

The role of molecular genetics in animal breeding: A minireview

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Abstract: Current animal breeding approaches are strongly associated with the development of sophisticated molecular genetics methods and techniques. Worldwide expansion of genomic selection can be achieved by the identification of genetic DNA markers and implementation of the microarray (“chip”) technology. Further advancement was associated with next-generation sequencing methods, high-throughput genotyping platforms, targeted genome editing techniques, and studies of epigenetic mechanisms. The remarkable development of “omics” technologies, such as genomics, epigenomics, transcriptomics, proteomics and metabolomics, has enabled individual genomic prediction of animal performance, identification of disease-causing genes and biomarkers for the prevention and treatment and overall qualitative progress in animal production.

Keywords: copy number variation; gene ontology; genome-wide association; quantitative trait loci; RNASeq; SNP

Introduction

Molecular genetics can contribute to complex traits by identifying causal genes, understanding the regulatory mechanisms of gene expression, identifying genetic networks and pathways and understanding the role of “other” genomic structures and mechanisms, e.g., structural variations, microRNAs, metagenome and epigenome (Simianer 2019).

Reductions in sequencing costs and the creation of sophisticated whole genome assembly algorithms accelerated the number of successfully sequenced organisms. The reference genomes of all farm animal species are available through the NCBI Reference Sequence Database (RefSeq). The last assembly releases ARS-UCD1.2 (cattle), Oar_rambouillet_1.0 (sheep), ARS1 (goat), and Sscrofa 11.1 (pig) are a result of extensive collaboration between

academic groups and international consortia. The successful international 1000 Bull Genomes Project sequenced the entire genomes of 234 bovine individuals and provided the scientific community with a vast amount of data on single nucleotide polymorphism (SNP) variants. The Project enabled the creation of a database of the sequence variants of the founders of modern breeds for the large-scale sequence-level genome-wide association study (GWAS) and the use of these data to quickly identify mutations that are important for health, wellness and performance (Daetwyler et al. 2014).

RefSeq data can be used as reference sets to impute whole-genome sequences in the datasets of herd individuals with SNP array genotypes to allow cost-effective genotyping for the estimation of genomic breeding values. Illumina (San Diego, CA, USA) is the leading provider of sequencing and genotyping service and has cooperated with a vari-

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ety of international consortia to develop Infinium BeadChips with thousands of SNP loci representing genetic diversity. The BovineHD chip is the most comprehensive genome-wide genotyping array featuring 777 962 SNPs that uniformly span the entire bovine genome. Additionally, a 50K international goat SNP genotyping panel, an OvineSNP50 chip with more than 54 000 SNPs and a PorcineSNP60 chip with more than 62 000 SNPs enabled the interrogation of genetic variations across the genome for any breed to perform comprehensive whole-genome studies.

The animal quantitative trait loci (QTL) database is another crucial genomic resource that contains a total of 190 838 QTLs or associations from 2 293 publications for more than 690 different traits in cattle, chicken, horse, pig, and sheep. Animal QTLdb strives to collect all publicly available trait-mapping data, i.e., QTL (phenotype/expression, eQTL), candidate gene and GWAS data, and copy number variations (CNV) mapped to livestock animal genomes, to facilitate locating and comparing discoveries within and between the species. Database tools are continually developed to align various trait-mapping data to the map-based genome features, such as annotated genes (www.animalgenome.org/cgi-bin/QTLdb/index).

“Molecular breeding” and functional annotation of genomes

The emergence of genomics as a discipline in the 1980s led to the concept of marker-assisted selection, in which genetic variants and genes that influence agriculturally important quantitative traits are identified and used to increase the genetic response. A global search for QTLs started in all livestock species. Quantitative loci with large effects on economically important traits were initially mapped by linkage analyses and then genome-wide association studies ([Georges et al. 2019](#)).

A study by [Cai et al. \(2019\)](#) can be considered an example of a complex approach to the identification of causal candidate genes. In this study, 15 551 021 SNPs were found to be associated with the female fertility index in 5 038 Nordic Holstein cattle, and QTLs on six chromosomes were identified. The nearest genes to GWAS hits, gene-based analysis, and linkage disequilibrium information

were used to generate a list of potential candidate genes influencing fertility in cattle. Prior information on genes related to fertility was acquired from Gene Ontology terms, Kyoto Encyclopaedia of Genes and Genomes pathway analysis, mammalian phenotype databases, and publicly available RNASeq data and was used to refine the list of candidate genes associated with fertility. Variant annotations were used to investigate the candidate mutations within the prioritized candidate genes. Candidate genes with biological relevance to each of these seven QTLs were proposed based on multiple sources of information.

Another approach used custom sheep chips for genotyping SNPs located in the known QTL regions for mastitis resistance. A follow-up association study was based on phenotypic records of the incidence of clinical mastitis and its indicators, i.e., number of somatic milk cells, total viable count of bacteria in milk, and California mastitis test. Functional gene clusters of mastitis resistance were confirmed, and 14 relevant candidate genes involved in innate immunity were identified within the analysed regions ([Banos et al. 2017](#)).

[Aguilar et al. \(2017\)](#) identified genomic regions with CNV markers using the classical commercial Illumina BovineHD Bead Chip. Association analysis confirmed significant associations between CNV and breeding values for somatic cell scores in the Holstein population. Candidate genes included in the functional biological pathways of inflammation initiation, immune response, stress, and cell death were identified in these regions. The combination of CNV detection/association analysis based on two different algorithms helped to identify genes associated with a phenotypic change in somatic cell count.

Intramuscular fat (IMF) and fatty acid composition are characteristics used as important indicators for evaluation of high-quality meat and contribute to the economic benefits of meat production. In a study by [Kim et al. \(2019\)](#), QTL fine mapping of chromosome 12 was performed in an F2 inter-cross population from a cross between Yorkshire and Korean native pigs by adopting a combined linkage and linkage disequilibrium method using high-density SNP chips. Quantitative trait loci for IMF and oleic acid (C18:1) were located within chromosome 12. In addition, 31 candidate genes within the IMF QTL region and 28 candidate genes within the C18:1 QTL region were

selected. To understand the functions of these candidate genes at the molecular level, these genes were functionally categorized by gene ontology and network and pathway analyses.

According to a study by Killeen et al. (2014), differentially expressed genes (DEGs) are potential candidate genes for identification of genetic changes influencing cow fertility that may be included in future breeding programmes. Microarray analysis of the endometrial tissue detected 419 DEGs and quantified differences in fertility, which potentially influence the functions of the uterine endometrium. The authors emphasized that the described molecular mechanisms can influence the success of fertilization and reproductive indicators of breeding by controlling the endometrial function.

Sandri et al. (2015) used microarray expression profiling technology to study the entire leukocyte transcriptome in the peripheral blood of healthy lactating Holstein and Simmental cows with different breeding values (BV) for milk protein content. Specific metabolic and immunological pathways associated with a given BV were identified. Comparison of differentially expressed genes with various BVs is becoming a promising approach to the identification of molecular responses associated with production traits.

To determine the molecular pathways associated with genetic variability in intramuscular fat, Albrecht et al. (2016) compared the transcriptomes of the *musculus longissimus dorsi* muscle of the Japanese Black and Holstein-Friesian bulls. A total of 569 DEGs were identified, and the majority (433) of DEGs were upregulated in the Japanese cattle breed characterized by extreme capacity to store intramuscular fat. The data of this study are stored in the publicly accessible repository of the NCBI Gene Expression Omnibus database.

RNASeq transcriptome profiling of porcine lung in two pig breeds in response to *Mycoplasma hyopneumoniae* infection identified 2 250 and 3 526 DEGs in Jiangquhai and Duroc pigs involved in the immune-relevant pathways, including cytokine-cytokine receptor interactions and chemokine signalling. The biological functions of these important DEGs require additional confirmation and may be used as molecular markers to improve pig health (Ni et al. 2019).

MicroRNAs (miRNAs) are small noncoding RNAs involved in the regulation of gene expression. Wicik et al. (2016) identified various miRNA

molecules influencing mammary gland development in dairy versus meat breeds. The results of the microarray analysis were refined by quantitative PCR, according to which 54 differentially expressed miRNAs in heifers regulate signalling pathways of mammary gland development. These data suggest that the significant developmental potential of the mammary gland in dairy cattle, which leads to high milk productivity, depends on the specific patterns of miRNA expression.

The Functional Annotation of Animal Genomes project is a newly developed international initiative that globally coordinates functional annotations across domesticated species. Discovery of the gene functions directly associated with economically important traits in agricultural animals will facilitate the identification of biochemical and genetic markers that can be indexed in genetic improvement programmes. The following tasks are required:

1. Catalogue gene expression.
2. Link genes to function.
3. Discover and exploit epigenetic factors.
4. Standardize frameworks for functional genomics data.

Better transcriptomic, proteomic, and metabolomic technologies are needed to fully bridge the gap between DNA sequences and phenotypes, to help identify the genes, proteins, and epigenetic modifications controlling various phenotypes and to determine the changes in DNA responsible for various phenotypes (Rexroad et al. 2019).

Is genome editing the future of animal breeding?

Genome editing technology is at the forefront of novel tools in animal breeding. Advances in technology are accelerating; improving efficiency, eliminating off-target edits, and introducing multiplex editing events can make use of the technology more practical in livestock selection programmes (Fleming et al. 2018). Innovations in gene-editing systems, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated 9 (Cas9) system have dramatically increased the efficiency of production of genetically engineered (GE)

pigs (Ryu et al. 2018). The use of these engineered endonucleases provides higher success in genetic modifications and an opportunity to introduce site-specific modifications during embryogenesis, thus bypassing the need for somatic cell nuclear transfer.

Successful genome editing was used to produce polled Holstein cattle (Carlson et al. 2016) and to interrupt the myostatin and fibroblast growth factor 5 genes in goats (Wang et al. 2015) to improve animal performance. Kalds et al. (2020) mentioned that ovine and caprine genomes have been engineered using CRISPR-based systems for numerous purposes, such as generation of superior agricultural breeds, expression of therapeutic agents in mammary glands, and development of animal models for the studies of human genetic disorders and regenerative medicine. In a study by Bi et al. (2020), an *MSTN* frameshift mutation in exon 2 was introduced into Meishan pigs by zinc finger nuclease (ZFN) technology. Zinc finger nuclease-edited *MSTN*^{-/-} Meishan pigs were successfully produced by a cloning method of somatic cell nuclear transfer. Slaughter results indicated that lean meat yield was increased by 16.53% in approximately 80 kg (10-month-old) *MSTN*^{-/-} Meishan sows compared with that in the corresponding wild-type counterparts.

Nevertheless, generation of transgenic animals is exceptionally troublesome and expensive; only a few useful transgene constructs are available, and many consumers are afraid of genetically modified organisms. Jenko et al. (2015) proposed a strategy that combines genomic editing with genomic selection (promotion of alleles by genome editing; PAGE). Bulls are initially selected based on their genomic estimated breeding values. Before dissemination, the bull genome is edited to produce a number of causative variants to generate homozygous favourable alleles. Under optimal conditions, it is estimated that PAGE could increase the genetic response 2–4-fold over genomic selection alone (Georges et al. 2019).

Conclusion

Tremendous development in the field of molecular genetics and genomics resources in recent years has enabled a significant shift in the paradigm of classical animal breeding. Currently, a sophisticated combination of pedigree data and SNP ge-

notyping has provided breeders with accelerated genetic progress in their herds. Functional gene annotation with respect to both animal health and economically important traits will further expand the implementation of genetic markers in genetic improvement programmes. Although animal genome editing technologies still have their limitations that are difficult to overcome, they are expected to become an important agricultural strategy for improving animal well-being and global food production in the future.

Conflict of interest

The authors declare no conflict of interest.

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