Final report: HESTEFORSK Sykdomskontroll og forbedret dyrevelferd ved hjelp av genetiske markører: Osteochondrose og Birkelandfraktur

Short description of background and goals

The background for the project was to verify the findings of genes with a large effect, or Quantitative Trait Loci (QTL) for osteochondrosis dissecans (OCD) (Lykkjen et al., 2010) and for Birkelandfractur (POF) (Lykkjen et al., 2013) in the Norwegian Standardbred trotter population The first goal was to strengthen the material by Lykkjen, that contained about 240 horses, by collecting a material that altogether became about three times as large. Another goal was to publish two papers in international peer-reviewed journals, one that reports the findings for OCD, the other for POF

Results achieved in the project, relative to goals

In this project a total of 464 horses have been genotyped with a high density chip, fulfilling the first goal of the project.

With regard to the second goal, Knut Røed succeeded to grant a PhD student through NMBU for the project: Liv Østervik. The idea was that that the material should found the basis for her PhD thesis. This was reported to the Norwegian Research Council (NFR) in the progress report of autumn 2013, and accepted. It was considered a strengthening of the project. However, in May 2017, just after final reporting of this project to NFR, Østervig decided to quit at Vet, NMBU. This implied that the project suddenly lost its working power, meaning that finalizing the project had to be done by the project group (permanent staff) at NMBU. Since all are rather occupied, this work had to be done in between other tasks, but deliverance will be at least two scientific papers. Here, we report on two papers, as promised. The first paper reports our results regarding QTL for OCD and POF , while the other reports the finding of inbreeding depression for racing performance, obtained in the same material. Both papers exist as drafts, and a summary is given in what follows. The two papers will be submitted for scientific publication in 2018/2019.

Paper 1

Introduction: The goal of the first paper was to search for QTL-effects by utilizing data for horses born in two different time periods and chipped at different SNP-densities.

Material and methods: The data was that described by Lykkjen et al. (2010) and additionally the horses phenotyped and genotyped in this study.

The 240 horses born in 2006/2007 (Lykkjen et al, 2010) had been genotyped with the SNP50 BeadChip of Illumina, with a density of 54 602 SNP, while in this study horses were genotyped with the Affymetrix (now: Thermo Fisher) 670K chip. This implied that the 50K genotyped horses first had to be imputed to a 670K density. For this we used Beagle 5.0 (Browning et. al., 2018). Moreover, twenty horses had been chipped at both densities, and the imputation accuracy was evaluated for these by use of Plink (Purcell et al., 2007). For all the 20 horses, the identity by state (IBS) relationship coefficient between the imputed data and the genotype data obtained by the Affymetrix chip was > 0.99.

A total of 463 horses had phenotypes available for OCD, while the number of horses with phenotypes scored for POF in the fetlock joint on the hind legs (medial) was 414, and for POF (lateral) 289 phenotypes existed.

These phenotypes and the imputed genotypes were exposed to a QTL search by use of Gemma (http://www.xzlab.org/software.html). The analysis of variance was carried out with a linear mixed model that includes the information in the IBS matrix to utilize information about relationships between animals.

Results and discussion: Figure 1 shows a significant QTL on chromosome 28 for OCD, and verifies the finding on chromosome 28 of Lykkjen et al. (2010), while it does not support the findings of QTL on chromosomes 5, 10 and 27. Note also that a lot of SNP have a high test-statistic value on chromosome 28, which thus can be considered a real hit. The same pattern with a consistent peak is seen for POF (medial)on the same chromosome (Figure 2), although the test statistics does not reach the threshold value of significance. However, in contrast POF (lateral),

did not have a peak on chromosome 28. The current finding does not verify any of the putative QTL for POF reported by Lykkjen et al. (2013).

This means that we are left with the peak on chromosome 28 both for OCD and POF, and our approach is now to examine candidate genes to possibly identify the underlying causative gene. Note that, recently, others have also examined candidate genes for OCD on chromosome 28 in horses (Wypchlo et al, 2018), which will be reasonable to check out in our data.

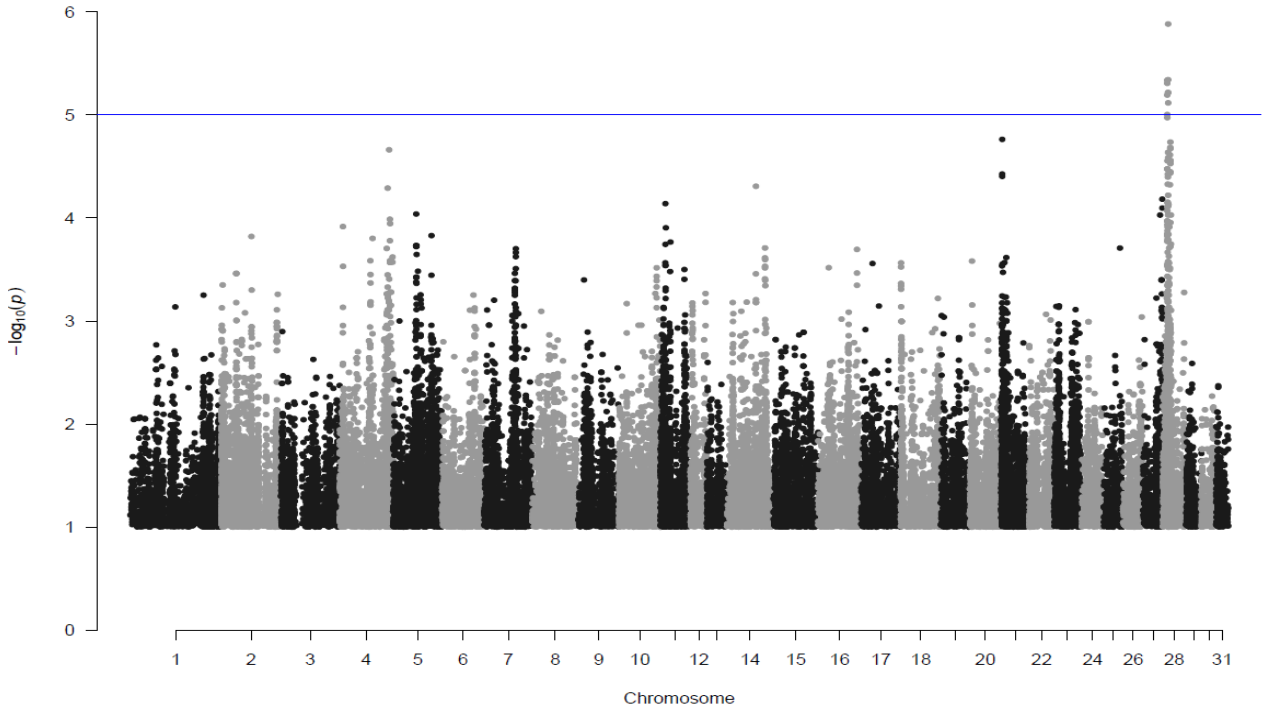

Figure 1: Manhattan plot for OCD in the hock joint, with indicated Bonferoni significance level.

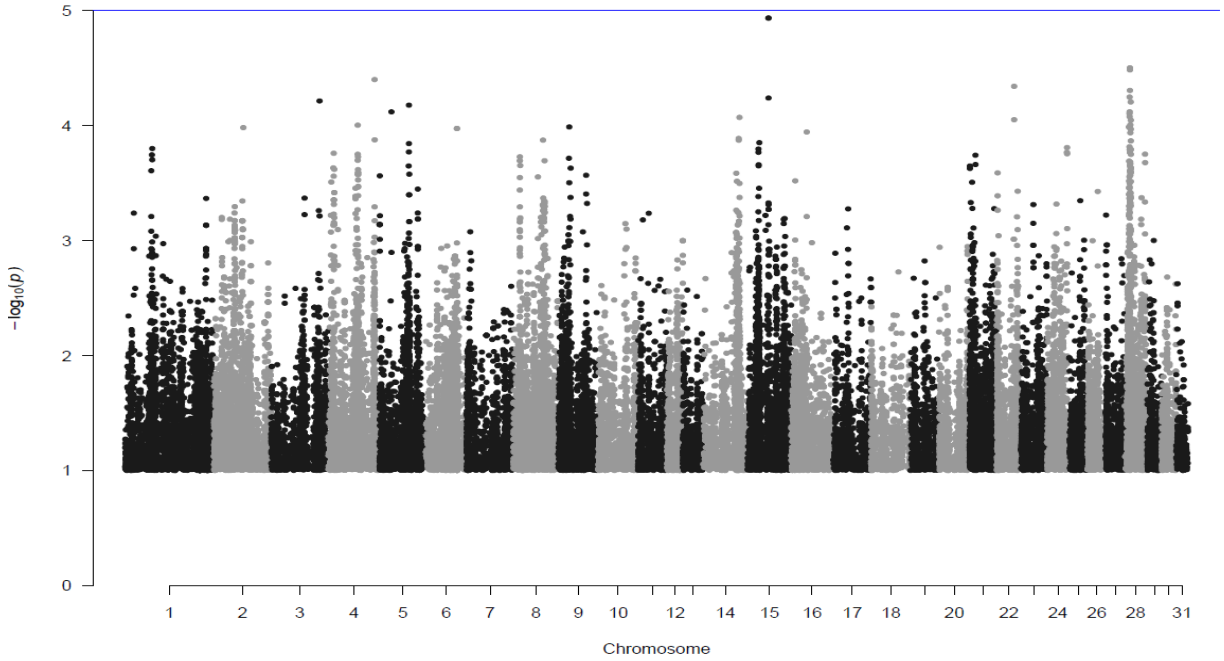

Figure 2: Manhattan plot for POF in the fetlock joint on the hind legs, medial, with indicated Bonferoni significance level.

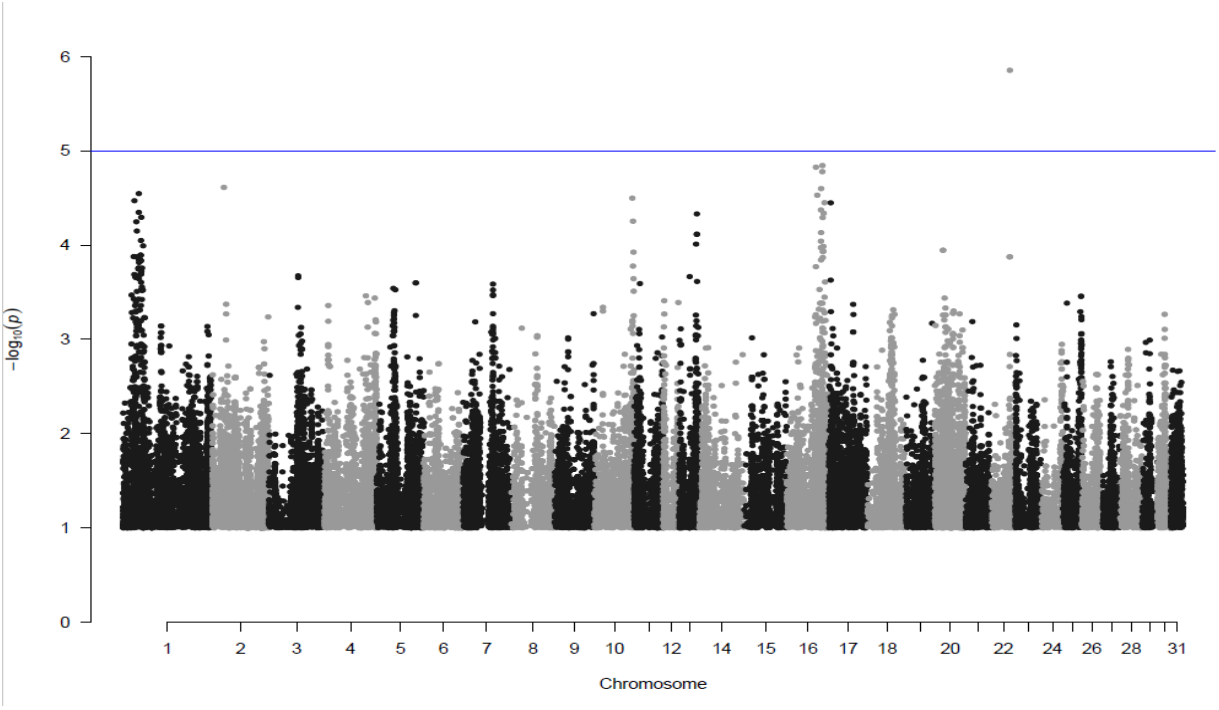

Figure 3: Manhattan plot for POF in the fetlock joint on the hind legs, lateral, with indicated Bonferoni significance level.

Paper 2

Introduction: The aim of the study was to examine whether there was an effect of inbreeding on racing performance of Standardbred trotters in Norway.

Material and methods: The study included a random sample of 1217 Norwegian Standardbred trotters born in 1988 (n=753), 2006 (n=363) and 2007 (n=101), previously described by Lykkjen et al (2014). Ancestors of the 1217 study horses were traced back to the founders to generate a pedigree file containing a total of 8483 horses. Approximately 80% of the horses born in 1988 were pure American Standardbred trotters compared with 45% of the horses born in 2006 and 2007 (Lykkjen et al, 2014), showing an increased French contribution over time.

For the 1217 study horses, the annual number of starts, earnings and number of disqualifications within Norway were made available. Data from test races were not considered. Records between three to five years of age were cumulated on an individual basis. For horses born in 1988, relevant records were from racing seasons 1991-1993, while horses born in 2006 and 2007 had their data from the seasons 2009-2011 and 2010-2012, respectively. A total of 807 horses had raced (66.3 %), and for these performance data were analyzed.

To normalize the data, starts was power transformed with the square root (t-starts). Earnings per start was derived and exposed to a fourth root transformation, to normalize the data. Finally inflation was accounted for by standardizing the variable within the two groups of horses, those born in 1988 and those born in 2006/2007, respectively; the final variable being; cumulated, transformed and standardized earnings per start (t-earnings). Cumulated disqualifications per start (CDS) is a proportion that had extreme values; 363 horses had never been disqualified and 9 horses were disqualified in all races. Therefore the CDS-data was empirically logit-transformed (t-disq) prior to analysis:

t-disq $= ln(\frac{CDS+0.5}{1-CDS+0.5})$

Based on the renumbered pedigree file, inbreeding coefficients were estimated for 8483 horses using PEDIG (Boichard 2002).

The three variables of performance; t-starts, t-earnings and t-disq were analysed separately with the following two linear models:

$$y_{ijk} = \mu + s_j + by_k + bFped_i + a_i + e_i \quad (1)$$

$$y_{ijk} = \mu + s_j + by_k + \mathrm{b}Fped_i + e_i \quad (2)$$

where y_i is one of the three performance variable for the i-th animal, μ is the mean, s_j is the fixed effect of the j-th sex (stallion (n=193), mare (394), gelding (220)), by_k is the fixed effect of the k-th birth year group (1988 or 2006/2007), F_{pedi} is the inbreeding coefficient of animal with associated regression coefficient b, a_i is the additive genetic effect ~ N(0, $\mathbf{A}\sigma^2_a$), with **A** denoting the relationship matrix as generated from the pedigree file and σ^2_a being the additive genetic variance, while e is the error term ~ N(0, $\mathbf{I}\sigma^2_e$), with **I** an identity matrix and σ^2_e the error variance. Calculations were carried out with ASReml-W 3.0.4, and the significance of the fixed effects was tested using incremental Wald *F*-statistics (Gilmour et al., 2009).

Results: The result from the analysis of variance for Models 1 and 2 are given in Table 1. For Model 1, F_{ped} had a significant effect on t-earnings with a p-value < 0.05. For Model 2 there was a significant effect of F_{ped} on t-disk (p-value < 0.05), and the effect of F_{ped} on t-earnings had a p-value = 0.051. All the performance traits were significantly influenced by sex for both models. There were no significant difference between birth periods for any of the performance traits. Thus, we have found that an increased inbreeding coefficient influenced the performance trait t-earnings negatively with both models. For both models, geldings were started more frequent, and mares the least. Geldings and stallions earned more money and were less frequently disqualified than mares (Table 2).

Table 1. Model 1 and Model 2 Wald F-statistics for 807 horses with recordings of performance traits born 1988 and 2006/2007 (birth year groups), respectively.

	Model 1			Model 2		
	t-starts	t-earnings	t-disq	t-start	t-earnings	t-disq
Fped	0.20	4.48*	1.81	0.55	3.89x	4.29*
Sex	3.45*	14.94***	8.50***	3.47*	14.40***	8.53***
Group	1.32	1.35	2.46	1.17	0.12	3.17

x p-value =0.051 *p-value<0.05 **p-value<0.025 ***p-value<0.001.

Table 2. : Model 1 and Model 2 estimates of fixed effects with their standard errors The regression coefficients estimate is given for 100% inbreeding.

	Model 1			Model 2		
	t-start	t-earnings	t-disq	t-start	t-earnings	t-disq
Average	4.58±0.21	0.20±0.16	-0.86±0.04	4.62±0.18	0.30±0.10	-0.86±0.30
Fped	1.18±2.62	-3.66±1.73	-063±047	1.80±2.44	-2.69±1.37	-0.85±0.41
Stallion	-0.17±0.18	0.07±0.10	0.07±0.03	-0.16±0.17	0.07±0.10	0.07±0.03
Gelding	0.00	0.00	0.00	0.00	0.00	0.00
Mare	-0.37±0.15	-0.35±0.09	-0.03±0.02	-0.37±0.15	-0.33±0.83	-0.03±0.02
1988	0.00	0.00	0.00	0.00	0.00	0.00
2006/2007	-0.18±0.15	-0.07±0.11	0.05±0.03	-0.17±0.14	0.03±0.08	0.06±0.02

Heritability of traits ranged from 0.05-0.24. The highest estimate (0.24±0.11) was obtained for t-earnings.

Discussion: The inbreeding coefficient affected the trait t-earnings negatively (Model 1; $P < 0.05$). Contrary to Model 1, Model 2 did not contain the additive genetic effect, and so when even not allowing for a genetic trend, as with Model 1, a negative effect of F_{ped} was obtained for t-earnings and also t-disk. The latter model is conservative, i.e. that a genetic trend for performance should be anticipated. Thus, our results points towards inbreeding depression for racing performance in Standardbred trotters. Models 1 and Model 2 gave, respectively, 0.22 and 0.16 standard deviations poorer performance for a horse that are 6.1 % inbreed (average in data), compared with a horse with an inbreeding coefficient equal to 0. This is a handicap on the race track, and should encourage breeders to choose stallions that sire offspring with a low inbreeding coefficient, i.e. to carry out outbreeding or crossbreeding. With crossbreeding, heterosis is expected (Falconer & Mackay 1996), meaning that crossbred animals will perform better than purebreds. This is consistent with Richard (2005) who found an effect of heterosis on racing performance in French data, where horses with 50% American- and 50% French lineages performed 0.38 standard deviations better than pure Americans. Otherwise, the inbreeding results reported here is consistent with the reported significant inbreeding depression for earnings obtained in Norwegian cold-blooded trotters (Klemetsdal 1998).

Conclusion: The Standardbred trotter in Norway has pedigree data of a depth that allows to estimate the effect of inbreeding on performance traits in trotters. The effect was negative, i.e.

that racing performance in Standardbred trotters is concluded depressed by inbreeding. In corollary, outbreeding or crossbreeding is encouraged, and contributes to the increasing contribution of French trotter in Norwegian Standardbred over time.

Description of the most important RnD challenges, and what environments that have carried out the activities

Paper 1: Blood sampling and radiography was done by Nils Ivar Dolvik and Sigrid Lykkjen. Preparation of DNA-samples for SNP analysis was done at BioBank AS, while SNP-genotyping was done at Cigene. Responsible for SNP typing was Dag Inge Våge. Imputation of data to high density was done jointly by Dag Inge Våge and Sigrid Lykkjen, as well as the statistical analysis with GEMMA.

Paper 2: Data was extracted by the Norwegian Trotting Association (DNT) and quality control was done by Nils Ivar Dolvik and Gunnar Klemetsdal. Calculation of individual inbreeding coefficients was done by Stine Samsonstuen and Gunnar Klemetsdal as was the statistical analysis of the racing performance data.

Short evaluation of the implementation of the project and use of resources

Resources have in main been used for phenotyping as well as genotyping. Number of phenotyped and genotyped animals are according to the number aimed at, described in the project application. Consequently the fulfillment of the goal in this regard is evaluated as reached.

The group decided to apply for a PhD student to strengthen the project, and the funding was seen as an assets for the PhD student. When the PhD student then quitted in May 2017, the project was back where it all started, meaning that the permanent staff had to carry out the analyses. Since, the results did not show a QTL for POF, we decided to merge the two promised publications, the one on OCD and the other on POF, into one. To fulfil the promise of two publications, Nils Ivar Dolvik and Gunnar Klemetsdal did Paper 2. By this, we are able to deliver two international peer-review paper from the project, and by that we consider to have reached the goal with respect to number of international scientific publication from the project.

Description of what importance/value the results are expected to have (e.g. for the research area, enhancement of competence, the industry and society)

OCD and POF increase the costs in the horse industry and affect animal welfare negatively, since many horses have to be operated. The diseases contribute to increased costs for treatment and restitution, reduced performance and income as well as reduced value of the animal. This as well as high frequencies of the diseases illustrate the importance of revealing the causative nature of both OCD and POF.

Selection can be used as a preventive means towards such diseases, either by selecting against the QTL, or by summing the effect of all alleles and utilize the resulting genomic breeding value. With the finding of only one QTL as herein, one would be interested in its effect and possibly to select against it, e.g. by denying an individual with the gene to be accepted to the stud book, or to deny it if its sire carries the QTL. An alternative would be genomic selection. However, with only a small number of animals genotyped, the accuracy of the breeding values will be limited, and this calls for international collaboration. Such an initiative through across-population genomic selection will make selection against diseases in horses possible, a species for which extensive disease recording seems far from realistic.
Our vision is that prolonged research on OCD and across-population evaluation is a natural next step, since continued knowledge building around OCD can be transferred as a model to other diseases, and even to other species, among these other companion animals.

Description of the plans for dissemination and use of results

The dissemination will be through scientific publications. We will also inform the responsible breeding organization, DNT, of our findings, when appropriate, as well as inform through Trav og Galopp-Nytt.

Description of what results is expected to be finished after the project has been reported

The two papers, and possibly several student theses and papers; the most relevant being related to imputation, quality control studies, population genetic studies as well as comparison of methods for QTL detection.

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