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A novel A-to-G mutation in circBDP1 alters adipocyte proliferation and differentiation and affects bovine carcass traits

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Fat deposition in cattle, influenced by factors such as genetics, age, sex, and nutrition, plays a crucial role in determining growth rates, beef yield, and meat quality. Among these, genetics is the predominant factor (Kim et al., 2020; Xing et al., 2025). Research indicates a high heritability for visceral (36%) and subcutaneous (57%) fat (Fox et al., 2012). Gene mutations that affect adipocyte function are significant contributors to variations in fat deposition and meat quality (Mannen, 2011; Mwangi et al., 2019; Sun et al., 2023; Dementieva et al., 2024). Therefore, investigating genetic variations in fat-related genes is essential for enhancing cattle performance.

Genome-wide association studies (GWAS) have identified genetic variants in non-coding RNAs (ncRNAs), including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), which have been primarily studied in humans (Ahmed et al., 2019; Liu et al., 2019; Hall et al., 2021). Meanwhile, there is a scarcity of research on connecting ncRNA variants to phenotypic traits in livestock (Jevsinek Skok et al., 2013; Shi and Sun, 2017; Li et al., 2018; Jin et al., 2019; Bruscadin et al., 2021). CircRNAs, which are formed through covalent binding of their 3' and 5' ends, can mediate transcription and post-transcriptional processes (Kelly et al., 2015).

Certain circRNAs have been shown to influence adipocyte development, such as circFUT10 (Jiang et al., 2020) and circINSR (Shen et al., 2021), by acting as sponges for miRNAs.

Given the pivotal role of circRNAs in adipose tissue development, identifying genetic variations in these molecules can facilitate the discovery of genetic markers for cattle breeding. Our previous study demonstrated that circRNA derived from *B* double prime 1 (*BDP1*) gene, termed circBDP1, promotes bovine adipocyte proliferation and differentiation (Zhang et al., 2022). Recognizing that genetic variations in ncRNAs can significantly impact their functional roles, this study aims to address the following questions: (1) Is there genetic variation in circBDP1? (2) Does genetic variation in circBDP1 influence bovine growth or carcass traits? (3) What are the functional implications of these genetic variations for circBDP1? The findings are expected to provide a foundation for advancing beef cattle breeding strategies.

By conducting circRNA sequencing and subsequent validation, we identified that bovine circBDP1, derived from exons 14–16 of the *BDP1* gene, promotes the proliferation and differentiation of pre-adipocytes (Zhang et al., 2022). Notably, we discovered an A>G mutation in exon 16 of circBDP1 (ENSBTAT00000000474.6:c.2254A>G), as recorded in the Ensembl genome browser. Using primers designed to target exon 16 along with the adjacent intronic sequences (Table S1), we successfully amplified and sequenced the resulting polymerase chain reaction (PCR) product, and sequence alignment demonstrated the presence of this variant in both Qinchuan

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cattle and the Shandong Black Cattle Genetic Resource (SDBCGR) population (Fig. 1).

The circBDP1-single nucleotide polymorphism (SNP) genotypes in Qinchuan and SDBCGR cattle were identified through the sequencing of PCR products and the Kompetitive allele-specific PCR (KASP) method. PCR product sequencing was conducted in a total of 122 Qinchuan cattle and 231 SDBCGR individuals. Additionally, the KASP method was utilized to determine the circBDP1-SNP genotypes in 384 SDBCGR individuals (Fig. S1).

Three genotypes of circBDP1-SNP were identified in the Qinchuan and SDBCGR cattle populations: AA, AG, and GG. Notably, only one individual with the GG genotype was detected in both breeds, resulting in a genotypic frequency of less than 0.01. The predominant genotype was wild-type AA, with a frequency exceeding 85%, and the major allele was A, exhibiting a frequency greater than 90% (Fig. S2).

The analysis of genetic parameters revealed high homozygosity at this locus, indicating low polymorphism (polymorphic information content (PIC)<0.25). This locus was found to be in Hardy-Weinberg equilibrium in both populations ($P>0.05$) (Table S2). Furthermore, the analysis of published global bovine genome sequences and data from the Bovine Genome Variation Database (BGVD) indicated that the G allele may have originated from Indian indicine cattle (Chen et al., 2018, 2020; Zhou et al., 2022).

Association analysis revealed that the circBDP1-SNP genotypes were significantly associated with the yield of cupim, hanging tender, chuck short rib, and rectum in female SDBCGR individuals, as well as the gross weight of male SDBCGR individuals and body length of Qinchuan cattle (Figs. 2a and 2b; Table S3).

Given that circBDP1-SNP is located within the circBDP1 gene and is significantly associated with

various carcass traits in SDBCGR cattle and the body length of Qinchuan cattle, this study generated circBDP1 overexpression plasmids containing different circBDP1-SNP genotypes to investigate their regulatory effects on adipose tissue development. To assess the impact of these genotypes on preadipocyte proliferation, 5-ethynyl-2'-deoxyuridine (EdU) labeling, quantitative reverse transcription-PCR (qRT-PCR), and flow cytometry were performed.

The EdU assay results indicated that there was no significant difference in the proportion of proliferating cells among the groups; however, the circBDP1-A group (which contains the wild-type A allele of the circBDP1-SNP locus) exhibited a higher trend compared to both the control group and the circBDP1-G group (containing the wild-type G allele) (Fig. 3a). Moreover, the qRT-PCR results demonstrated that circBDP1-A significantly enhanced the messenger RNA (mRNA) expression level of proliferating cell nuclear antigen (*PCNA*) compared to both the circBDP1-G and control groups (Fig. 3b).

Flow cytometry analysis revealed no significant differences in cell division between the circBDP1-A and control groups; however, the circBDP1-G group exhibited a significantly lower number of cells in the division phase compared to both the control and circBDP1-A groups, while the number of cells in the G1 phase was significantly higher in the circBDP1-G group. These findings suggest that circBDP1-G inhibits cell cycle progression (Fig. 3c).

We further examined the impact of the mutation on preadipocyte apoptosis. The flow cytometry results demonstrated robust cell growth across all three groups, with approximately 90% of the total cell population classified as normal. The proportion of early apoptotic cells was significantly lower in both the circBDP1-A and circBDP1-G groups compared to the

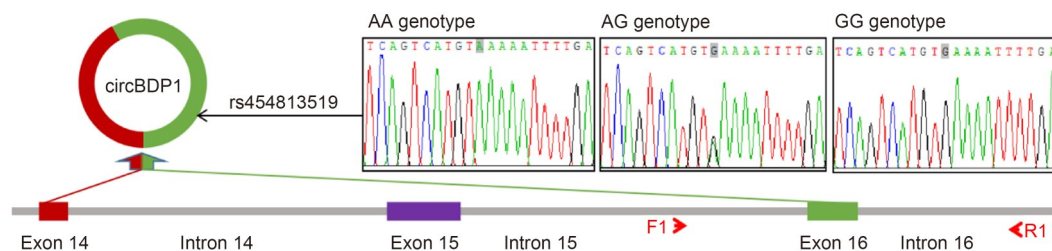


Fig. 1 Location and genotypes of circBDP1-SNP. The gray line represents the DNA sequence; the box with a different color represents the different exons of the bovine *BDP1* gene; the black arrow indicates the location of rs454813519 on the circBDP1; the chromatograms show different genotypes of rs454813519. circBDP1: circRNA derived from B double prime 1 (*BDP1*) gene; SNP: single nucleotide polymorphism; F1: forward primer; R1: reverse primer.

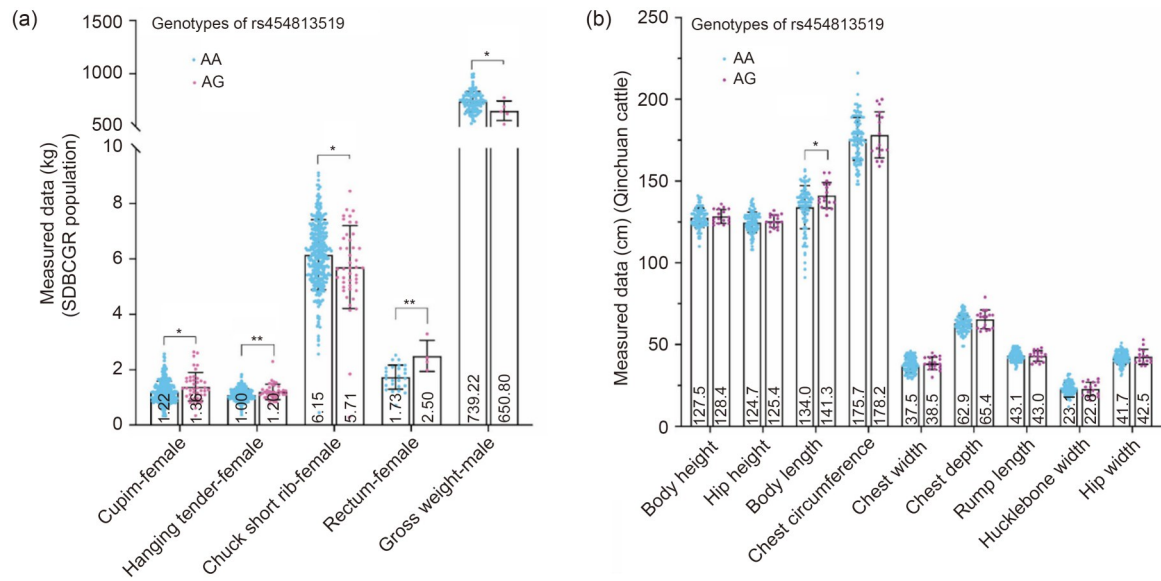


Fig. 2 Association between circBDP1-SNP genotypes and carcass traits of Shandong Black Cattle Genetic Resource (SDBCGR) population (a) and body measurements of Qinchuan cattle (b). All values are expressed as mean±standard error of the mean (SEM), $n=122$ (a) or 231 (b). * $P<0.05$, ** $P<0.01$. CircBDP1: circRNA derived from B double prime 1 (*BDP1*) gene; SNP: single nucleotide polymorphism.

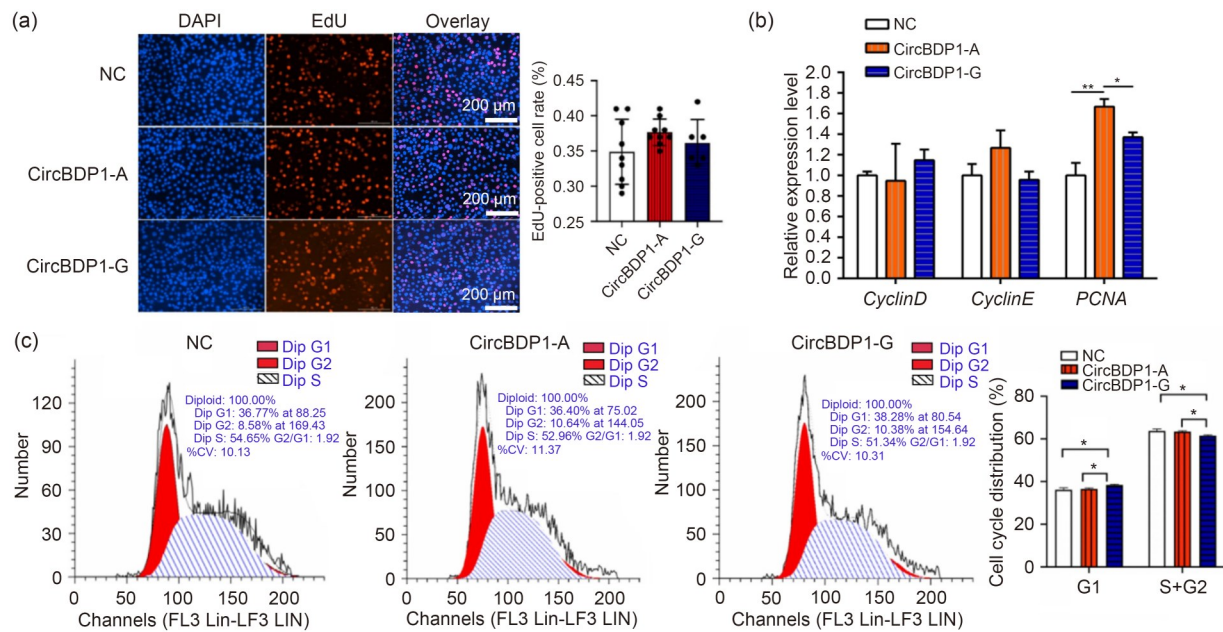


Fig. 3 Effects of circBDP1 with different genotypes of circBDP1-SNP on preadipocyte proliferation. (a) 5-Ethynyl-2'-deoxyuridine (EdU) assay results. (b) The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) results. (c) Flow cytometry results. All values are expressed as mean±standard error of the mean (SEM), $n=3$. * $P<0.05$, ** $P<0.01$. CircBDP1: circRNA derived from B double prime 1 (*BDP1*) gene; SNP: single nucleotide polymorphism; DAPI: 4',6-diamidino-2-phenylindole dihydrochloride; PCNA: proliferating cell nuclear antigen; NC: negative control.

control group, and notably, it was significantly lower in the circBDP1-A group than in the circBDP1-G group (Figs. S3a and S3b). Interestingly, the circBDP1-A group showed a higher number of late apoptotic cells compared to the other two groups.

Further analysis revealed no significant differences in the overall proportion of apoptotic cells (B2+B4) among the three groups, suggesting that while the mutation influenced the apoptotic process, its effect was not pronounced (Figs. S3a and S3b). The qRT-PCR

results indicated that the mRNA expression level of the apoptosis-inhibiting gene B-cell lymphoma 2 (*Bcl-2*) was significantly higher in the circBDP1-A group compared to both the control and circBDP1-G groups. This suggests that circBDP1-A may play a role in inhibiting cell apoptosis. Meanwhile, circBDP1-G did not demonstrate a significant effect on apoptosis (Fig. S3c).

Preadipocytes were induced to differentiate following the overexpression of circBDP1-A and circBDP1-G. To assess the mRNA and protein expression levels of differentiation marker genes, RNA samples were collected after 2 and 4 d of differentiation. The results indicated that the expression levels of adipocyte differentiation-related marker genes decreased with the A to G mutation, suggesting that adipocyte differentiation was impaired as a result of this mutation (Fig. S4). In conclusion, the mutation from circBDP1-A to circBDP1-G may influence phenotypic traits by modulating cell proliferation, apoptosis, and differentiation levels.

In summary, the circBDP1-SNP variation within the circBDP1 was significantly associated with carcass traits in a genetic resource population of Shandong black cattle. This variation diminished the stimulatory effect of circBDP1 on the proliferation and differentiation of preadipocytes, and thus it holds potential as a DNA marker for molecular-assisted selection in beef cattle breeding.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

Data are available upon request from corresponding authors.

Acknowledgments

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Author contributions

Enhui JIANG and Chiyuan ZHANG performed the methodology, validation, and data curation. Zhuoyuan HE and Yuli

ZHANG contributed to the validation. Yuta YANG contributed to the writing–review & editing and data curation. Chuanying PAN, Fugui JIANG, and Enliang SONG contributed to the writing–review & editing. Sihuan ZHANG and Xianyong LAN contributed to the project administration and funding acquisition. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Enhui JIANG, Chiyuan ZHANG, Zhuoyuan HE, Yuli ZHANG, Yuta YANG, Chuanying PAN, Fugui JIANG, Enliang SONG, Sihuan ZHANG, and Xianyong LAN declare that they have no conflicts of interest.

All animal experiments were maintained according to the Regulation for the Administration of Affairs Concerning Experimental Animals (State Council of China, 2017 Revision), and our study was approved by the Northwest A&F University Institutional Animal Care and Use Committee (No. NWAUFU-314020038).

Declaration on the use of generative AI tools

During the preparation of this work, no generative AI tools were used in the preparation of this manuscript.

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Supplementary information

Figs. S1–S4; Tables S1–S3; Materials and methods