764. Recent advances in genomics of equine health, welfare and performance

S.C.M. Mikko

Swedish University of Agricultural Sciences, Dept of Animal Breeding and Genetics, P.O. Box 7023, 750 07 Uppsala, Sweden; sofia.mikko@slu.se

Abstract

Equine research and breeding have encountered major changes due to the rapid development of molecular genetic technology. Today, there are 254 equine traits listed in Online Mendelian Inheritance in Animals (OMIA), while the *Horse*QTLdb lists 2,605 QTLs representing 64 traits. Strong selection increases the risk of inbreeding and multiplication of deleterious variants. Analysis of genomic regions under selection, and trio sequencing could complement Genome-wide Association Studies (GWAS) in the search for genetic background of traits and diseases. The increased possibilities to test for a variety of disease-causing mutations, or performance-associated QTLs, support breeders in informed breeding decisions. SNP genotyping will most likely be implemented for horse parentage analysis in the near future. This large amount of genomic data will open new possibilities to address research questions, and will make it possible to carry out marker-assisted and/or genomic selection also in horses.

Introduction

Equine research and breeding currently encounter major changes due to the rapid development of molecular genetic technology. The horse was among the first mammals to be sequenced, and the trace files, and first gene build of the EquCab2 assembly, was released in already in 2007 (Wade *et al.* 2009). This resulted in an increase of likely causal variants detected and reported to the database Online Mendelian Inheritance in Animals (OMIA) (Figure 1).

The availability of the horse genome assemblies likewise increased the number of QTLs reported to the Horse Quantitative Trait Locus Database (*Horse*QTLdb) (Figure 2; Hu *et al.* 2007, 2022). In 2018 the EquCab3.0 assembly was released providing the community with updated genomic data (Kalbfleisch *et al.* 2018), which resulting in even more variant- and QTL-associations reported.

Equine genomic research has created new possibilities to effectively identify and study genetic risk factors for hereditary diseases in order to develop new and efficient treatments. The gain in genomic understanding about complex traits has further facilitated the selection process for performance in sport horses. Breeding

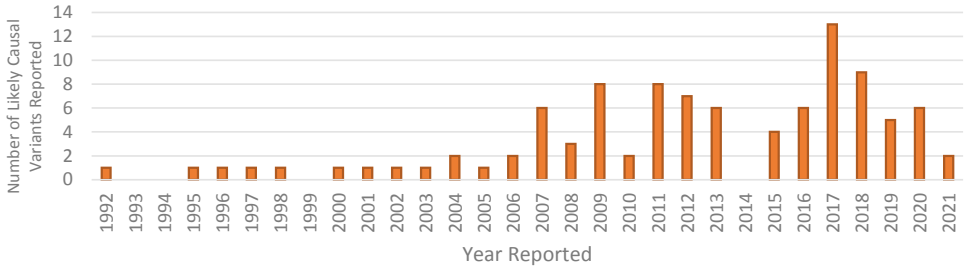

Figure 1. Number of likely causal variants reported in OMIA per year.

R.F. Veerkamp and Y. de Haas (eds)
Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP)
DOI: 10.3920/978-90-8686-940-4_764,

organizations are obliged to monitor mutations unfavourable to animal welfare, and should be proactive in providing breeding advise promoting sound and healthy individuals. Offering a scientifically supported platform will ensure that the Animal Welfare Act and breeding advice are adapted to current and future technological developments in genomics. However, for horse breeding in practice, the new technologies present both opportunities and risks with the entire genome of a horse sequenced in an effort to detect disease-causing mutations, and at the same time reveal information about other genetic variants in this particular individual. Hence, we are facing a new era where breeders will need and demand assistance and guidance by geneticists, veterinarians and other professionals to interpret the plethora of data available so they can make informed choices in their breeding scheme. The structure of the equine breeding business mainly differs from the breeding of production animal with very few large internationally operating commercial breeding companies. Instead, the equine breeding mostly relies on smaller private companies, or single breeders gathering a quite disparate group of people with a diverse base of knowledge. To produce competitive and healthy horses, breeders therefore need reliable information based on scientific evidence.

Strong selection pressure, as in selective breeding of elite performing horses, will result in chromosomal regions with lower genetic variation, that could be referred to as 'regional inbreeding'. Identification of genetic markers or causative mutations in such regions provide new selection tools in horse breeding. By calculating breed divergence, we revealed long homozygous regions in equine chromosomes, comprising genes possibly important for performance in sport horses (Ablondi *et al.* 2019a). Further, we revealed significantly divergent selection pressure on chromosome regions comprising genes controlling cell signalling in the central nervous system in horses not bred for show jumping, compared to show jumping horses. Our analysis discovered positive selection on chromosome regions with genes involved in mobility, relaxed locomotion and sensitivity in non-show jumping horses. In show jumping horses, positive selection, to a larger extent involve regions with genes associated with mentality, postsynaptic signalling, reward system, neuromuscular control of coordination, muscle growth and -function (Ablondi *et al.* 2019b).

Important monogenic and multifactorial diseases, and traits in the horse

Strong selection increases the risk of inbreeding and multiplication of deleterious disease-causing variants, that do not only negatively affect health and welfare of individual horses, but also have negative economic consequences for breeders. Knowledge of association between traits and genotypes with major influence will therefore aid breeders to produce healthy, sustainable, and better performing horses.

Currently, OMIA lists in total 254 Equine traits, and disorders (https://omia.org/home/). The absolute majority of them are health related (82%), followed by exterior (8%), and reproduction (6%), while there are fewer growth, performance, or behaviour traits reported. A likely causal variant is known in 99 of

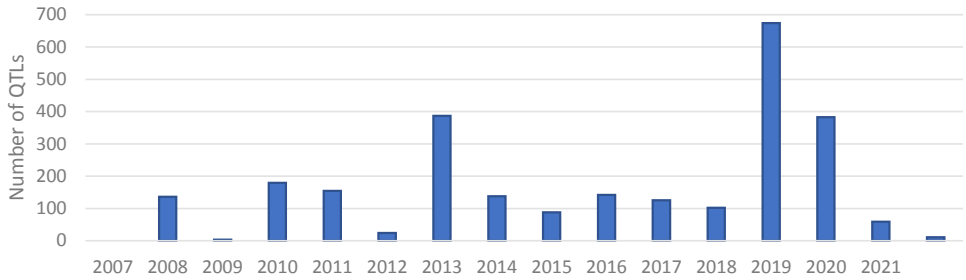

Figure 2. Number of QTL/associations published and reported in the *Horse*QTLdb per year.

these traits or disorders, where 54% of them represent coat colours, 41% are disease-causing variants, and 4% of the variants are associated with performance traits. Among the most recent studies of important monogenic diseases are Fragile Foal Syndrome (Ablondi *et al.* 2022), Dwarfism and joint laxity in Frieasian horses (Leegwater *et al.* 2016), and Ocular Squamous Cell Carcinoma (Chen *et al.* 2021).

Since 2007, 2,605 QTLs/associations representing 64 traits, have been reported to the *Horse*QTLdb (https://www.animalgenome.org/cgi-bin/QTLdb/EC/index). Also in this database, health traits are the most common (48%), followed by growth traits (24%), performance traits (17%), and exterior (6%). Reproduction and behaviour traits are the least reported in the *Horse*QTLdb. Among the most recent studies of important Equine QTLs are; Osteochondrosis (Drabbe *et al.* 2022), Height at Withers (Vosgerau *et al.* 2022), and gait and racing performance (Ricard and Duluard 2022). The difficulties in recording behaviour phenotypes probably reflect the small number of genetic associations reported in both OMIA and *Horse*QTLdb.

Inherited congenital disorders are not always reported by the breeders to the breeding association, and the frequencies of traits and disorders differ largely between breeds, why it is challenging to calculate prevalences. Breeds with an economic value may be studied more often than native and feral horse breeds kept mainly for conservation purposes. In race- and sport horses, a functional and healthy musculoskeletal system is critical for their use, while endangered breeds might instead face fertility problems due to inbreeding. Such factors will influence what specific traits and disorders will be studied, and will not always reflect the prevalence or importance of a trait. In general, health, size and performance traits are the most studied reflecting the functional use of horses.

From disease to genetic testing

To find an assumed genetic association, it is of utterly importance to obtain a detailed clinical description reflecting the correct disease classes associated with the genotypes. A GWAS could be performed using any kind of large-scale genotyping methodology; SNP arrays, 'Genotyping by Sequencing' (GBS, Elshire *et al.* 2011), or 'Skim Sequencing' (Golicz *et al.* 2015). These technologies are also useful for detection of signatures of selection, or specific haplotypes carrying disease-causing mutations. In rare diseases with few cases available, and diseases with autosomal recessive inheritance, causative variants can be detected by Whole Genome Sequencing (WGS) of family trios where candidate variants with the expected genotypes are filtered bioinformatically. In a current project we use WGS of family trios to unravel mutations causing microphthalmia where one or both eyes are underdeveloped or missing. Once a candidate variant is detected it is crucial to validate the finding in a larger sample set, and by functional studies.

Today, the increased possibilities to test for a variety of disease-causing mutations, or performance-associated QTLs, support breeders in informed breeding decisions. Such genetic tests will indeed prevent production of disease-affected foals while unaffected carriers can still be kept in the breeding pool and maintain the genetic variation of the population. Large scale genotyping, or WGS is likely to replace today's genetic tests on individual or panels of mutations. Inevitably, this technology-driven efficient mutation identification will affect the future of horse breeding.

Genomics and the breeders' perspective

Despite the continuous increase in the amount of equine genomic data, genomic selection has not yet been fully implemented in horse breeding. The main reasons are high costs, difficulties to obtain large enough reference populations, and a high effective population size or short linkage disequilibrium in many horse breeds, compared with for example cattle where genomic selection has been applied for many years. For more than 20 years, a few microsatellites have been the standard markers for parentage analysis of horses, however SNP genotyping will most likely be implemented for parentage in the near future. Such a large

amount of genomic data from a huge pool of horses comprising many breeds around the globe, will open new possibilities to address research questions, and will make it possible to implement marker-assisted and/or genomic selection also in horses.

References

Ablondi M, *et al.* (2019a). Signatures of selection in the genome of Swedish Warmblood horses selected for sport performance. *BMC Genomics* 20:717:1-12. https://doi.org/10.1186/s12864-019-6079-1.

Ablondi, M., *et al.* (2019b). Genomic divergence in Swedish Warmblood horses selected for equestrian disciplines. *Genes* 10(12):E976. https://doi.org/10.3390/genes10120976.

Ablondi M., *et al.* (2022) Performance of Swedish Warmblood fragile foal syndrome carriers and breeding prospects. *Genet Sel Evol* 54(4):1-16. https://doi.org/10.1186/s12711-021-00693-4

Chen, L., *et al.* (2021). A novel DDB2 mutation causes defective recognition of UV-induced DNA damages and prevalent equine squamous cell carcinoma. *DNA Repair (Amst)* 97:103022:1-9. https://doi.org/10.1016/j.dnarep.2020.103022.

Drabbe, A., *et al.* (2022). Genome-Wide Association Analyses of Osteochondrosis in Belgian Warmbloods Reveal Candidate Genes Associated With Chondrocyte Development. *J Eq Vet Sci* 111:103870:1-6. https://doi.org/10.1016/j.jevs.2022.103870

Elshire, R.J., *et al.* (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One* 6(5):e19379. https://doi.org/10.1371/journal.pone.0019379.

Golicz, A.A., *et al.* (2015) Skim-Based Genotyping by Sequencing. In: Jacqueline Batley (ed.), Plant Genotyping: Methods and Protocols, Methods in Molecular Biology, vol. 1245, Springer Science & Business Media, New York, USA. https://doi.org/10.1007/978-1-4939-1966-6_19.

Hu Z-L., and Reecy J.M. (2007). Animal QTLdb: Beyond a Repository - A Public Platform for QTL Comparisons and Integration with Diverse Types of Structural Genomic Information. *Mamm Gen,* 18(1):1-4. https://doi.org/10.1007/s00335-006-0105-8

Hu Z-L., *et al.* (2022). Bringing the Animal QTLdb and CorrDB into the future: meeting new challenges and providing updated services. *Nucl Acid Res,* 50(D1):D956–D961. https://doi.org/10.1093/nar/gkab1116.

Kalbfleisch, T.S., *et al.* (2018). Improved reference genome for the domestic horse increases assembly contiguity and composition. *Commun Biol* 1(197):1-8. https://doi.org/10.1038/s42003-018-0199-z

Leegwater, P.A., *et al.* (2016). Dwarfism with joint laxity in Friesian horses is associated with a splice site mutation in B4GALT7. *BMC Genomics* 17:839. https://doi.org/10.1186/s12864-016-3186-0.

Online Mendelian Inheritance in Animals, OMIA. Sydney School of Veterinary Science, 11 March 2022. Available at: https://omia.org/

Ricard, A., and Duluard, A. (2021). Genomic analysis of gaits and racing performance of the French trotter. *J Anim Breed Genet* 138(2):204-222). https://doi.org/10.1111/jbg.12526

Vosgerau, S., *et al.* (2022). Genetic and genomic characterization followed by single-step genomic evaluation of withers height in German Warmblood horses. *J Appl Genet.* Epub ahead of print. https://doi.org/10.1007/s13353-021-00681-w.

Wade, C.M., *et al.* (2009). Genome Sequence, Comparative Analysis, and Population Genetics of the Domestic Horse. *Science* 326:865-867. https://doi.org/10.1126/science.1178158