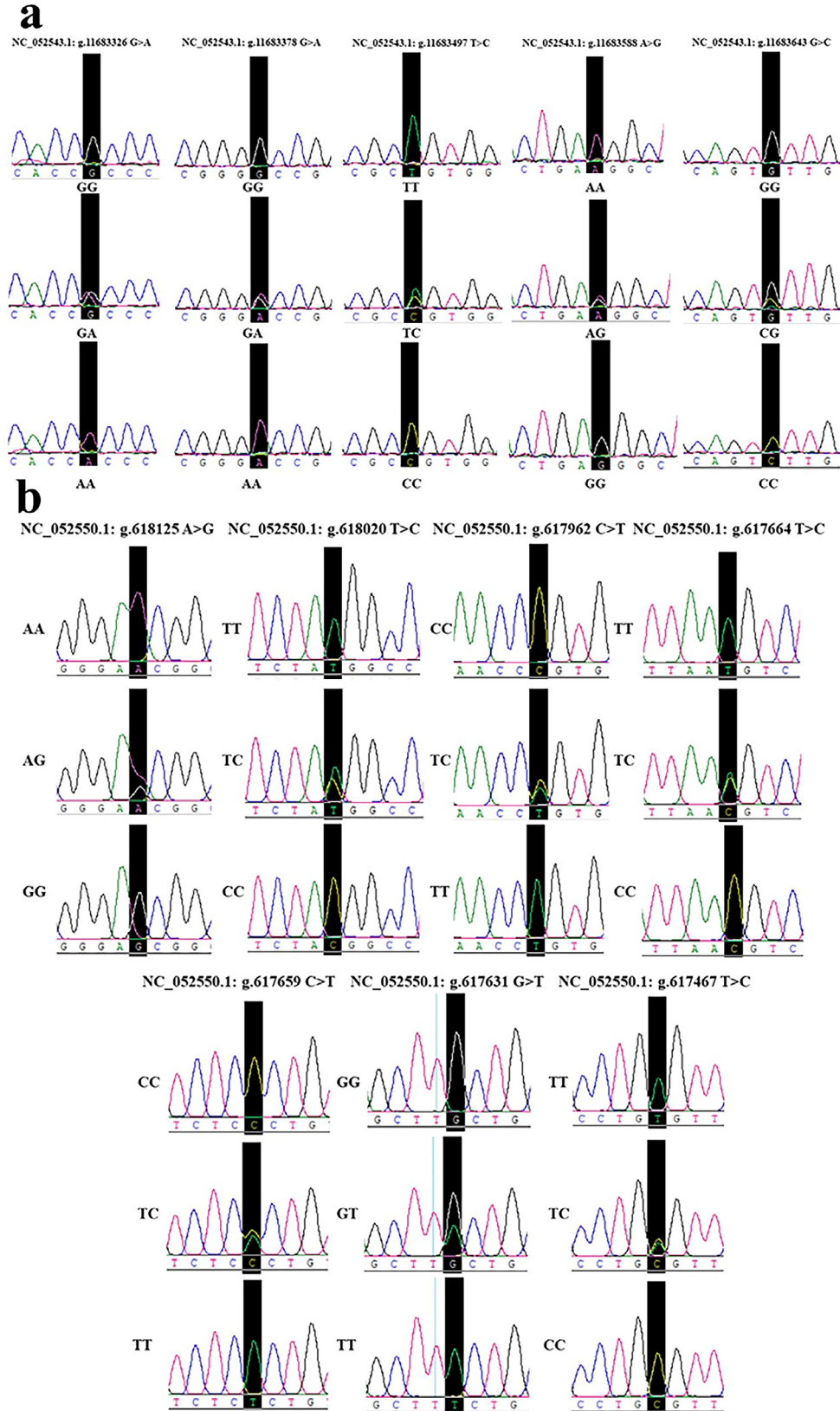
Figure 3. The peak maps of all 3 genotypes of SNP sites in *CRELD1* and *DNAJC30*. (A) SNP sites of *CRELD1* in this study. (B) SNP sites of *DNAJC30* in this study. Abbreviations: *CRELD1*, cysteine rich with epidermal growth factor like domains 1; *DNAJC30*, DnaJ heat shock protein family member C30; SNP, single nucleotide polymorphism.

2020; Zhang et al., 2020; Wang et al., 2021; Li et al., 2022; Yang et al., 2022). However, it still needs further research to unravel the underlying mechanisms of chicken carcass traits.

Previous studies have reported that *CRELD1* is highly expressed in muscle tissue including skeletal muscle and myocardial tissue (Rupp et al., 2002; Beckert et al., 2021). Importantly, *CRELD1* plays an

Table 2. Genotype and allele frequency, and diversity parameters of SNPs in *CRELD1* and *DNAJC30*.

Gene	SNPs[1] ID (Abbreviation)	Genotype frequency			Allele frequency		P_w[2]	Ho[3]	He[4]	PIC[5]
		Wild type	Heterozygote	Mutant type	Wild type	Mutant type				
CRELD1	NC_052543.1: g.11683326 G>A (C-SNP1)	0.28	0.48	0.24	0.52	0.48	0.72	0.50	0.50	0.37
	NC_052543.1: g.11683378 G>A (C-SNP2)	0.50	0.41	0.09	0.71	0.29	0.97	0.59	0.41	0.33
	NC_052543.1: g.11683497 T>C (C-SNP3)	0.09	0.40	0.51	0.29	0.71	0.80	0.59	0.41	0.33
	NC_052543.1: g.11683588 A>G (C-SNP4)	0.09	0.40	0.51	0.29	0.71	0.92	0.59	0.41	0.33
	NC_052543.1: g.11683643 G>C (C-SNP5)	0.56	0.36	0.08	0.74	0.26	0.54	0.62	0.38	0.31
DNAJC30	NC_052550.1: g.618125 A>G (D-SNP1)	0.22	0.26	0.52	0.35	0.65	0.00	0.55	0.45	0.35
	NC_052550.1: g.618020 T>C (D-SNP2)	0.29	0.30	0.41	0.44	0.56	0.00	0.51	0.49	0.37
	NC_052550.1: g.617962 C>T (D-SNP3)	0.64	0.22	0.14	0.75	0.25	0.00	0.63	0.37	0.30
	NC_052550.1: g.617664 T>C (D-SNP4)	0.08	0.23	0.69	0.20	0.80	0.00	0.69	0.31	0.27
	NC_052550.1: g.617659 C>T (D-SNP5)	0.09	0.22	0.69	0.20	0.80	0.00	0.68	0.32	0.27
	NC_052550.1: g.617631 G>T (D-SNP6)	0.72	0.18	0.09	0.82	0.18	0.00	0.70	0.30	0.26
	NC_052550.1: g.617467 T>C (D-SNP7)	0.66	0.17	0.17	0.74	0.26	0.00	0.62	0.38	0.31

[1]SNPs: single nucleotide polymorphism sites.
[2]P_w= the χ 2 test of Hardy−Weinberg equilibrium. $P_w < 0.05$ means Hardy-Weinberg disequilibrium (HWD) and $P_w > 0.05$ means Hardy−Weinberg equilibrium.
[3]Ho: observed heterozygosity.
[4]He: expected heterozygosity.
[5]PIC: polymorphism information content. Suggested PIC value classes are as follows: PIC>0.5 means high polymorphism, 0.25<PIC< 0.5 means moderate polymorphism, and PIC <0.25 means low polymorphism.Abbreviations: *CRELD1*, cysteine rich with epidermal growth factor like domains 1; *DNAJC30*, DnaJ heat shock protein family member C30; SNP, single nucleotide polymorphism.

important role in controlling the abundance of ionotropic acetylcholine receptors in C2C12 myoblasts (D'Alessandro et al., 2018). Mitochondrial function is important for basic cell metabolism including muscle cells and adipocytes (Jokinen et al., 2017; Migliavacca et al., 2019). As a member of HSP, *DNAJC30* is a key gene in mitochondrial function which is required for damaged mitochondrial complex I subunits exchange and ATP synthase complex function (Tebbenkamp et al., 2018; Stenton et al., 2021). These 2 genes have gradually become research hotspots in human disease phenotypes but there is no research in poultry science.

Interestingly, we found that both *CRELD1* and *DNAJC30* are the differentially expressed genes in the muscle tissue between XH chicken and WRR chicken based on our previous work. It indicated that *CRELD1* and *DNAJC30* may play some role in chicken muscle development. Besides, the interaction network between *CRELD1* and *DNAJC30* is highly associated with muscle development according to the enrichment analysis. Interestingly, some lipid deposition-related terms are also enriched in addition to muscle-related terms, which further suggests that *CRELD1* and *DNAJC30* are likely to be associated with chicken carcass traits.

The bioinformatics analysis results are consistent with the relative gene expression pattern in different tissues between WRR and XH. We found that *CRELD1* and *DNAJC30* are expressed in almost all chicken tissues, but these 2 genes are differentially expressed in breast muscle, leg muscle, and abdominal fat between XH and WRR. In WRR, *CRELD1* and *DNAJC30* show a higher expression level of breast muscle and leg muscle but show a lower expression level of abdominal fat compared to XH. XH is one kind of Chinese indigenous chicken, which has a slower muscle growth rate and more fat deposition compared with commercial chicken breeds like WRR (Tian et al., 2021). Based on these results, we demonstrate that *CRELD1* and *DNAJC30* may play some role in chicken muscle development and fat deposition, which may have an impact on chicken carcass traits.

To further confirm the relationship between *CRELD1* and *DNAJC30* genes and chicken carcass traits, we detect SNP mutation in *CRELD1* and *DNAJC30* and performed association analysis with chicken carcass traits. Our results show that a total of 4 of 5 SNP sites in *CRELD1* and 3 of 7 SNP sites in *DNAJC30* are significantly associated with carcass traits. These SNP sites could serve as candidate sites for marker-assisted breeding in chicken carcass traits and confirm the underlying functions of *CRELD1* and *DNAJC30* for regulatory muscle development and fat deposition. Notably, C-SNP1 located in intron 5 of *CRELD1* is associated with most carcass traits including BMW, BW, DW, SL, and SG. SL and SG are
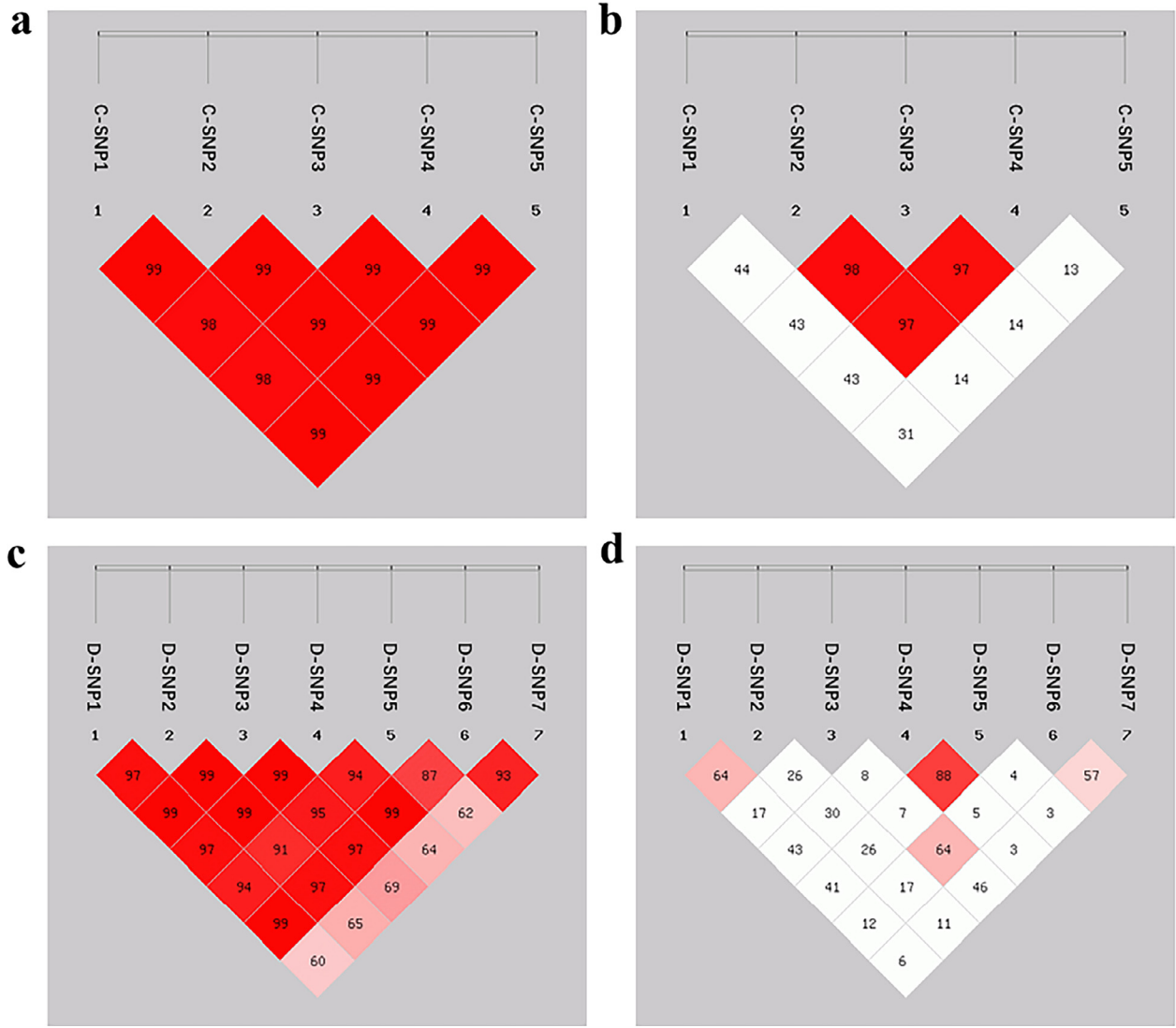
Figure 4. Genetic analysis of linkage equilibrium on SNP sites in *CRELD1* and *DNAJC30* genes. (A) *D'* of SNP sites in *CRELD1*; (B) r^2 of SNP sites in *CRELD1*; (C) *D'* of SNP sites in *DNAJC30*; (D) r^2 of SNP sites in *DNAJC30*. Abbreviations: *CRELD1*, cysteine rich with epidermal growth factor like domains 1; *DNAJC30*, DnaJ heat shock protein family member C30; SNP, single nucleotide polymorphism.

correlated with other muscle and fat-related carcass traits according to our correlation analysis results of chicken carcass traits. Multiple studies have reported that shank traits may be related to fat deposition and muscle development (Ye et al., 2014; Luo et al., 2016;

Chen et al., 2021). Other SNP sites in *CRELD1* are only associated with 1 or 2 carcass traits. The SNP sites of *DNAJC30* are also only associated with 1 or 2 carcass traits. It may be due to the smaller number of individual chickens in this population. Nevertheless, we
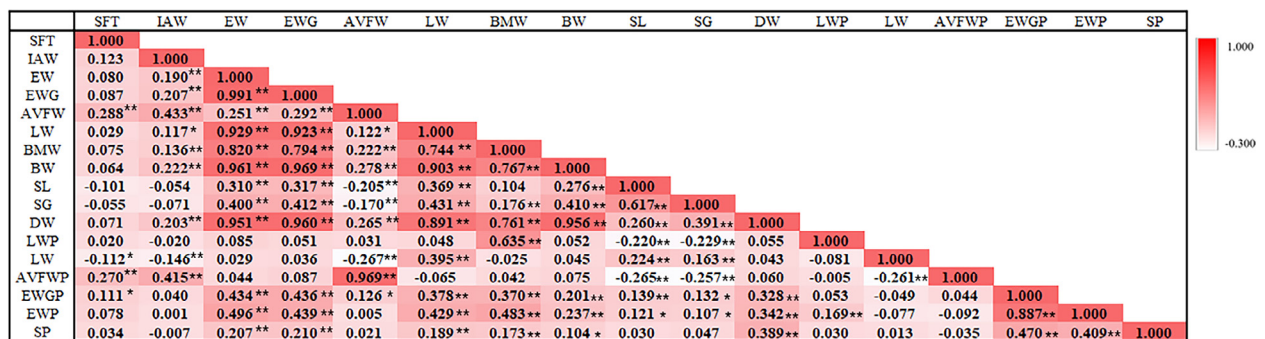

Figure 5. Pearson's correlation analysis of carcass traits. The result was shown as a heat map. Symbol "*" and "**" indicate a significant difference at $P < 0.05$ and $P < 0.01$ (2-tailed), respectively.

Table 3. Association analysis of SNPs in *CRELD1* and *DNAJC30* with chicken carcass traits (MEANS ± SD).

Gene	SNPs abbreviation	Carcass trait	Genotype			Available Sample	*P*-value
			Wild type	Heterozygote	Mutant type		
CRELD1	C-SNP1 G>A	BMW (g)	104.51 ± 17.87[b] (n = 108)	111.08 ± 16.59[a] (n = 173)	107.88 ± 18.79[ab] (n = 90)	371	0.0094
		BW (g)	1834.86 ± 229.65[b] (n = 108)	1922.08 ± 211.63[a] (n = 177)	1901.80 ± 227.52[a] (n = 91)	376	0.0051
		DW (g)	1621.50 ± 218.01[b] (n = 108)	1700.90 ± 197.55[a] (n = 175)	1672.34 ± 224.16[ab] (n = 90)	373	0.0090
		SL (cm)	7.49 ± 0.83[ab] (n = 108)	7.62 ± 0.55[a] (n = 177)	7.32 ± 1.01[b] (n = 91)	376	0.0104
		SG (cm)	11.90 ± 1.11[ab] (n = 108)	12.02 ± 0.90[a] (n = 177)	11.66 ± 1.02[b] (n = 91)	376	0.0188
	C-SNP2 G>A	EWGP (%)	79.71 ± 2.65[a] (n = 182)	80.01 ± 1.84[a] (n = 137)	78.71 ± 3.39[b] (n = 34)	353	0.0217
	C-SNP3 T>C	EWGP (%)	78.78 ± 3.30[b] (n = 36)	80.01 ± 1.86[a] (n = 134)	79.71 ± 2.65[a] (n = 183)	353	0.0287
	C-SNP4 A>G	EWGP (%)	78.71 ± 3.39[b] (n = 34)	80.01 ± 1.85[a] (n = 136)	79.70 ± 2.65[a] (n = 183)	353	0.0222
		SG (cm)	11.48 ± 0.94[b] (n = 35)	11.94 ± 0.98[a] (n = 152)	11.94 ± 1.01[a] (n = 189)	376	0.0316
DNAJC30	D-SNP1 A>G	BMWP (%)	8.73 ± 0.85[a] (n = 81)	8.61 ± 0.78[ab] (n = 97)	8.44 ± 0.79[b] (n = 193)	371	0.0167
		SG (cm)	11.63 ± 1.03[b] (n = 82)	11.98 ± 1.03[a] (n = 95)	11.98 ± 0.96[a] (n = 196)	373	0.0200
	D-SNP2 T>C	BMWP (%)	8.70 ± 0.83[a] (n = 106)	8.56 ± 0.79[ab] (n = 114)	8.44 ± 0.78[b] (n = 151)	371	0.0425
		LWP (%)	17.45 ± 0.86[b] (n = 106)	17.73 ± 0.89[a] (n = 114)	17.50 ± 0.87[b] (n = 151)	371	0.0344
	D-SNP6 G>T	IAW (mm)	27.09 ± 9.83[ab] (n = 267)	23.93 ± 10.66[b] (n = 70)	29.02 ± 9.67[a] (n = 34)	371	0.0228

[ab]The different letters stand for significant differences ($P < 0.05$), while the same letter indicates no difference ($P > 0.05$). Abbreviations: BMW, breast muscle weight; BMWP, breast muscle weight percentage; BW, body weight; *CRELD1*, cysteine rich with epidermal growth factor like domains 1; *DNAJC30*, DnaJ heat shock protein family member C30; DW, dressed weight; EWGP, eviscerated weight with giblet percentage; IAW, intermuscular adipose width; LWP, leg weight percentage; SG, shank girth; SL, shank length; SNP, single nucleotide polymorphism.

first proved that *CRELD1* and *DNAJC30* have the potential to become candidate genes for muscle development and fat deposition to affect chicken carcass traits. However, there are still many unanswered questions about the underlying molecular mechanism of the function of *CRELD1* and *DNAJC30*. Future studies on the current topic are therefore recommended.

CONCLUSIONS

In summary, this is the first investigation to analyze the function of *CRELD1* and *DNAJC30* in chicken. Our results demonstrated that *CRELD1* and *DNAJC30* are related to muscle development and fat deposition in chicken according to bioinformatic analysis and tissue gene expression profiles. SNP mutation of *CRELD1* and *DNAJC30* are significantly associated with chicken carcass traits. Our study identifies several SNP sites in *CRELD1* and *DNAJC30* for molecular-marker-assist breeding to improve chicken carcass traits.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2022.102324.

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