

Genetic Bases and Molecular Breeding of Key Economic Traits in China Dairy Cattle: A Progress Report

Running head: Molecular breeding of dairy cattle

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Abstract:

Animal growth is a coordinated developmental process that requires altering the expression of hundreds to thousands of genes to modify many biochemical and biological signaling cascades. The genetic analysis of economic traits and molecular breeding research are hotspots for animal husbandry and, in the case of Chinese dairy cattle, much progress has been made in recent years. Actually, the level of information of molecular events at the transcriptional, biochemical, hormonal, and metabolite levels underlying animal development process has increased considerably. The current review summarizes progress in research into the genetic basis of economic traits and molecular breeding of dairy cows, dual-purpose cattle and dairy buffalo, to better understand the molecular switch involved in development process with important consequences from a breeding point of view.

Keywords: chinese dairy cattle; key economic traits; genetic mechanism; animal breeding; research progress

1. Introduction

Advances in molecular biology technology and the continued breeding of dairy cattle (including dual-purpose cattle and dairy buffalo), have contributed to significant progress in China[1]. For example, the role of the anti-aging gene Sirtuin 1[2] may be critical to the survival of dairy cows, cattle and dairy buffalo. Sirtuin 1[3] levels may need to be assessed with relevance key regulatory mechanisms and key gene functions that determine the molecular switch involved in the aging process[4, 5] that may affect milk production and breeding programs. Heat and cold stress[6] affects Sirtuin 1 with core body temperature effects that may determine milk production and breeding of dairy cattle and buffalo. Activators and inhibitors of Sirtuin 1 may play key gene functions that regulate the breeding programs[7]. Genetic mechanisms relating to key economic traits, mining of genetic markers and verification of gene functions have enabled germplasm innovation and the cultivation of new breeds. The current focus of genetic improvement is to select desirable genetic traits, integrate a suite of traits from different donors, or alter the innate genetic traits of a species[8]. With the development of genome

sequencing and metabolic detection technologies, multi-omic integrative analysis of genomes, transcriptomes, and metabolomes has greatly facilitated the deciphering of genetic basis and molecular breeding[1, 9]. The current review summarizes progress in research into the genetic basis of economic traits and molecular breeding for the grouping of three types of bovines: dairy cattle, dual-purpose cattle, and milk buffalo to provide reference materials for future research directions.

2. Advances in molecular breeding of Chinese dairy cows

With the development of molecular biology, biostatistics, molecular genetics and marker-assisted breeding technology in recent years, assisted breeding using experimental methods of molecular biology is becoming a new and feasible breeding method[10, 11]. The Molecular breeding of dairy cattle has centered around the balanced breeding concept in which functional traits such as reproduction, body shape, health and longevity[12, 13] are incorporated into the breeding program. Identifications of genes and genetic markers relevant to milk production

are expected to accelerate genetic analysis and breeding programs[14]. At present, for molecular breeding of dairy cows, some genes have been confirmed to have significant genetic effects on their functional traits.

2.1 Reproductive traits

With the further development of breeding technology, genome-wide association studies (GWAS) have provided a new way to find genes associated with complex traits, and have begun to replace the traditional QTL linkage mapping analysis and become a more powerful tool for gene detection. Many subsequent GWAS studies have been conducted in cattle to explore the improvement of reproductive traits in cows. Chen et al. studied the effect of serving sire on female reproductive traits of Holstein cattle from the genomic perspective through GWAS, indicating that sire selection plays an extremely important role in maternal conception rate and have contributed to the identification of the loci, *WVOW*, *TFB2M* and *SMYD3*, which regulate calving in Holstein cattle[12]. In addition, the genes, *SOD2*, *SNRPA1*, *TGFBR1*, *SLC39A11*, *PDE5A*, *HPSE* and *PRPF4B*, were found to influence reproductive traits[14] and *PLAG1*, *AMHR2*, *SP1*, *KRT8*, *KRT18*, *MLH1* and *EOMES* to affect the reproductive capacity of heifers[15].

2.2 Milk production traits

As the most important economic trait in dairy industry, milk production of dairy cows is affected by major and minor genes. GWAS studies have shown that the loci, *FAM135b*, *C2CD2*, *GOLGA4*, *ARFGEF1*, *CTDSOL*, *TSPEAR*, *SUPT3H*, *ATAD2B*, *KLHL29*, *FKBP2*, *STOML2* and *ECI2*, are related to milk production[14]. Single nucleotide polymorphisms (SNPs) refer to the DNA sequence polymorphism caused by single nucleotide variation at the genome level, including the conversion or transversion of segmented bases. SNPs are abundant in many species and has important marker value for modern molecular breeding programs, genetic map construction and quantitative genetics studies. Recent studies have shown that SNP have become one of the ideal genetic markers. Indeed, Lo et al. also proposed that SNPs of the G64695500A, G490025A, rs381960368 and rs801171594 loci can be used as molecular markers related to milk production traits of dairy cows[16].

Given the importance of milk-producing traits in dairy cow, understanding their genetic basis is critical to implementing effective genetic improvement programs. For example, Liu et al. found that the *IGF1R* gene influences milk yield, fat and protein content in Chinese Holstein cattle[17]. A combination of genetic analysis and functional verification has identified the *DDIT3* gene as regulatory of milk production, the *HTR1B* gene as influencing fatty acid content[18], the *SLC27A6* gene as being involved in lipid metabolism[19] and the *EEF1D* can promote milk fat synthesis by regulating PI3K-AKT signaling in mammals, which plays an important role in mammary gland development and milk fat synthesis[20]. Comprehensive analysis of genomic DNA methylation and gene expression profiles has revealed that *DOCK1*, *PTK2* and *PIK3R1* are candidate genes related to milk production[21].

2.3 Inflammatory response and stress resistance

Mastitis is an important disease associated with reduced milk production, changes in milk composition and quality, and is considered to be one of the most expensive diseases in the dairy industry. At present, the research on the factors and pathways related to the regulation of inflammation at the molecular level is increasing. A combination of bioinformatics analysis and mechanistic studies has found by Wang et al. that target genes of long non-coding RNAs (lncRNA), tcon_00039271 and tcon_00139850, regulated inflammation-related signaling pathways (Notch, NF- κ B, MAPK, PI3K-Akt and mTOR) and affected susceptibility to *Escherichia coli*-induced mastitis[13]. Furthermore, micro-RNAs, miR-320a, miR-19a, miR-19b, miR-143, miR-205 and miR-24, may be blood biomarkers for *Staphylococcus aureus*-induced mastitis[22]. And

lncRNAs, PRANCER and TNK2-AS1, can be regarded as stable markers associated with bovine *Staphylococcus aureus* mastitis[23]. In addition, miR-125b is pro-inflammatory factor and its silencing alleviates bovine mastitis[24].

2.4 stress resistance

Stress has negative effects on livestock productivity, which may be exacerbated by climate change. Emerging diseases are often linked to a positively associated with climate change and the survival of microorganisms and/or their vectors. Therefore, the application of emerging molecular tools, on the one hand, by identifying the genes associated with them, helps us better understand the biological processes and mechanisms affected by intense and sustained natural or artificial selection in livestock populations. On the other hand, improving current methods of animal selection and developing climate-resilient varieties may contribute to the sustainability of livestock production systems in the future. The loci, *FAM107B*, *PHRF1*[25], *HCRT1*, *AGRP*, *PC*, *GUCY1B1*, *EIF2A*, *HSPA1A*, *HSP90AA1* and *HSF1*, have been shown by GWAS analysis to be candidate genes related to heat stress and the *HIF1A* gene to be associated with cold stress[15]. Loci, *ST3GAL4*, *ALAD*, *NOD1* and *ITGB2*, were associated with the immune response. Further findings from GWAS have demonstrated the following associations: *ITGA9*, *ACAT2* and *PLAC8* were associated with adaptation to specific environments and production systems; *NDUFB3*, *RGS3*, *UBD*, *DIS3L2*, *NRXN2*, *PEX14*, *SPTLC2*, *AQP1* and *PTPN9* with climate (tropical humidity or harsh environment) adaptations; *IL22RA1*, *CALHM3*, *SNW1*, *PLXNA4*, *ABCA9*, *DDOST*, *ATP1B3*, *ALDH6A1* and *ADCCK1* with clinical mastitis; *SCS*, *ITGA9*, *FKBP1b*, *ACAT2*, *AMZ2*, *MRPL18*, *HDHD3*, *GNAS*, *VSX2*, *PLAC8*, *PXDC1*, *REG3G*, *DNAJB5* and *PRDX5* with body temperature during heat stress. By genotyping the genome reduction sequence data of the Shanghai Holstein dairy cow population, it was found that *EXOSC10*, *MASP2* and *CSPP1* could affect the with longevity[14].

3. Advances in Molecular Breeding of dual-purpose cattle in China

Research into dual-purpose cattle (milk and meat) mainly concerns Sanhe, Xinjiang brown, grassland red and China Simmental cattle. Due to the limited market demand and unclear breeding direction, the basic population and genetic progress of dual-purpose cattle are relatively slow.

3.1 Sanhe cattle

Sanhe cattle, a dual-purpose cattle breed, originated in Chinese Inner Mongolia, has a high ability to resist cold stress. The definition of new traits for genetic and genomic selection to improve climate resilience in cattle can be achieved by exploring livestock-related metabolomic analysis and biological mechanisms. Yan et al.[26] identified the Mongolian hornless allele in Chinese Sanhe cattle breeds and the 2 bp deletion in the Friesian hornless allele which is a specific marker for the hornless gene. And Hu et al.[27] found 19 metabolites related to cold stress in inner Mongolian Sanhe cattle which is involved in the metabolic regulation of fat metabolism, amino acid metabolism and intestinal microbiota metabolism in response to cold in Sanhe cattle.

3.2 Xinjiang brown cattle

Similar to other dual-purpose cattle breeds, Xinjiang brown cattle breed take in to account both dairy cattle and beef cattle trait to achieve comprehensive breeding purpose. The unique characteristics of dual-purpose cattle need to be preserved in subsequent breeding in order to be able to produce a variety of products that can be adapted to market demand. Recent study has also focused on dual-purpose traits. In terms of reproductive traits, the bull's age, year of collection and frequency of collection were found to have a significant impact on semen quality in Xinjiang brown cattle in studies between 2006 and 2019[28]. In terms of

meat quality, Sun *et al.* found that the E2JW locus in exon 2 of the leptin gene which correlated positively with muscle fiber diameter and cystine content and showed a weak negative correlation with methionine content. Thus, there is potential for use of the E2JW locus as a molecular marker locus affecting quality traits in Xinjiang brown cattle [29]. In addition, transcriptomic and proteomic comparisons of Kazakh and Xinjiang brown cattle revealed 12 differentially expressed genes of troponin I1, crystallin alpha B, cysteine, and glycine rich protein 3, phosphotriesterase-related, myosin-binding protein H, glutathione s-transferase mu 3, myosin light chain 3, nidogen 2, dihydropyrimidinase like 2, glutamate-oxaloacetic transaminase 1, receptor accessory protein 5, and aspartoacylase with an impact on the *longissimus dorsi* muscle. The relevant genes were involved in fatty acid degradation and histidine metabolism [30]. The relationship between the 67 bp structural variation of the *ADIPOQ* promoter region and lactation traits in Xinjiang brown cattle has also been the subject of discussion and was found to correlate with 305-day milk yield, fat yield and milk fat percentage in Xinjiang brown cattle [31].

3.3 Chinese Red Steppes cattle

Chinese Red steppes cattle is a Chinese indigenous cattle breed from northeast China, which is raised as dual-purpose cattle. They have unique characteristics, such as disease resistance and better meat quality than other native Chinese cattle. Due to its rich genetic resources, so Chinese Red Steppes cattle has been widely concerned by the cattle genetics and breeding researchers in China. For example, comparative analyses of genome-wide patterns of alternative splicing in the *longissimus dorsi* of Japanese Wagyu and Chinese Red Steppes cattle [32] have shown that *MCAT*, *CPT1B*, *HADHB*, *SIRT2* and *DGAT1* are alternatively spliced candidate genes affecting bovine lipid metabolism and that *NR4A1*, *UQCC2*, *YBX3/CSDA* and *ITGA7* are related to muscle development. A study of polymorphisms in 20-month-old grassland red cattle ($n = 118$) indicated that SNPs in the *MED4* gene had an impact on meat quality [33]. Moreover, comprehensive analyses of miRNAs and their target mRNAs in immature and mature testis tissues of Chinese grassland red cattle, identified differentially expressed genes and miRNAs related to male reproductive capacity [34].

3.4 Chinese Simmental cattle

Chinese Simmental cattle is a dual-purpose breed, but much of the research has focused on meat quality. For example, multi-omic analyses have identified a series of candidate genes (*ANGPTL4*, *FABP4*, *GNA11*, *CAST*), metabolic pathways (tricarboxylic acid cycle, glycolysis and gluconeogenesis, glyoxylate and dicarboxylic acid metabolism, pyruvate metabolism and purine metabolism) and molecules (L-carnitine, cis-aconitic acid, 2-furoic acid, citraconic acid, itaconic acid, adenine, glutathione, linolenic acid and lactic acid) considered to be related to marble score (MS), water-holding capacity (WHC) and shear force (SF) in Chinese Simmental cattle [35, 36]. Studies on polymorphisms of the *MAT2B* gene in 127 Chinese Simmental cattle have indicated that the I2-2382G>T locus is a molecular marker for meat quality [37]. In addition, 10 SNPs in the promoter region of the *SDC3* gene were identified in a study of 135 Simmental cattle. A correlation was found between the g.123236647C>G and g.123236666C>G SNPs and marbling grade of Chinese Simmental cattle ($p < 0.05$) and between the g.123236666C>G locus and fat coverage and color ($p < 0.05$). Linkage and haplotype analyses revealed that *H2H2* correlated with marbling and fat color ($p < 0.05$) [38]. Growth curves of 808 Chinese Simmental were constructed using body weights at 0, 6, 12 and 18 months. GWAS demonstrated that 22 SNPs, including *PLIN3*, *KCNS3*, *TMCO1*, *PRKAG3*, *ANGPTL2*, *IGF-1*, *SHISA9* and *STK3*, were related to growth and development. The loci affect different components of the overall process of growth and development, with *KCNS3* affecting intramuscular fat (IMF) content, *IGF-1*, *TMCO1*, *PRKAG3* and *SHISA9* affecting muscle development and

ASPH affecting carcass development and meat quality [39, 40]. A total of 364 variants were found by GWAS to influence bone weight in Chinese Simmental cattle. Genes, such as *LAP3*, *MED28*, *NCAPG*, *LCORL*, *SLIT2* and *IBSP*, previously reported to be associated with animal carcass characteristics, body measurements and growth characteristics were more precisely identified as being involved with bone weight [41]. Further GWAS, based on a random regression model, revealed 37 SNPs associated with body weight, including *FGF4*, *ANGPT4*, *PLA2G4A* and *ITGA5* [42].

4. Advances in Molecular Breeding of China dairy buffalo

Compared with cow's milk, buffalo milk has properties that contain high levels of fat, lactose, protein and calcium content. It has antioxidant activity and is a good source of minerals such as potassium and phosphorus. Given these characteristics, buffalo milk has been used with considerable success for dairy products. Therefore, the research on milk production traits of buffalo is increasing gradually. Functional and correlation analyses have identified *FATP1* [43], *LXRα* [44], *PPARγ* [45], *ELOVL6* [46], *HSD17B4* [47], *BTN1A1*, *XDH*, *PLIN1*, *PLIN2* [48, 49], *ABCD1* [50] and *INSIG2* [51] as having regulatory roles in milk fat synthesis and lactation. In particular, *DGAT1* [52, 53], *FASN* [54] and *PPARGC1A* [55] influenced milk production, as did *Casein* [56] and *OAT* [57]. Omics-based studies have also been used to investigate milk production. Transcriptomics showed that differential expression of genes involved in pathways for milk protein, fat synthesis and the immune response influenced buffalo milk fat content [58]. Metabolomics identified breed-specific metabolic markers for milk quality that could be used to produce buffalo with high milk fat [59]. A genome-wide selection response analysis of Chinese crossbred buffalo has revealed candidate genes related to production traits [60].

5. Conclusion

Breeding underpins the development of the bovine industry. Progress through traditional methods is slow due to the singleton births and long generation intervals of cattle. However, an approach incorporating molecular breeding has the potential to speed up the process. Many advances have been made in terms of molecular breeding, through the employment of genetic mechanism analysis, genetic marker mining and key gene function verification for Chinese dairy cows, dual-purpose cattle and dairy buffalo and the important economic characters of the genetic gene of cows effect has been verified, however, research involving Holstein, Juanshan, dual-purpose cattle or dairy buffalo is affected by many factors, such as group size and regional influences, and the cow function character belongs to the continuous variation of properties, regulatory mechanism is complex, and acceptor gene and tiny effect polygene common regulation, this needs to dig and identify new cow function candidate genes, and then analyze its genetic effect validation of biological function at the same time, To find the key genes that actually have a genetic effect. Existing experimental techniques have been little applied to large livestock and large-scale trials are required for appropriate verification. Molecular aspects of dairy traits, especially concerning network regulatory mechanisms and key gene functions, also need further research and in-depth analysis. Continuous improvements in breeding techniques, informed by molecular approaches represented by multi-omics, are likely to contribute to significant advances in the full exploitation of the economic traits of Chinese dairy cattle, dual-purpose cattle and dairy buffalo.

6. Author contribution

Fen Li and Xue Feng were writing-original draft preparation, investigation, and validation. Ruirui Li, Bingqin Du, Xiaoshu Xue, Honghong Hu, Junxing Zhang and Hui Sheng were revision and

supervision. Yun Ma: conceptualization. All authors contributed to the article and approved the submitted version.

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