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Final report

Identification of Genes with a Negative Effect on Equine Health and Welfare: *A framework based on whole-genome sequencing and bioinformatics for horses*

Project number: H-18-47-406

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Part 1: Detailed summary (Svenska)

Projektets övergripande syfte var att förbättra hästarnas hälsa och välbefinnande genom att identifiera orsakande mutationer för ärftliga sjukdomar och underlätta informerad avel av friska hästar. Initialt fokuserade vi sjukdomarna mikroftalmi och WFFS som nyligen observerats bland svenska varmblodshästar. Vi kombinerade helgenomsekvensering (WGS) av åtta individer med mikroftalmi och deras föräldrar samt fyra kontroller, bioinformatisk filtering, klinisk diagnos av sjukdomsfenotyper och våra kunskaper om genetik i avelsprogram. I projektet utvecklade vi ett generellt ramverk för att finna genetiska varianter som orsakar sjukdomar med enkel mendelsk nedärvning. Målen med studien var:

1. kliniskt definiera monogena sjukdomar med autosomalt recessiv nedärvning,
2. utveckla ett allmänt ramverk för att identifiera sjukdomsorsakande mutationer med en bioinformatikpipeline för helgenomsekvensering,
3. ta fram en plan för avelsrådgivning i olika scenarier av arvsmönster och sjukdomens svårighetsgrad.

Projektet blev framför allt försentat p.g.a. Covid-19 pandemin då det var svårt att samla in prover. Dessutom gick laboratorieverksamhet på sparlåga då resurser som instrument och förbrukningsmaterial prioriterades till Covid-diagnostik. Det visade sig dessutom att vår modellsjukdom, mikroftalmi, hade ett mer komplext nedärvningsmönster än vår hypotes om autosomalt recessiv nedärvning. Det har hittills gett oss fler frågor än svar men också en

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vägledning om hur vi kan gå vidare i forskningen om denna sjukdom. Det gör vi nu i två nya forskningsansökningar.

Preliminära resultat tyder på att individer med mikroftalmi är mer relaterade till varandra än till hela SWB-populationen. Vår härstamningsanalys pekar på ett fåtal drabbade kandidatfamiljer. Sex av de sju fäderna som sekvenserades, delade samma anfader, inte mer än fyra generationer tillbaka. Cirka 5-10 SWB-fall av mikroftalmi rapporteras varje år. Vid ett autosomalt recessivt nedärvningsmönster, kan anlagsbärarfrekvensen uppskattas till cirka 10%, vilket är en nivå där fall vanligtvis dyker upp, och åtgärder bör tas inom avelsföreningarna för att förhindra ytterligare spridning. Vidare ser vi en signifikant skev könskvot mellan drabbade föl, där >70% av fallen är ston (z-värde=6,8, p<0,0001). De totalt 64 insamlade fallen fördelar sig lika mellan uni- och bilaterala fall. I alla unilaterala fall där lateralitet är känd, är vänster öga affekterat, oavsett kön.

Vår första strategi med trio-WGS var att filtrera efter autosomalt recessivt nedärvda genetiska varianter. Inledningsvis utförde vi WGS av fyra fall och kompletterade senare triosekvenseringen med ytterligare fyra fall. Ändå fick vi inte några riktigt konklusiva resultat. Troligen beror detta på att mikroftalmi har en mer komplex nedärvning än den hypotetiska autosomalt recessiva. Triosekvensering är inte helt optimal för andra arvsmönster än autosomalt recessiv, eftersom filtreringen inte är så effektiv för att utesluta icke-orsakande varianter. Med tanke på det upptäckta skeva könsförhållandet bland de affekterade fölen, modifierade vi ändå filtreringen för att inkludera andra arvsmönster. Analyser av dessa varianter pågår, och vi behöver ytterligare andra metoder för att undersöka det till synes komplexa arvsmönstret av mikroftalmi från häst. Därmed har vi nu inlett ett internationellt samarbete och lämnat in två forskningsansökningar för att lösa mysteriet med nedärvd mikroftalmi hos häst.

Vi använde den autosomalt recessivt nedärvda dödliga sjukdomen *Warmblood Fragile Foal Syndrome* (WFFS) som ett exempel på hur man hanterar en sådan sjukdom i ett avelsprogram beroende på arvsgång, anlagsbärarfrekvens och selektionstryck. Våra simuleringar tyder på att de höga anlagsbärarfrekvenserna som observerats för WFFS-allelen bland varmblodiga ridhästar, överensstämmer med en balanserad selektion (Ablondi et al. 2022). Sådana simuleringar kan vara användbara vid avelsrådgivning i populationer med olika arvsmönster, populationsstrukturer och selektionstryck. I exemplet WFFS har vi redan varit involverade i rådgivning om hur man använder anlagsbärande avelshingstar. Vi planerar att ytterligare utveckla sådana simuleringar för att kunna ge bra genetisk rådgivning till olika avelsorganisationer. Råden kan t.ex. se mycket olika ut mellan en liten ras som riskerar en hög inavelsgrad, jämfört med en större ras med stor genetisk variation.

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Part 2: Main report (max. 10 pages)

Introduction

The overall aim of this project was to improve equine health and welfare by identifying causative mutations for inherited diseases to aid informed breeding of healthy horses. We used a comparative genomics approach, combining whole-genome sequencing (WGS) and bioinformatics with state-of-the-art clinical diagnosis of disease phenotypes. In the project we developed a general framework for variant-detection of equine monogenetic diseases using WGS of family trios consisting of affected offspring, and healthy parents, consistent with an autosomal recessive inheritance. In this way, affected offspring should be homozygous for recessive deleterious variants, not present in the reference genome, and the parents should be heterozygous for the same variant. The initial focus was to investigate the eye diseases microphthalmia, and Warmblood Fragile Foal Syndrome (WFFS), recently observed among Swedish Warmblood horses. The objectives of the study were:

1. clinically define monogenic diseases with autosomal recessive inheritance,
2. develop a general framework for identifying disease causing mutation with a bioinformatics pipeline for whole-genome sequencing,
3. develop a plan for breeding advise in different scenarios of inheritance patterns, and severity of the disease.

Material and methods

Ethical approval

An ethical application was filed and approved (Dnr 5.8.18-05055/2019). The ethical approval is valid for collection and storage of blood and tissues from both healthy and diseased horses, as well as tissues from euthanized horses. Blood and tissue samples are stored in Hästbiobanken at SLU, which means that samples can later be used in other projects.

Material and Methods

In total more than 60 cases of microphthalmia/anophthalmia have been identified among Swedish Warmblood horses. We received blood samples from eight affected foals – four unilateral and four bilateral, including eight mothers, and four fathers. We were unable to get hold of blood samples from three of the fathers, while two of the cases shared the same father (Table 1). Hair samples are available from another eleven cases, and either hair and/or blood samples are available from 35 parents of affected foals. These samples were used to validate candidate variants.

Four eyes from three foals (Foal-2, both eyes; Foal-32, left eye; and Foal-34, left eye) were enucleated and collected. The eyes from Foal-2 were collected in formalin, while in the case of the eyes from the unilateral affected Foal-32 and Foal-34, one half of the eyes were stored in formalin for histopathology, and the other half in RNAlater for possible analysis of gene expression. All eyes collected in formalin were examined by light-microscopy.

Eight cases, and twelve carriers were whole genome sequenced using the instrument Illumina NovaSeq at the SNPSEQ facility, SciLifeLab in Uppsala. Foal-1, and its parent were also sequenced using Oxford Nanopore Technology (ONT) to detect structural, and/or epigenetic variants. (Table 1).

To model the management of a lethal allele in SWB, we used stochastic genetic simulation to investigate the effect of balancing selection and selection against carriers of a recessive lethal allele (*Warmblood Fragile Foal Syndrome*, WFFS), using a population modelled on SWB horses. Simulations were performed with R (R Foundation for Statistical Computing. R: A

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language and environment for statistical computing. Vienna: R Core Team; 2018), and the AlphaSimR package (Gaynor, Gorjanc, and Hickey, 2020).

Table 1. *List of the eight microphthalmia affected foals with blood samples available, the status of their disease and availability of samples from parents. Whole genome sequence (WGS) coverage of parents is shown within brackets.*

Cases	Type of microphthalmia	WGS coverage (NovaSeq/ONT)	Father	Mother
Foal-1	Unilateral	24X/20X	Father-1 (32X/8X)	Mother-1 (29X/8X)
Foal-2	Bilateral	20X/NA	Father-2 (14X/NA)	Mother-2 (16X/NA)
Foal-3	Unilateral	20X/NA	Father-3, only hair sample available, no WGS	Mother-3 (20X/NA)
Foal-5	Bilateral	14X/NA	Father-5 (>30X/NA)	Mother-5 (22.8X/NA)
Foal-30	Bilateral	>30X/NA	Father-30, no sample available	Mother-30 (>30X/NA)
Foal-32	Unilateral	26X/NA	Father-32, no sample available	Mother-32 (23X/NA)
Foal-34	Unilateral	>30X/NA	Father-34, WGS available, (20X/NA)	Mother-34 (20X/NA)
Foal-35	Bilateral	>30X/NA	Father-5 (>30X/NA)	Mother-35 (>30X/NA)

Pipeline for genome variant detection and prediction the effects of variants on genes

A new pipeline for genome variant detection and prediction of their effect was developed and realized. We chose the GATK best practices workflow – short variant discovery (SNVs + InDels >50bp) for cohort analysis (*Figure 3*). This workflow is designed to operate on a set of samples constituting a study cohort. This allow us to achieve the same results as joint calling in terms of accurate genotyping results, without the computational bottleneck of exponential runtimes, and with the added flexibility of being able to re-run the population-level genetic analysis at any time as the available cohort grows.

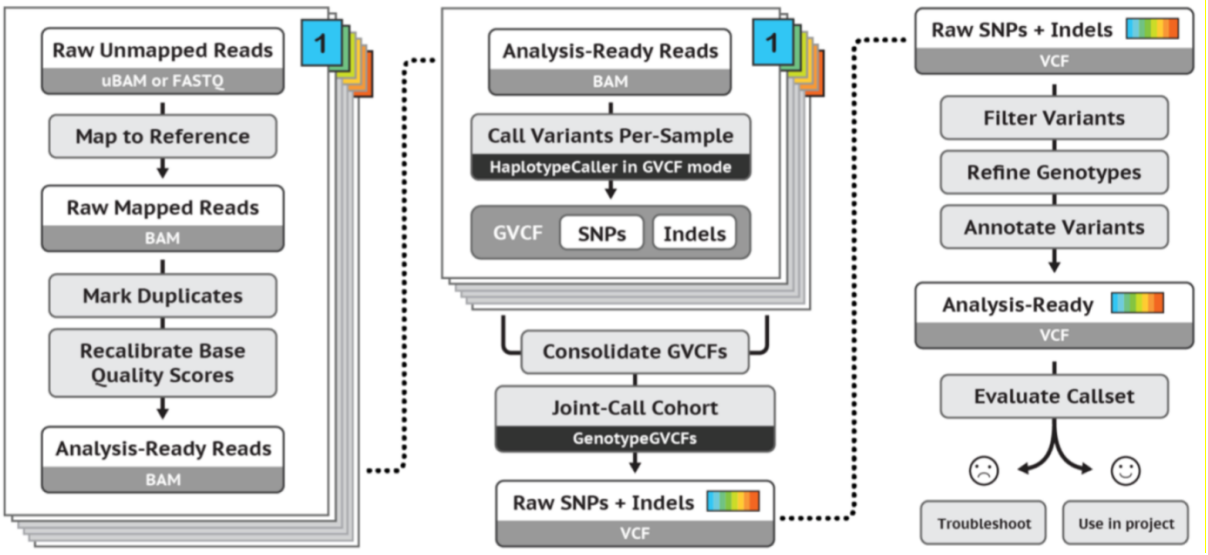

Figure 1. Short variant discovery (SNPs + InDels) for cohort analysis [GATK best practices workflow, https://gatk.broadinstitute.org/].

For the prediction of the effect of genomic variants we used the Ensembl *Variant Effect Predictor* (VEP) bioinformatics tools. VEP determines the effect of the variants (SNPs, insertions, deletions, CNVs or structural variants) on genes, transcripts, and protein sequence, as well as regulatory regions [https://www.ensembl.org/info/docs/tools/vep/index.html]. To predict the effects of coding variants on protein function we used *Sorting Intolerant from Tolerant* (SIFT) bioinformatics tools. SIFT predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids

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[https://sift.bii.a-star.edu.sg/]. The SIFT score ranges from 0.0 (deleterious) to 1.0 (tolerated). Variants with scores <0.05 are considered deleterious and may affect protein function.

Pipeline for structural variant discovery

A new pipeline for the structural variant discovery and prediction of the effects of variants on genes was developed and realized. As a workflow for the new pipeline, we chose the Delly Germline Structural Variant calling tool which was designed for cohort analysis of structural variants in samples for high-coverage genomes. (Figure 4).

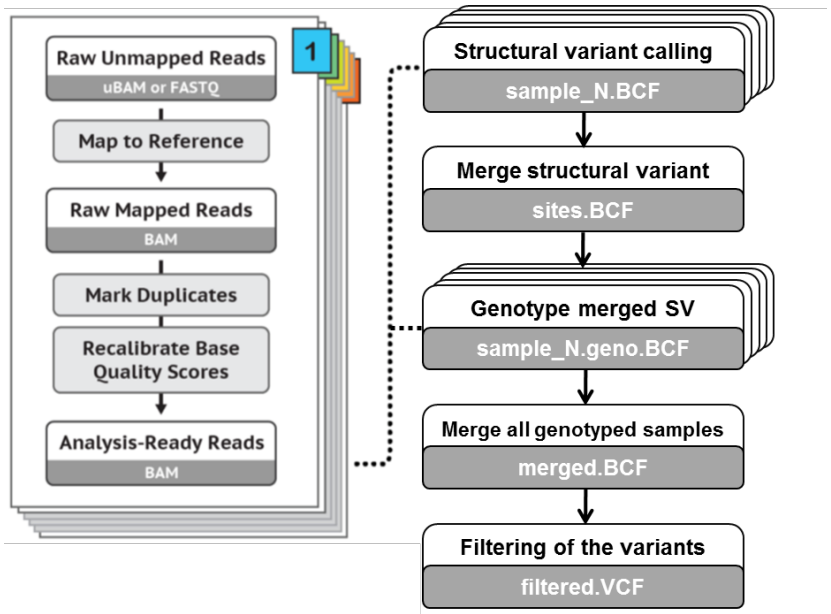

Figure 2. Cohort analysis of structural variant in samples for high-coverage genomes using Delly Germline Structural Variant calling, https://github.com/dellytools/delly).

This workflow is designed to operate on a set of samples constituting a study cohort, in the same way as the pipeline described above for SNVs and InDels. Our designed Delly pipeline allow us to discover all types of structural variants, such as large deletion, inversions, translocations, duplications, and copy number variations. Also with this pipeline, Ensembl Variant Effect Predictor (VEP) bioinformatics tools were used to predict the consequence of variants.

Sample processing and analysis

In total, we processed 24 samples with our two new pipelines based on GATK and Delly. The samples included five trios, three duos, and four control samples. The control samples comprise WGS data from three individual SWB stallions and one pool of 20 SWB stallions (Table 1). The WGS samples were mapped on the reference genome of the horses (*Equus Caballus*) EquCab3.0 (https://www.ncbi.nlm.nih.gov/assembly/GCF_002863925.1/). After processing, all discovered variants were filtered by genotypes using custom R scripts and were analyzed in VEP and SIFT. The reference and variant alleles are denoted “0”, and “1”, respectively. Thus, an individual homozygous for the variant allele has the genotype “1/1”. All filtering was as well done for three groups of cases; 1) variants common to all eight cases, 2) variants found in unilateral cases, and 3) variants found in bilateral cases. The number of variants with different types of filtering for SNVs and InDels, are shown in Table 2. Likewise, the number of variants with different types of filtering for SVs InDels, are shown in Table 3.

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Table 2. *SNV and InDel genotypes of the eight microphthalmia affected foals and their parent were filtered according to different putative inheritance patterns. The four control samples were filtered as "Not 1/1" and missed positions as "./.". A) The number of variants is common between all eight cases. B) The number of variants is common between all four unilateral cases. C) The number of variants is common between all four bilateral cases.*

A) All cases	**Offspring**	**Father**	**Mother**	**Controls**	**Incl missed positions**	**Total number of variants**
Autosomal recessive	1/1	Not 1/1	Not 1/1	Yes	Yes	5,308
Dosage effect on ECAX	1/1	1/1	Not 1/1	Yes	Yes	541
B) Unilateral						
Autosomal recessive	1/1	Not 1/1	Not 1/1	Yes	Yes	13,704
Dosage effect on ECAX	1/1	1/1	Not 1/1	Yes	Yes	1,165
C) Bilateral						
Autosomal recessive	1/1	Not 1/1	Not 1/1	Yes	Yes	11,645
Dosage effect on ECAX	1/1	1/1	Not 1/1	Yes	Yes	1,206

Table 3. *SV genotypes of the eight microphthalmia affected foals and their parent were filtered according to different putative inheritance patterns. The four control samples were filtered as "Not 1/1" and missed positions as "./.". A) The number of variants is common between all eight cases. B) The number of variants is common between all four unilateral cases. C) The number of variants is common between all four bilateral cases.*

A) All cases	**Offspring**	**Father**	**Mother**	**Controls**	**Incl missed positions**	**Total number of variants**
Autosomal recessive	1/1	Not 1/1	Not 1/1	No	Yes	1,815
Dosage effect on ECAX	1/1	1/1	Not 1/1	Yes	Yes	97
B) Unilateral						
Autosomal recessive	1/1	Not 1/1	Not 1/1	No	Yes	1,843
Dosage effect on ECAX	1/1	1/1	Not 1/1	Yes	Yes	102
C) Bilateral						
Autosomal recessive	1/1	Not 1/1	Not 1/1	No	Yes	1,827
Dosage effect on ECAX	1/1	1/1	Not 1/1	Yes	Yes	98

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Results and discussion

Analysis of microphthalmia cases

In cases where samples were not available, pedigree information was collected to identify inheritance patterns, and potential ancestral founders. The pedigree analysis so far conducted in this study, all point to a few affected candidate sire family lineages with potential common ancestors discovered many generations back. In fact, six out of the seven sires sequenced, shared the same male ancestor, no more than four generations back. Most of the other cases, point in the same direction, although some do share a common ancestor, more than four generations back. Preliminary results indicate that cases are more related to each other than to the whole SWB population. Assuming an autosomal recessive inheritance, and the number of about 5-10 cases of microphthalmia reported among SWB each year, the carrier frequency could be estimated to about 10%, which is a level where usually cases are appearing in the population, and action should be taken within the breeding associations to prevent further spreading of the deleterious gene variants. Carrier sire lineages are also present in microphthalmia foals in other European studbooks than SWB. We have therefore initiated collaboration with colleagues in relevant countries, and together with them we have filed two different grant applications (Formas/Weave; 2023-00980 & SHF; H-23-47-767).

With the increasing number of cases reported, we do see significant skewed sex ratio of affected foals, with >70% of cases being females (z-value=6.8, $p<0.0001$). This has now been considered when filtering for causative variants. Although WGS trio sequencing is not as powerful for other inheritance patterns than autosomal recessive, there is still a possibility to perform some other filtering steps. Furthermore, out of 64 cases, 32 are bilateral, 31 are unilateral, and in one case this information was lacking. In all unilateral cases where laterality is known, the left eye is affected regardless of sex.

Clinical examinations and histopathology

Clinically, all four eyes enucleated from three foals were diagnosed with severe microphthalmia, in the first case bilaterally and in the two unilaterally with a fellow eye that was considered normal on ophthalmic examination (*Figure 1*).

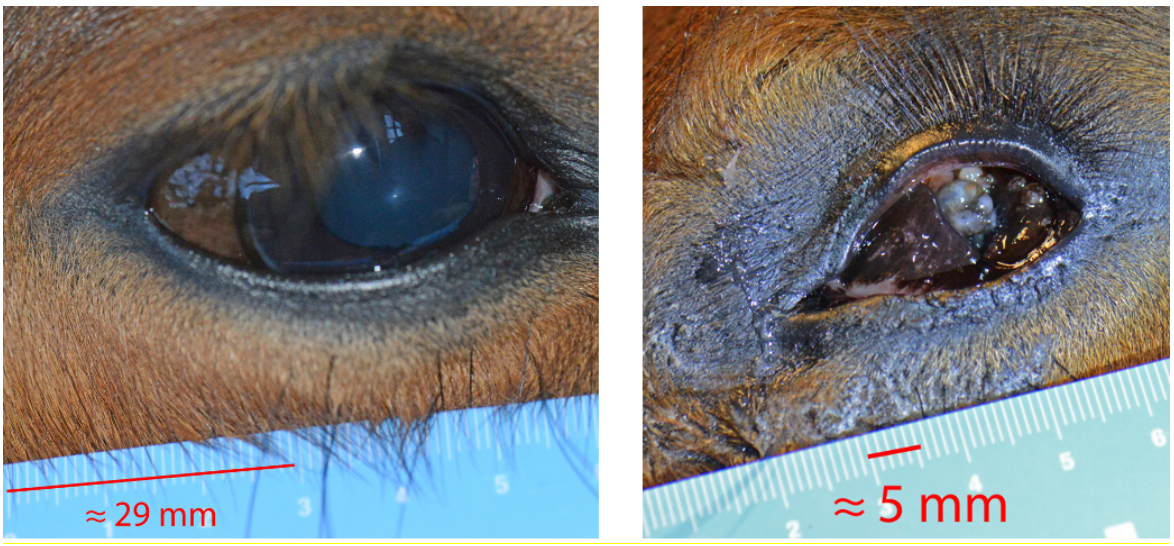

Figure 3. Right and left eyes, respectively, from a foal with left-sided microphthalmia. The right eye was considered normal in size and functional on ophthalmic examination, whereas the left eye was considerably reduced in size and had a profoundly abnormal appearance (the left eye is the greyish, lobulated structure in the abnormally shortened palpebral fissure).

Light-microscopic findings in the enucleated globes were similar in all three eyes: severe microphthalmia, ocular dysplasia and lenticular metaplasia (*Figure 2*). There were no signs of

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intraocular inflammation or infection. Similar congenital microphthalmia has been reported in Rocky Mountain foals (Grahn et al. 2008), and a population of White Tail Deer in the USA (Clarke et al. 2018).

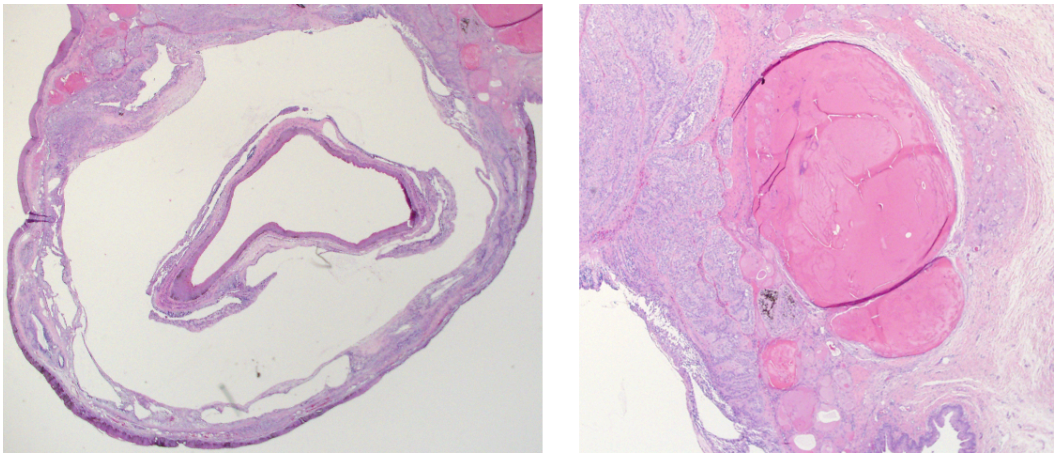

Figure 4. Light-microscopy of a globe from the foal with bilateral microphthalmia. The left panel shows the folded, primitive retina in a cavity interpreted as the malformed globe. The right panel shows aggregates of lens protein and lens epithelium embedded in the surrounding connective tissue.

Whole Genome Trio Sequencing

Our initial strategy with the WGS of trios, was to filter for autosomal recessive inheritance. Initially, we performed WGS of four cases. Four candidate gene variants were detected in the Foal-1 family and were selected for validation by Sanger sequencing. Although three of the variants were confirmed in Trio-1, they were not conclusive for the other cases. We later complemented the trio sequencing with another four cases. Still, no conclusive results were achieved.

Trio sequencing is not optimal for other inheritance patterns than autosomal recessive, as the filtering is not that efficient to exclude non-causative variants. Considering the discovered skewed sex ratio among the microphthalmia cases, we anyway modified the filtering to include other inheritance patterns. One of them being some kind of dosage effect where the sire can carry one copy on the X chromosome without being affected, while the mother must be heterozygous. Analysis of these variants are on-going, and we need yet other methods to investigate the seemingly complex inheritance pattern of equine microphthalmia. Thus, we have now initiated international collaboration and filed two grant applications to solve the mystery of equine micropthalmia (see above).

Population genetic simulations as a guide for breeding advise

We used the autosomal recessive lethal disease Warmblood Fragile Foal Syndrome (WFFS) as an example on how to consider a congenital disease in a breeding scheme depending on inheritance pattern, carrier frequency, and mode of selection pressure. Our simulations suggest that the high carrier frequencies observed for the WFFS allele are consistent with balancing selection. The simulations were used to evaluate the prospects for selection against carrier stallions and indicate that this is feasible with little impact on genetic gain (Ablondi et al. 2022, listed in Part 3: Result dissemination). Such simulations could be useful for breeding advise concerning different inheritance patterns and population structures. In the example of WFFS, we have already been involved in the advice of how to use WFFS carrier breeding stallions in Warmblood breeds.

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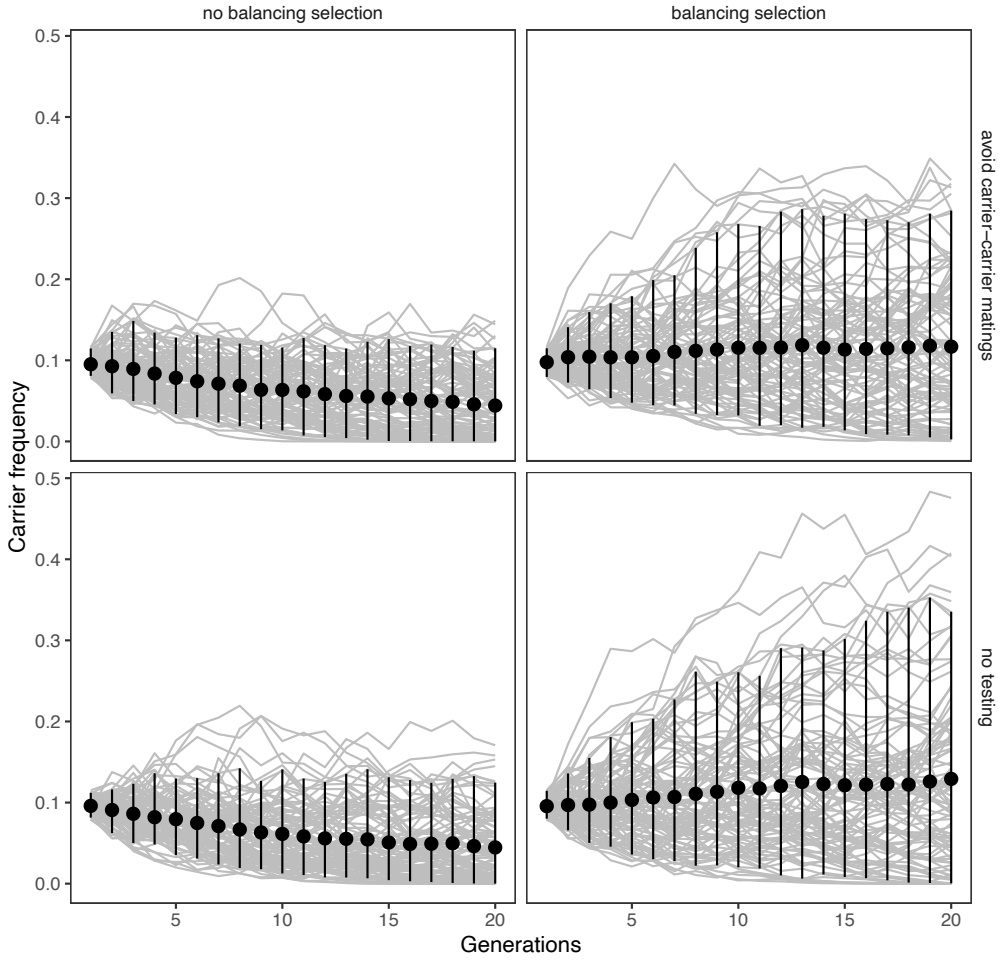

Figure 5. Frequency of the carriers of the recessive lethal allele in simulated breeding programs. Frequency of carriers over 20 generations of breeding without (panels to the left) and with balancing selection (panels to the right), in scenarios where genetic testing is used to avoid mating between carriers (top panels) or in a scenario where the lethal allele is unknown, and no testing is available (bottom panels). The points show the mean frequency of carriers over simulation replicates, with error bars showing the 5 and 95 percentiles, and the grey lines in the background showing the individual simulation replicates.

Conclusions

Equine microphthalmia is characterized by one or two undeveloped eyes. In all unilateral cases, the left eye is affected. More than 70% of all cases are females. Carrier stallions and their sons are unaffected, while their daughters can be affected.

Whole Genome Sequencing of eight cases (four uni-, and four bilateral cases), and their parents revealed common genetic variants with putative functional effect in 15 annotated genes when filtered for autosomal recessive inheritance. Among uni- and bilateral cases, functional variants were found in 57 and 43 unique genes, respectively. Likewise, there were 1815 large structural variants (SVs) common to all eight cases. Among those, 28 and 12 were unique in uni-, and bilateral cases, respectively. Validation of these findings as well as filtering for other inheritance patterns is on-going.

Our pipelines for filtering autosomal recessive inherited genetic variants are up and running and could be used for any new disease appearing in a population. The pipelines will be publicly available in GitHub, as soon as our results are scientifically published.

Our simulation studies show that we can offer sound breeding advise depending on inheritance pattern, and population structure.

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Relevance for the practical horse sector incl. recommendations

Describe how the project results can be used in the practical horse sector, what is needed for the results to be implemented, and (if applicable) what needs further investigation after the project.

Whole genome sequencing provides new opportunities for identifying disease-causing mutations and gaining increased knowledge of the underlying genetic causes and additional risk factors behind hereditary diseases in horses. The change in technology, and the lower cost of WGS will change the everyday life of the horse breeder.

Initially, we have focused on finding the causative mutation/s responsible for equine microphthalmia. In the future, our framework used for finding causative genetic variants, could be applied also for other polygenic and complex diseases. We will evaluate the possibility to add putative microphthalmia causative variants to the SNP-chip to be used for parentage analysis, which is soon likely to replace today's genetic tests on individual mutations. The main objective of the project is to improve equine health and provide a scientifically substantiated platform that can be used to ensure that the Animal Welfare Act, and breeding advice, are adapted to today's and the future's technological development.

When a new inherited disease is discovered in a breed, or have increased in frequency in the population, anxiety and fear arise among breeders. They want to know where the mutation came from, if their breeding stallion or mare is a carrier, or if there are any stallion families they should avoid. Inherited diseases, like microphthalmia, are not only negatively affecting health and welfare of individual horses, but also have negative economic consequences for breeders. Financial loss can be substantial when mares resorb or abort their pregnancies, the newborn foal must be euthanized, or if stallions are not chosen by a mare owner because he is a non-affected carrier, even if the mare to be covered is homozygous normal. In addition, when causative mutations are unknown, it may lead to decreased genetic variation if horses are excluded from breeding, based merely on breeders' fear and lack of information. Therefore, the results of this project and the possibility to test for microphthalmia causing mutations, will aid breeders in informed breeding decisions. With an ambition to achieve increased genetic variation and the productions of competitive as well as healthy horses, breeders need reliable information based on scientific evidence. Preserving and increasing genetic diversity in horses have long-term implication for future breeding goals.

There are many unknown deleterious mutations in the horse genome with potential to spread in the population if they are linked or associated to other traits selected for in sport horses where breeding generally involves strong selection pressure for performance. This may therefore lead to inbreeding and negative effects on health and welfare, i.e., when disease-causing mutation hitch-hike with genes with positive effect for performance. A deleterious, recessive mutation may in such cases, spread and remain undetected for many generations until reaching a large enough carrier frequency. If the mutation becomes widespread, there will be a higher risk of mating between two carriers. It is therefore of utterly importance to quickly identify disease causing mutations and aid breeders in making informed decisions. Hence, the findings from this study will benefit breeders of all horse breeds, not only the Warmblood breeds investigated in this specific study.

The acquired knowledge from this project will be a guide as how to meet the change from today's estimated breeding values, DNA-tests of a few genes to sequence information from the entire genome. Inevitably, this technology-driven efficient mutation identification will affect the future of horse breeding. It is therefore important to ensure that Animal Welfare Act, guidelines, and breeding managements are adapted to a situation where individual horses entire genome and if not all, but many disease mutations are known. Not the least considering that

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there may already be examples where DNA-tests of individual diseases have led to a reduction in the breeding population because carriers are not used in breeding. In such cases the result will be a reduction of genetic variation, and an increased level of inbreeding with a higher risk for other hereditary diseases where no DNA-tests is yet available. Another problem is when DNA-tests are performed for only one of many risk factors for complex diseases, and breeders base their breeding entirely on this information. This can lead to unwanted consequences where the frequency of the other risk factors can increase, and those combinations together create a greatest risk for disease.

In summary, we have partly established a logistic and scientific platform on how to use pre-breeding DNA-tests. Such tests will help to avoid production of disease-affected foals and compromised animal welfare, while carriers for these conditions may still be kept in the breeding pool and maintain the genetic variation in the population. The International Society of Animal Genetics (ISAG) will soon implement SNP-chip genotypes for parentage in horses. This will create an excellent platform for large scale screening of genetic variants associated with congenital diseases. In the future, our new information could be included in a scenario of genomic selection.

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Part 3: Result dissemination

Scientific publications, *published*	Michela Ablondi, Martin Johnsson, Susanne Eriksson, Alberto Sabbioni, Åsa Gelinder Viklund & Sofia Mikko. 2022 "WFFS carrier effect on performance traits in Swedish Warmblood horses and future breeding prospective" BMC Genetics Selection and Evolution 54:4 pp1-16. https://doi.org/10.1186/s12711-021-00693-4
Scientific publications, *submitted*	*Author(s), title*
Scientific publications, *manuscript*	Iryna Shutava, Björn Ekesten, Tomas Bergström, Suvi Mäkeläinen, Carl-Johan Rubin, Sofia Mikko. "Inheritance of Equine Microphthalmia", *in preparation*
	Iryna Shutava, Sofia Mikko: The bioinformatic pipeline will be publicly available in GitHub upon scientific publication of our results
Conference publications/ presentations	An abstract was submitted to the Havemeyer workshop that was planned in July 2020, but was cancelled due to the Corona Pandemic
	"Will selection for elasticity maintain the allele causing fragile foals?" Oral poster presentation at the Virtual ISAG2021 Conference. https://www.isag.us/2021/
	Sofia Mikko. 2022 "Recent advances in genomics of equine health, welfare and performance" Proceeding of 12th World Congress on Genetics Applied to Livestock Production (WCGALP), 3-8 July 2022 https://wcgalp.com/, https://doi.org/10.3920/978-90-8686-940-4
	Michela Ablondi, Susanne Eriksson & Sofia Mikko. 2022 "Haplotype blocks and heterozygosity rich regions on ECA2 in Swedish Warmblood horses" Proceeding of 12th World Congress on Genetics Applied to Livestock Production (WCGALP), 3-8 July 2022 https://wcgalp.com/, https://doi.org/10.3920/978-90-8686-940-4
	Sofia Mikko "Equine Microphthalmia – A developmental disease with skewed inheritance pattern" Poster at Carenet's networking meeting September 23, 2022, http://www.slu.se/carenet
	Iryna Shutava, Björn Ekesten, Carl-Johan Rubin, Suvi Mäkeläinen, Tomas Bergström, Jens Tetens & Sofia Mikko. "Whole Genome Trio Sequencing to Reveal the Genetics of Equine Microphthalmia", Poster & oral presentation, ISAG2023 Conference, Cape Town, South Africa, 3/7 July 2023. https://www.isag.us/2023/
Other publications, *media etc.*	
Oral communication, *to horse*	"Welfare from a breeding point of view: WFFS & other hereditary diseases in sport horses", 2 Dec 2018, World Breeding Federation for Sport Horses Annual Meeting, Budapest, Hungary, http://www.wbfsh.org/GB/General%20Assembly/General%20Assembly%202018.aspx

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sector, students etc.	”Genomics in horse breeding”, 29 May 2019, World Trotting Conference, Stockholm, http://www.wtc2019.se
	”Genetiska profiler för SWB-hästar”, 13 March 2019, Forskningsseminarium, Strömsholm
	”Molecular Genomics in Swedish Warmblood Horses”, 6 Feb 2020, HGEN department seminar, Uppsala
	”Hur avel skapat sporthästen”, Paddock-program at EuroHorse 2020, during Gothenburg Horse Show, Feb 2020
	“Horse breeding goes Genomic”, 13 May 2020, pedagogic lecture to be evaluated as Associate Professor, SLU, Uppsala
	“WFFS carrier effect on performance traits in Swedish Warmblood horses”, 21 April 2021, Digital seminar for horse researchers at Veterinary & Animal Science Faculty, SLU
	“Molekylär hästgenetik & -genomik”, 20 May 2021, SWB avelsutskott, digital webinar
	“Olika gener- vad betyder det?, 18 Sept 2021, ST webinar
	“Forskningsnytt inom hästgenetik & -genomik”, 23 Jan 2022, SWB judges at young horse evaluation & stallion performance test, digital seminar
	”Effekten av WFFS-anlaget på prestationegenskaper hos SWB-hästar och avelsstrategier, 4 March 2022, Strömsholm. Åsa Gelinder Viklund presented since Sofia Mikko was double booked.
	”WFFS carrier effect on performance traits in Swedish Warmblood horses”, 21 April 2021, digital seminar
	Sofia Mikko. “Recent advances in genomics of equine health, welfare and performance” Invited speaker, WC-GALP 3-8 July 2022 https://wcgalp.com/
	“Forskningsnytt inom hästgenetik & -avel”, 22 Oct 2022, SWB Mälardalen 50 år jubileumsgala
	“How to breed a champion” – Hästavel på DNA-nivå, 30 Oct 2022, SWB/ST avelskonferens 30 Oct 2022, Upplands Väsby
	“Sofia Mikko’s lab”, 10 Nov 2022, HGEN Department Research Day, Krusenberg, Knivsta
	“Identification of Genes with a Negative Effect on Equine Health and Welfare: *A framework based on whole-genome sequencing and bioinformatics for horses”,* 30 Nov 2022, Norsk Hestesenter Avelswebinar
	Information material handed out to the public in the SLU boot at EuroHorse 2023, during Gothenburg Horse Show, Feb 2023
Student theses	
Other	

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