


CONNECTION BETWEEN GENE MARKERS WITH MILK PRODUCTION TRAITS OF UKRAINIAN DAIRY COWS

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 Supporting Information

ABSTRACT: The purpose of this study was to obtain information on the genotyping of cows of Ukrainian dairy breeds for the presented loci *capa-casein* gene (CSN3), thyroglobulin (TG-5) gene, leptin (LEP), pituitary-specific transcription factor (Pit-1), and beta-lactoglobulin (BLG) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and to test their association with milk production. The influence of the genotype on milk yield, fat and protein content in the milk of cows of Ukrainian selection has been established. The highest levels of milk productivity traits were expected from animals with the CT genotype for the LEP gene, AA for the CSN3 gene, CC for the TG5 gene, and AA and BB for the Pit-1 gene. But at the same time, milk obtained from cows with CSN3 BB genotype were characterized by the best cheese suitability. The effect of the homo- or heterozygous state of BLG on the signs of milk production was less noticeable, and the homozygous TT genotype for the TG5 gene was not found in any of the studied breeds. The results obtained can be used in the practical work of breeding farms along with traditional methods of selection, control and preservation of the genetic diversity of specific herds at an optimal level.

Keywords: *Capa-casein*, Marker genes, Polymorphism, Thyroglobulin, Leptin, Pituitary transcription Factor, Beta-lactoglobulin

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INTRODUCTION

In recent decades, advances in DNA-based marker technology have been able to identify regions of the genome (namely, quantitative trait loci, QTLs) underlying complex traits such as milk yield. Instead of traditional animal breeding programs based solely on phenotype and pedigree information, the application of identified QTLs to genetic evaluation provides great potential to improve selection accuracy, thereby could accelerate genetic improvement in animal productivity (Jiang et al., 2010; Kumar, 2017; Kramarenko et al., 2019).

The introduction of the molecular genetic methods in animal husbandry is associated with the development of PCR technology, made it possible to conduct a quick analysis of the connection between allelic variants of genes with productivity. With the development of DNA-based marker technology, it has become possible to identify regions of the genome (i.e., loci of quantitative traits, QTLs) that are associated with complex traits. The inclusion of the detected QTLs in the genetic evaluation allows a clear increase in selection accuracy, thereby accelerating the genetic improvement of the productive qualities of the animals. Numerous studies have been published in recent decades to identify QTLs for signs of dairy livestock dairy productivity. Advances in the detection of causal genes for complex such are slow, as association mapping results in large confidence intervals. In particular, the region in which QTL is mapped may contain a large number of possible candidate genes (Wang et al., 2014; Kusza et al., 2015). Genome-wide association studies (GWAS), based on high-throughput single nucleotide polymorphisms (SNPs) genotyping technologies, open up ample opportunities to study genes associated with signs of livestock dairy productivity, as confirmed by a number of domestic and foreign scientific studies (Arora and Bhatia, 2004; Soltani-Ghombavani et al., 2013; Zhou et al., 2019).

Breeding farm animals is an integral part of a complex breeding system. When evaluating animals of dairy breeds, not only a high level of milk productivity, but also quality indicators of milk are of great importance. Increasing yield, fat content in milk, live weight of cows is considered the classic direction of work of genetic scientists. But at the same time, there are few works, especially domestic ones, devoted to the study of marker genes associated with the protein content in milk. Although the protein content and amount in milk, its structure is of great economic importance to the processing industry. Since the consumption of raw materials, time and energy resources for the production of dairy products depend on the fat content in milk, in addition, the protein content indicator largely determines the quality of finished products (Yurnalis et al., 2013; Lu et al., 2021).

The association of polymorphism in the 3' region of the oxidized low density lipoprotein receptor 1 (OLR1) gene with milk fat and protein in active Holstein cows of Irish selection was studied by Soltani-Ghombavani et al. (2013). Thus, scientists have found that the OLR1 is the main receptor on the cell surface for the oxidized low-density lipoprotein, actively participates in the metabolism of lipoproteins and affects the protein content in milk. According to the authors,

the *OLR1* gene may be a marker gene that is associated with the production of protein content in milk. Because the genetic linkage phase can be different across breeds and populations, the use of previously identified markers to conduct marker selection is problematic, especially when marker density was low during discoveries. Consequently, high-density SNP GWASs are essential for understanding the genetic architecture of important and complex traits in cattle breeds.

A genome-wide association of milk research was carried out by Chinese scientists in Xinjiang Brown cattle of a combined direction of productivity. In their studies, the scientists evaluated five milk productivity measures: milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP) and protein percentage (PP) in Chinese cattle breed Xinjiang Brown. The authors found two very significant SNPs associated with milk composition characteristics. One SNP is associated with a percentage of fat and is located in the cadherin-2 (*CDH2*) gene at 29.1 Mbp on BTA 24. The cadherin-2 is a protein coding gene and is involved in adipogenesis. Depressing the *CDH2* to block the epithelial-mesenchymal reaction can weaken the production of fat content in milk. Another milk-related SNP that the authors identified was significantly associated with protein content in milk and mapped at 75.8 Mbp on *Bos taurus* (BTA 7), which is in a gene called the Gamma2 receptor gamma-aminobutyric acid type A subunit (*GABRG2*). *GABRG2* primarily promotes the activity of the gamma-aminobutyric acid-controlled chloride ion channel (GABA) and is involved in the activity of the GABA-A receptor and promotes the production of the amount of protein in milk (Zhou et al., 2019; Zhang et al., 2022). Scientific research on the assessment of annual genetic progress and economic efficiency becomes the basis for the development of methods for optimizing long-term breeding programs for dairy cattle, while improving methodological approaches to solving this problem. Wang et al. (2014) established a significant association between SNPs in the histidine ammonia-lyase gene (*HAL*) gene and signs of cow dairy productivity of the Chinese Holstein breed, indicating the potential role of *HAL* variants in these signs. These identified SNPs may be genetic markers used in genomic selection schemes to accelerate the genetic growth of dairy livestock productivity traits.

Thus, domestic and foreign experience shows that the effectiveness of selection depends on many genetic, paratypical and economic factors (Ng-Kwai-Hang et al., 1984; Lund et al., 2011; Kramarenko et al., 2019; Lu et al., 2021; Pedrosa et al., 2021). The possibility of purposefully creating a highly productive animal population is significantly correlated with the presence of information about genes that control signs of productivity. In this regard, the task of identifying and using marker genes responsible for the manifestation of economically valuable signs becomes urgent.

The composition of milk is an important breeding characteristic of dairy breeds of cattle, especially in the conditions of modern animal husbandry; therefore, the task arises to analyze the influence of polymorphism of protein and lipid metabolism genes on the formation of milk productivity indicators in cows of the dairy direction of domestic breeding productivity.

MATERIALS AND METHODS

Materials

To conduct the study, experimental groups were formed from breeding cattle of the dairy direction of productivity - Ukrainian Red Dairy (URD, n = 32 heads), Ukrainian Black-speckled Dairy (UBSD, n = 32 heads), Ukrainian Red-speckled Dairy (URSD, n = 28 heads) breeds of the leading enterprise in the south of Ukraine PSP "Kolos-2011" Ochakov region.

Ethical regulation

The rules for handling animals in experiments comply with European legislation on the protection and comfort of animals kept on farms (Directive No. 95/58 EU "On the Protection of Farm Animals" of the Council of the EU of 20.07.1998, as amended by EU Regulation No. 806 / 203 of 14.04.2003, No. 91/630 EU "Minimum standards for the protection of pigs" of 19.11.1991, as amended by the EU Regulation. Pilot study protocol for blood sampling in cows approved by the local commission on bioethics of the National University of Life and Environmental Sciences Ukraine on Good Clinical Practice (GCP) for the protection and humane treatment of experimental animals.

Methods

The characterization of the polymorphism of the genes studied was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Grodzicker et al, 1974; Alexander et al., 1988; Pedrosa et al., 2021). Genomic DNA was isolated from the peripheral blood of animals according to the method of Kostyunina et al. (2020) and using the standard commercial kit "DNA Sorb B" manufactured by Amplissens (Russia), according to the manufacturer's recommendations (Pedrosa et al., 2021). The DNA concentration was tested by electrophoresis in a 2% agarose gel. To carry out the polymerase chain reaction, a reaction mixture of 10 µL was used in operation: dH₂O - 4.3 µL, PCR buffer 5 (15 mM Mg²⁺-1.0 mL) - 2.0 µL; Deoxynucleotide triphosphates (dNTP) mixture of 10 (2 mM each) - 0.8 µL; two primers (70 ng each) - 0.8 µL; Taq polymerase (1 ml/1000 U) - 0.1 µL; DNA 50-100 ng - 2.0 µL.

Restriction products were separated by electrophoresis in 2% agarose gel followed by staining in ethidium bromide solution. Visualization was carried out on a transilluminator in UV light, followed by electropherogram photography by a digital camera. The size differentiation of amplicons was carried out using the molecular weight marker Gene Ruler TM 50 bp DNA Ladder, SM0378 (Fermentas®, Lithuania; Oztabak et al., 2008). The temperature regime and the number of PCR amplification cycles for each gene were determined separately. For the analysis of the polymorphism of the structural loci *k-Cn*, *βLG*, *TG*, *Pit-1*, and *LEP*, restriction enzymes matched for each locus were used; typed immediately after PCR analysis (Grobet et al., 1998).

Statistical analysis

Statistical data processing was carried out in the standard package Microsoft Excel-2013.

RESULTS AND DISCUSSION

Identification of selection signatures allows a better understanding of the evolutionary processes, functions and organization of genes in the genome. Of particular note are genes, certain allelic variants of which are associated with the quality of raw materials. In cattle, genes whose polymorphism is associated with dairy productivity are known and investigated by us (Figure 1).

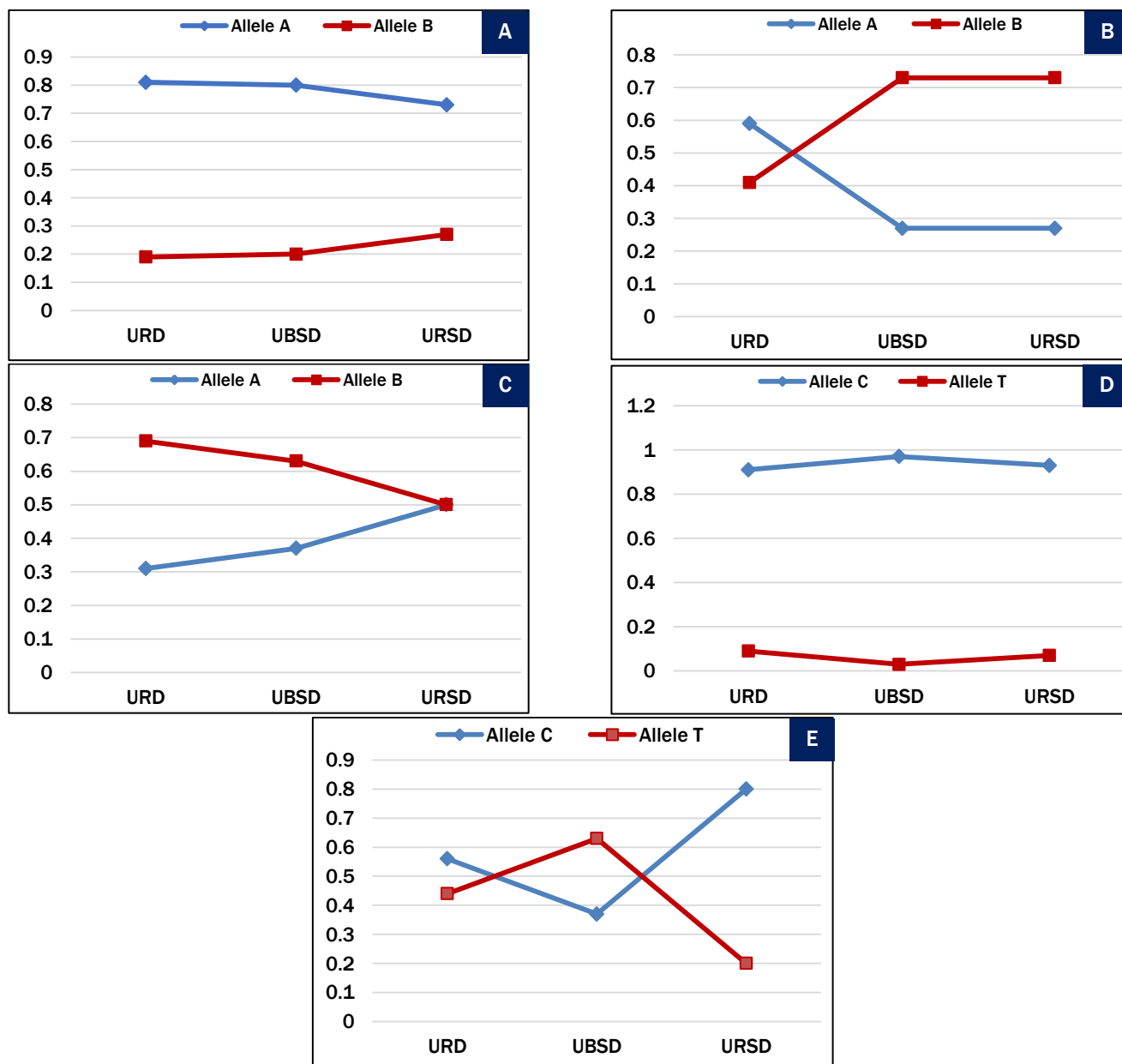


Figure 1 - Distribution of allelic variants frequencies by loci in cows. A: Capa-casein gene (CSN3); B: Beta-lactoglobulin (BLG); C: Leptin (LEP); D: pituitary-specific transcription factor (Pit-1); E: TG5 thyroglobulin (TG-5) gene. Ukrainian Red Dairy (URD): n=16, Ukrainian Black-speckled Dairy (UBSD): n=15; Ukrainian Red-speckled Dairy (URSD): n=15.

The studied genes are divided into several groups: A) milk protein genes, which affect the protein content in milk, its technological properties, quality and yield of protein-containing products; B) lipid metabolism genes synthesized in adipocytes, are responsible for regulating the body weight of the animal, its consumption of feed and its fat deposits, and are also involved in the synthesis of milk fats and genes of regulatory systems, which generally affect the productive performance of the body and serve as a somatic regulator of animal growth (Khatib et al., 2007; Miluchová et al., 2018).

That's how, capa-casein (CSN3) is connected with milk protein and its coagulation characteristics, including the role of a stabilizing factor in the formation of micelles, blocking their aggregation. And when it is dissolved, milk coagulates, casein precipitate formation and clot formation and affects the mass share of fat in milk, the volume of milk yield. Beta-lactoglobulin (BLG) is associated with a higher concentration of fat and protein in milk and has a significant effect on the creation of active immunity in calves. Meanwhile, as a pituitary-specific transcription factor (Pit-1) is known for the function of increasing milk yield and fat yield. Leptin provides formation of fat deposits and increases productivity in

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