

Genetic analysis of conformation traits in Icelandic horses with focus on head morphology and body length

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Genetic analysis of conformation traits in Icelandic horses with focus on head morphology and body length

Genetiska analyser av exteriöra egenskaper hos islandshästar med fokus på huvud och kroppslängd

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Credits: 30 ECTS

Course title: Degree project in Animal Science

Course code: EX0558

Programme: Agricultural Science programme - Animal Science

Level: Advanced, A2E

Place of publication: Uppsala

Year of publication: 2016

Cover picture: Wikipedia Commons

Title of series: Examensarbete / Swedish University of Agricultural Sciences,
Department of Animal Breeding and Genetics, 509

Online publication: <http://stud.epsilon.slu.se>

Keywords: Icelandic horses, conformation, SNPs, association analysis

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Abstract

In the breeding evaluation of Icelandic horses there are eight breeding goals that refer to conformation, for example head carriage. Genetic evaluation of the horse is an important part for succeeding with breeding goals and this information is taken from assessments from breeding field tests, including conformation of the horses. These assessments can then be used for retrieving an estimated breeding value (EBV) through Best Linear Unbiased Prediction method (BLUP) with a multivariate animal model.

With this in mind a Genome-Wide Association Study (GWAS) was done, primarily focus set on the follow-up of results from previous studies (Shrestha et al. unpublished; Wellbring 2011) and the same material was used as the previous study used, horse owner information and genotypes as well as head morphology and body length as phenotypes. However, one distinct difference was made for this study, this time direct measurements were used instead of EBV's when using the traits head morphology and body length. Direct measurements give more accuracy than EBV's as traits within association studies. Direct phenotypes were collected with a questionnaire which was sent out to the 178 Icelandic horse owners. To further follow up the results from the previous study, a secondary study was done through t-tests. For the GWAS analysis, chosen phenotypes from the questionnaire were picked out and the results gave significant associations to the following phenotypes: appearance of jaws, muzzle circumference, jawbone circumference and head length) and one borderline significant association (height at withers). From the secondary study one significant p-value was obtained when comparing means for measurements on genotype GG/AA in the t-test for group 1 (body length: Chromosome 1). The significant p-value was 0.007 and where the mean also was 143.213.

Sammanfattning

I avelsvärdering av islandshästar finns åtta avelsmål som avser exteriören, till exempel huvudhållning. Avelsvärdering av hästen är en viktig del för att lyckas med exteriören av hästarna. Dessa bedömningar kan sedan användas för att hämta ett uppskattat avelsvärde (EBV) genom metoden vid namn, Best Linear Unbiased Prediction (BLUP) med en multivariant djurmodell.

Med detta i åtanke utfördes en Genome-wide association study (GWAS), i första hand lades fokus på uppföljning av resultat från tidigare studier (Shrestha et al., opublicerat; Wellbring 2011) och samma material användes som den tidigare studien använde, hästägarinformation och genotyper samt huvudform och kroppslängd som fenotyper. Emellertid skapades en distinkt skillnad speciellt för denna studie, den här gången användes direkta mätningar istället för EBV's vid användning egenskaperna huvudform och kroppslängd. Direkta mätningar ger mer precision än EBV's som fenotyper inom associationsstudier. Direkta fenotyper samlades in med en enkät som skickades ut till 178 isländska hästägare. För att ytterligare följa upp resultaten från tidigare studier, gjordes en ytterligare studie genom t-test.

För GWAS analysen plockades fenotyper ut från enkäten och resultaten gav signifikanta samband till följande fenotyper: utseende av käkar, mulens omkrets, käkbenets omkrets och huvudets längd. En borderline signifikant association hittades vid fenotypen: mankhöjd. Från den sekundära, ytterligare studien med t-test erhöles ett

signifikant p-värde vid jämförelse av medel för mätningar på genotypen GG/AA i t-test för grupp 1 (kroppslängd: Kromosom 1). Det signifikanta p-värdet var 0,007 och medelvärdet var 143,213.

Introduction

Many horse breeds of different sizes and shapes have been developed through decades since domestication, by selection for different use of the horses. For example the robust North Swedish horse compared to the American miniature horse that has a height at withers of on average one meter (Brooks et al., 2010). Today's usage of the horse is mainly through sports and for recreational purposes, but the historical use of the horse has been for assistance of the human in their labour, such as in agricultural purposes (Lawrence, 2014). With regard to the wellbeing of the horse in general, conformation, including body shape and size are important traits to take into consideration when breeding horses (Lawrence, 2014; Brooks et al., 2002). Body shape and size has been perceived to be important traits and believed to have relation to a numerous of performance abilities such as the horse's conformation and racing ability (Koenen et al., 1995). Longevity is always a key to a functional, improved generation when breeding in general, the right conformation of a horse will give a correctness in gaits as well as a good racing horse (Saastamoinen et al., 2000; Koenen et al., 1995).

The Icelandic horse is able to perform additional gaits to walk, trot and canter. If the horse can perform tölt it is said to be four-gaited, while if it has the ability to also perform pace, it is called five-gaited (Liedberg, 2013). It has been discovered that whether an Icelandic horse can perform all gaits or not depends on their genetic make-up (Andersson et al., 2012). If an Icelandic horse is heterozygous (CA) for the gene *DMRT3*, it will be four-gaited and if the horse is a homozygote with the genotype AA it also has the genetic abilities to perform pace and thus be five-gaited. This is based on the finding of a premature stop codon in the *DMRT3* gene that was shown to effect the pattern of locomotion in horses (Andersson et al., 2012).

The mutation in the *DMRT3* gene is important for the gaits but the conformation of the horse also matter (Andersson et al., 2012). According to Andersson et al. (2012) it is a disadvantage that the scientific knowledge of the locomotion performance and conformation is limited. The authors then discuss the importance of quantitative estimations could be a contributing key to further this kind of research.

Literature review

Breeding and importance of conformation

Approximately five thousand years ago domestication of the horse started in small scale (Dunlop and Williams 1996). Breeding in a selective, planned way is presumed to have started shortly thereafter. The locomotion of the horse was from the beginning and still remains a popular usage of the domestic horse in terms of for example transportation for the human, racing; the first scientific report on gaits in the horse was done by the French, they were the pioneers in this area (Back & Clayton, 2012). Later on, after World War II, interest was rising in some countries for using horses for other purposes than for transportation or warfare. This time for sports, in racing, the

rest of the world followed shortly after. The so-called electronic revolution was bursting at this time and so the equestrian locomotion research could therefore evolve much easier with new electronic help such as studies that are conducted through computer programs as of today (Back & Clayton 2012).

The performance ability is a complex trait that is influenced by the horse's conformation and consequently differs in different horse breeds as well as between individuals, but a common ground is that the conformation will matter in breeding a horse for a certain purpose. Franke (1935) see Weeren & Crevier-denoix (2006), was the first to mention influence of conformation on stride length, which is strongly connected to performance in the horse. Franke (1935) was later on to be contradicted, but could give an incentive to research that conformation indeed has played a main role in the horses' performance. A previous study found a positive correlation between different conformation parameters and kilometre time in Standardbreds (von Lengerken & Werner, 1969; see Weeren & Crevier-denoix, 2006).

Kristjansson et al. (2013) mention that it has been seen that Icelandic horses have evolved into a taller, square-shaped horse in recent years compared to the more rectangular horse historically. This could be a contributing reason to why the Icelandic horse is such a popular riding horse with its special gaits, such as tölt (Kristjansson et al., 2013). The Icelandic horses are popular not only in its indigenous surroundings but in over 35 countries across the world and their popularity is just growing due to as mentioned earlier, being a good riding horse, but also for its pleasant temperament (Kristjansson et al., 2013). This includes a specific and well thought over breeding goal for Icelandic horses with much regard to conformation, more specifically eight conformation traits. This is an aid in breeding for a successful riding horse and also to help further successful breeding for the numerous of gaits that the Icelandic horses usually possess. The breeding goals for Icelandic horses are nationally accepted, which means that Icelandic horses should be bred according to these goals, which also include seven traits for riding abilities. With that in mind, breeding a sound and well-functioning horse is being thought of here (Kristjansson et al., 2013).

Heritability of head and body length

Conformation is an important trait of the International Icelandic horse breeding goal (Albertsdottir et al., 2008). To genetically evaluate the traits for breeding goals, breeding field tests are assessed and assembled; a multivariate animal model is then used to estimate breeding values (EBVs) (Mrode, 1996).

The two traits, head morphology and body length seem to connect a moderate to high heritability: in a study on Pantaneiro horses (Miserani et al., 2002). In this study the heritability for body length was high, 0.72 (Miserani et al., 2002) and heritability on head morphology for Icelandic horses has been shown to be 0.33 (Albertsdottir et al., 2008). Also Albertsdottir et al., (2008) discusses the heritability for conformation traits for the Icelandic horse as a whole to have a range from 0.22 – 0.46. There is obviously a fairly wide range of this heritability. Therefore, accordingly to Shrestha et al. (unpublished) determination of a significant association on head morphology is important. The trait, body length with its high heritability (0.72) was also mentioned in a study by Wellbring (2011) and that it gives an incentive to pursue further investigation on possible meaning. The study by Wellbring (2011) found a borderline

significant association on body length and it was suggested that this measurement of the complex phenotype of body size is an important trait due to its connectivity to other trait developments, such as gaits.

Head, body size & genetics

Breeders select for complex traits like body size and skeletal conformation to improve marketability, function, soundness and performance in the show ring. The horse, like other domesticated species, has been moulded through selection within breeds into diverse skeletal morphologic types, often to fit specific functions (Brooks et al., 2010). Conformation seems to have an important role in horses and their performance, thus breeding for the right looks would then be imperative. According to numerous studies where body measurements have been investigated genetically, two genes are often mentioned, *LCORL* and *HMGA2*. These genes are believed to affect conformation traits, not only in horses but also in humans and dogs. These two genes along with three other genes, *NCAPG*, *ZFAT* and *LASPI*, have been found to not only affect the conformation of the horse but more specifically a significant effect on the horses body size (Makvandi-Nejad et al. 2012; Signer-Hasler et al. 2012; Petersen et al. 2013; Tetens et al. 2013).

The GWAS of conformation traits in horses by Signer-Hasler et al. (2012) found some regions with significant associations with conformation traits, where regions on chromosomes ECA3 and ECA9 associates with for example expression of the head as in head length and length of the back in horses. They also detected other interesting findings on chromosome 6 which were correctness of gaits which, is found to be biomechanically related to body size. By biomechanically Signer-Hasler et al. (2012) mean simply that a horse that is too tall could easier loose balance than a smaller horse and this is to take into account in horse breeding. These findings on ECA 3, 9 and 6 will matter and possibly affect the conformation traits mentioned above along with correctness of gaits (Signer-Hasler et al., 2012).

Association between phenotype and genotype

Association is explained within genetics as finding a connection between a genetic marker and a certain phenotypic trait. Therefore, it is important to identify the genotype of the location (in the genome), where the genetic factors underlying the trait analysed are located. The genotype data can be retrieved through genotyping using Single Nucleotide Polymorphisms chip (SNP chip) (Long et al., 2007).

Using SNPs for Genome-Wide association studies

SNPs are variations in a DNA sequence, where the position of a single nucleotide is known (Bush et al., 2012). The density of SNPs in the horse genome is 1/300-400 bases, they are suitable to use for associations studies because they are informative, easy to genotype and are spread all over the genome (G. Andersson, personal communication, may 21, 2016) SNPs are considered an especially common genetic variation as they are found in a large proportion in for example the human populations (Altshuler et al., 2010). SNPs usually contain two alleles, thus within a population there will be two possibilities of a base-pair occurring per one SNP-location (Bush et al., 2012). The rate of how often a SNP will occur is referred to as: minor allele frequency, which is the frequency of the less commonly occurring allele (Bush et al., 2012). For example a SNP with a minor allele (C) frequency of 30% indicates that

30% of a population has the C allele in contrary to the more common allele (the major allele), which in hand can be found in 70% of the population (Bush et al., 2012).

Genome-Wide association studies

A certain way of finding a possible variation in allele frequency between horses in the selected SNPs on a chip is called GWA's. This is used to test if any statistical associations can be found between a genetic marker and the certain phenotype (Pei et al., 2010). Association analysis can be exerted through a number of different computer programs (Pei et. al., 2010), one example is PLINK, which is used for present study (Purcell et al. 2007). Significant statistical associations can be tested for through multiple-tests, how many tests to do is usually decided through the amount of SNPs being used for the project at hand (Purcell et al., 2007). A level of significance, also called " α " will be set in beforehand, for example when choosing 0, 05 the number of SNPs that can be expected to end up being significantly associated is 5 out of a 100. When exerting multiple tests, for example 50 000 tests, correction for so called "false/positive" probability is required. This due to that the chance is high that randomly generated significant associations could occur. This could affect the end result, which with no correction for "false/positive" would then be invalid and untrue. This is done easily through Bonferroni correction, where the level of significance is pushed down through using the formula: $\alpha=0,05/k$. Where k stands for number is the number of statistical tests conducted. An alternative method to adjusting the false/positive significance level (α) is through Permutations. Here random reassigning of the phenotypes of each individual to another individual is done in the dataset. The original dataset of genotype-phenotype relationship is broken up and a random, new dataset is constructed with randomly placed phenotype. Each random reassignment of the data signifies one possible sample of individuals with similar results. This random, new dataset is tested for the number acquired that is equal or even superior results, compared to the first outcome with original complete relationships.

This is then repeated, for example 1000/phenotype and will generate a distribution (empirical). Thus a permutation process with 1000 repetitions will be divided with number of superior results and give a new probability value (p-value), for example: 0, 0001. This is the new, corrected level of significance ($\alpha=0,0001$). (Bush et al., 2012).

Aim of study

The aim of this study was to follow-up previously found GWAS results between head morphology and body length in Icelandic horses (Shrestha et al. unpublished), through additional genotyping and collection of more phenotypic data, including direct measurements of the head and body of the horses. The previous study by Shrestha et al. (unpublished data) aimed for identification of the chromosomal regions of which are associated with head morphology (chromosomes 4 & 20) and body length (chromosome 1) in Icelandic horses. Shrestha et al. (unpublished) used estimated breeding values (EBVs) as phenotypes for head morphology (349 Icelandic horses) and individual measurements (direct values) for body length (64 Icelandic horses), in total 413 Icelandic horses. This previous study used genotype data from SNP-chip Illumina SNP50 and SNP70. The idea of the current study was to instead of using breeding values for head morphology as phenotypes, use individual measurements (direct values) for both head morphology and body length in a Genome-Wide Association Study (GWAS). This was done through sending out a

questionnaire containing questions mainly about head morphology and body length to 178 of the Icelandic horse owners that participated in the previous study (Shrestha et al., unpublished). Since genotypic data already existed (SNP-chip Illumina SNP50 and SNP70) from this previous study by Shrestha et al. (unpublished) no genotyping for this part of current study was done, only new phenotypes as above explained was retrieved.

Another aim was to genotype Icelandic horses, never used in the previous study by Shrestha et al. (unpublished), using the SNP-chip, Illumina SNP50. Genotyping was done for the significant markers identified in the GWAS performed by Shrestha et al. (unpublished). Updated breeding values for head and direct measurements for body length (World Fengur, Icelandic horse studbook, 2015) was compared using the Student's t-test.

Material and methods

The material used for this study originated from two previous studies done on Icelandic horses (Shrestha et al., unpublished; Wellbring 2011).

Two sub studies were made for present study with two different large groups of horse material (including DNA and horse owner information). The two sub studies were referred to as sub study 1 and sub study 2 within present study.

Sub study 1 consisted of a GWAS and was performed through using a different set of phenotypes than in the previous study by Shrestha et al. (unpublished), this time with direct measurements instead of EBV's. The direct measurements of head morphology and body length (178 Icelandic horses), retrieved from a sent out questionnaire was included in an association study with the same Illumina 50K SNP and 70K SNP chip for chromosome 4 and 20 (head) as well as chromosome 1 (body length) as in the study by Shrestha et al. (unpublished).

The 70K SNP chip was excluded due to failure of the TaqMan-assay. Thus with only 50K SNP chip for chromosome 4 (head) and chromosome 1, the GWAS was performed through using the Whole genome association analysis toolset in PLINK. The questionnaire that was sent out to 178 Icelandic horse owners were the same horse owners that was included in the previous study by Shrestha et al. (unpublished) and Wellbring (2011). The same genetic data (from the pre-existing 178 horses used for questionnaire) that was used by the previous study (Shrestha et al., unpublished) was used when utilizing PLINK.

Sub study 2 consisted of performing Student's t-test combining retrieved updated breeding values with genotypes of 256 Icelandic horses, both retrieved specially for current study. A combination t-test using breeding values and genotypes from the current study (referred to as group 1) combined with the breeding values and genotypes used in the previous study (group 2) (Shrestha et al., unpublished).

Although the same Illumina 50K SNP chip when genotyping was used from the same chromosomal regions of 4 (head) and 1 (body length) was used as through this whole study.

The new parts for this present study only includes new direct measurements/phenotypes (sub study 1) and new genotypes with concurring breeding values (sub study 2). The Illumina 50K SNP chip is consistently used through the whole study in both sub study 1 and 2. The 70K SNP chip for chromosome 20 was as

mentioned below excluded from the study due to TaqMan assay failure, thus genotypes from these genetic data could not be retrieved.

Analysis – DNA and Genotyping

Roots from mane or tail hair of Icelandic horses were used when retrieving DNA from the 256 Icelandic horses that was used in sub study 2. Standard procedure was used for hair preparation, where Chelex (100 µl), 100 Resin (BioRad Laboratories, Hercules, California, USA) and proteinase K (7 µl; 20mg/ml) (Merck KGaA, Darmstadt, Germany) was added to the sample of hair. Incubation for 1 hour of the sample mix at 56 °C and inactivation of the proteinase K for 10 minutes at 95 °C was done. Genotyping was done with these sample mixes, now containing extracted DNA, through using StepOnePlus Real-Time PCR System (Life Technologies) with custom made TaqMan SNP Genotyping Assay for two SNP markers. These two SNPs were: BIEC2-881469 on a region on chromosome 4 with position: 103,101,920bp (C/T) and BIEC2-90672 in a region on chromosome 1 with position: 184233074bp (G/A).

TaqMan analysis

When genotyping DNA from Icelandic horses, custom designed TaqMan SNP Genotyping Assays was used. When applying the procedure of TaqMan-assay analyses for this study three regions on different chromosomes were used, chromosome 1 (body length); (Wellbring, 2011), chromosome 4 (head morphology) and chromosome 20 (head morphology) (Shrestha et al., unpublished). Unfortunately, due to TaqMan assay failure for chromosome 20, genotypes could not be retrieved. In the previous study by Shrestha et al. (unpublished), a region on chromosome 4 was borderline associated with SNP: BIEC2-881469 on position: 103,101,920. A borderline significant association with SNP: BIEC2-90672 on position: 184233kb was obtained in a region on chromosome 1.

Sub study 1

GWAS

The first sub study was a GWAS. A questionnaire, (see appendix 1) was sent out to 178 Icelandic horse owners. All of the owners had already participated in one of the previous studies (Wellbring, 2011; Shrestha et al., unpublished). Genotype data from the 50K (chromosome 4 & 1) and the 70K SNP chip (chromosome 20) already existed from the previous study by Shrestha et al. (unpublished). Along with the genotype data these horses had breeding values for head morphology, but no direct measurements. Instead of breeding values for head, this present study asked for measurements of various parts of the head along with updated measurements of body length including height at withers in the questionnaire. With the answers from the questionnaire, an association study was conducted. The studbook database for Icelandic horses, WorldFengur (Lorange, 2011) was used to find updated owner information.

Questionnaire

The questionnaire consisted of a first part purely with information about the horse such as name, WorldFengur identification number and family history. The questionnaire consisted of a total of 32 questions (including subqueries) regarding mainly the horse's conformation (see appendix 1). Some (14) queries concerned the

conformation in parts of the horse such as head, for example whether the horse had a fleshy or bony appearance. Measurements of parts of the head as well as the body were pervading through the questionnaire. An association study was done within the parameters of receiving answers from the questionnaire. Answering frequency of the whole questionnaire (including all queries) and means from measurements of the 14 queries, chosen for the Association study was calculated with the help of Microsoft Excel.

Statistical Analysis

All answers from the questionnaire were entered in Microsoft Excel (see appendix 2). The specifically chosen 14 phenotype data was analyzed in the Whole genome association analysis toolset, PLINK 1.7 (Purcell et al. 2007). Missingness rate excluded SNPs that were missing more than 5 % of data. Population stratification test with Identity-By-State (IBS) clustering was included in Quality Control to perform complete linkage clustering of individuals on the basis of autosomal Genome-wide SNP data, see appendix 6 for Multidimensional scaling (MDS)-plot of population stratification test. Multidimensional scaling is done for the purpose of defining if there lies a stratification within the population that is used for the study at hand. Where a (MDS)-plot is used to visualize this the genetic distances within the population (Purcell et. al., 2007).

Bonferroni correction was done in PLINK after QC. This correction adjusts the probability values (p-value) due to the fact that when performing multiple statistical tests a possible risk lies that the type I error can occur (Armstrong et al., 2014). The type I error is when a p-value shows a significant difference when there in fact is no difference (Armstrong et al., 2014).

In all association tests through PLINK, permutations was used to correct for multiple testing with reference to the raw p-value and the amount of permutations, used per trait was 1000. The selected traits with measurements are listed below.

(The abbreviations in the list can be understood further in appendix 1 in the questionnaire. The numbers and letters (with colours) belonging to these abbreviations are coherent to the questions in the questionnaire):

- 5a) head shape
- 5b) Appearance of jaws
- M) Ear length
- N) Lower eyes
- O) Distance nostrils
- P) Ears distance
- Q) Left eye to middle
- R) Right eye to middle
- S) Length nostril
- A) Muzzle circumference, just above nostrils
- B) Jawbone circumference, just above eyes upper circumference
- C) Head length
- A) Height withers
- D) Body length

Sub study 2

Additional genotyping of two SNPs

To follow up the significant results from the GWAS done by Shrestha et al. (unpublished), 203 Icelandic horses were genotyped for a SNP on chromosome 4 (CHR 4) and 64 Icelandic horses were genotyped for a SNP on chromosome 1 (CHR 1). Breeding values for head and direct values for body length were retrieved from WorldFengur (Lorange, 2011). The averages were compared with current study using Student's t-test, which was done in an online statistical tool, Vassar Stats: Statistical Computation Web Site (Lowry, 1998). A confirmation of these t-test's p-values was done with a second round of performing the same tests in Microsoft Excel.

The choice of using Student's t-test for this follow-up of the significant results was based on the information in the Handbook of Biological Statistics by McDonald (2014). Where the statement that calculating the relative efficiency of other biological statistical tests, such as used in this study, Student's t-test is the most reliable and sensitive for this purpose. McDonald (2014) also mentions in the handbook; when the study contains one group of nominal variables (such as genotypes) and one group of measurement variables (such as estimated breeding values, EBV's), this test is the one to use.

Two groups of horses were used for this analysis. The first group (group 1) included 163 Icelandic horses genotyped for the SNP on chromosome 4 (head morphology) and 93 Icelandic horses genotyped for the SNP on chromosome 1 (body length). The second group (group 2) contains data from the previous studies where 203 (CHR 4) and 64 (CHR 1) Icelandic horses were genotyped (Shrestha et al. unpublished; Wellbring 2011). In total sub study 2 included 366 (163 + 203) horses with breeding values for head and 157 (93 + 64) horses with a direct value for body length.

T-test - analysis

Ten t-tests were performed. Breeding values for head morphology and direct values for body length were compared for the two different groups mentioned earlier (group 1 and 2). These independent 2-sample t-tests were performed through the online statistical tool, Vassar Stats: Statistical Computation Web Site (Lowry, 1998) as well as confirmed with a second performing of the same t-tests in Microsoft Excel.

The t-tests were performed using updated breeding values (head morphology, CHR: 4) and direct measurements (body length, CHR: 1).

Chromosome 4 was labelled with the subgroups: **A₁** and **A₂**.

A₁: t-test was done using only the breeding values and genotypes (group 1) retrieved from the current study (n=163).

A₂: t-test using breeding values and genotypes from the current study (group 1) combined with the breeding values and genotypes used in the previous study (group 2) (Shrestha et al., unpublished) (n=163+203=366).

Chromosome 1 was labelled with the subgroups: **B₁** and **B₂**.

B₁: t-test was done based on the direct measurements for body length and the genotypes (group 1) retrieved from the current study (n=93).

B2: t-test using the body length measurements and genotypes (group 1) from the current study together with genotypes and measurements from previous studies (group 2) (Shrestha et al., unpublished; Wellbring, 2011) (n=93+64=157).

RESULTS

Sub study 1

Answering frequency and means for the questionnaire

The answering frequency for the questionnaire was 30.9% (46/149). Out of originally 178 sent out questionnaires to horse owners with updated addresses, one of the horses died during the time that the questionnaire had been sent out, which then leaves 177 horse owners in total that was mailed questionnaires. 28 mails came back because the owners of these addresses had not updated that they had moved, which leaves 149 in total (177-28=149). Out of those 46 answers were received back either by mail or e-mail. Means (measure of spread in statistics), modes (the statistical mode: the most frequently occurring value of a list of provided numbers) and standard deviations (STDEVP= standard deviation for an entire population) of all existing data posts for the measurements of the 14 phenotypes, chosen for the Association study was calculated. See table 1.

Table 1. Means of the measurements from the 14 phenotypes chosen for the Association study

Phenotype	Question #	means	mode	STDEVP
head shape	5a)	3,693	4	0,943
Appearance of jaws	5b)	1,905	1	0,84
Ear length	M)	15,855	17	2,314
Lower eyes	N)	17,527	18	2,382
Distance nostrils	O)	9,598	10	1,307
Ears distance	P)	17	15	3,155
Left eye to middle	Q)	8,849	9	0,661
Right eye to middle	R)	8,85	9	0,725
Length nostril	S)	6,561	7	0,825
Muzzle circumference	A)	46,264	46	5,317
Jawbone circumference	B)	90,1705	93	6,62
Head length	C)	47,06	44	5,691
Height withers	A)	138,337	138	6,835
Body length	D)	148,415	150	9,308

All means, modes and standard deviations were calculated in Microsoft Excel

Presenting standard deviation for all horses with data posts (STDEVP) within this table

For phenotype "Appearance of jaws": written answer was converted into a scale from 1-3 (1: bony, 2: normal, 3: fleshy)

Style of headlines for phenotypes are consistent with the lists of these phenotypes within current study

GWAS

Head morphology

All 14 phenotypes that were used in the association test and the p-values from the association tests are presented in Table 2. The genotype data contained 54 602 SNP's, after quality control (QC) in PLINK, all tests only contained SNPs with a minor allele frequency greater than the minimum 5 % (MAF = 0.05), leaving 38 106 SNP's with supplementary check and correction for population stratification see Appendix 2). Bonferroni correction gave a significance level of $1.31E-06$ after calculation with 38 106 SNP's. Quality parameters were with a missingness rate of 0.05. The p-values retrieved from the association test in PLINK has been permuted. In Table 3, the top significant p-values are presented (see appendix 5 for each phenotype with top 10 p-values before permutation).

Table 2. P-values for all tested phenotypes

Phenotype	Question #	P-value	EMP2	Chr	BP
head shape	5a)	2,05E-06	0.3926	4	17882739
Appearance of jaws	5b)	4,58E-07	0.01698	X	20743698
Ear length	M)	1.748E-05	0.3237	X	74815307
Lower eyes	N)	1,27E-06	0.7722	X	74815307
Distance nostrils	O)	1.501E-05	0.2627	13	31034068
Ears distance	P)	1.766E-05	0.8541	13	31034068
Left eye to middle	Q)	1.149E-07	0.4316	4	63246545
Right eye to middle	R)	4.884E-08	0.5265	X	74815307
Length nostril	S)	3,81E-09	0.1159	1	121557963
Muzzle circumference, just above the nostrils	A)	1,15E-13	0.03896	30	26321284
Jawbone circumference, just above eyes, upper circumference	B)	5,77E-13	0.03497	8	47054647
Head length	C)	1,74E-10	0.03596	8	47054647
Height withers	A)	5,42E-13	0.05794*	8	47054647
Body length	D)	4,61E-09	0.08492	8	47054647

P-values for all phenotypes that were tested in PLINK for association before permutation. # = Question number from questionnaire (see appendix 1). Top 1 p-value on chromosome = top 1 chromosome for each phenotype SNP = SNP location on chromosome P-value = top 1 p-value for that top1 chromosome, for each phenotype.

Table 3. Phenotypes that generated significant associations

Phenotype	Question#	P-value	EMP2	CHR	BP
Appearance of jaws	5b)	0,000000458	0,01698	X	20743698
Muzzle circumference	A)	1,15E-13	0,03896	30	26321284

Jawbone circumference	B)	5,771E-13	0,03497	8	47054647
Head length	C)	1,744E-10	0,03596	8	47054647
Height at withers*	A)	5,42E-13	0.05794*	8	47054647

#= question number in the questionnaire (see appendix 1) *= borderline significant
 permuted P-value EMP2= Significant p-values with permuted p-values P-value= P-value
 before permutation SNP= SNP location on chromosome BP= base pair location.

Phenotypes with significant association

Appearance of jaws

The horse owner's objective view of the horse's appearance of jaws was asked for. Multiple choices were presented as if the jaws looked fleshy, thin or bony. One significantly associated SNP was identified, (Figure 3). The top raw p-values are presented in Table 3 where the chromosome that had most significance on "jaw appearance" (EMP= 0. 0169) is the X chromosome. The marker is located 0. 7 Mb from the gene *MAGEB10* that encodes a melanoma-associated antigen protein family. It is expressed in testis and tumour cells (Melin et al., 2016). As this is a sex chromosome, further validation of the obtained significant association is required.

Muzzle circumference

Data needed for this phenotype was the lower circumference of the horse's frontal head/face (in cm) measured just above the nostrils. The significant marker is located on chromosome 30 (EMP₁= 0. 03896) (Table 3). The marker is located nearby the *PTPRC* gene, which encodes for a protein that is a member of the tyrosine phosphatase (PTP) family, commonly known to be signaling molecules that regulate a variety of cellular processes such as regulation of T cell receptor signaling pathway (Kaplan et al., 1990).

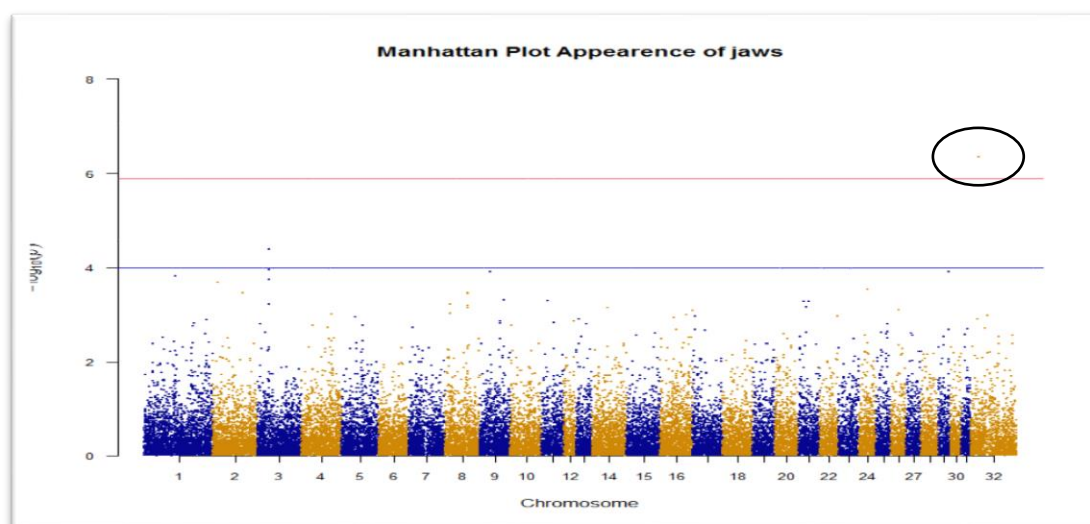


Figure 3. Manhattan plot for appearance of jaws: CHR X. The red line indicates the Genome-Wide significance line with Bonferroni correction ($-\log_{10}(1.32e-6)$; $p < 1.3110^{-6}$). Blue line= suggestive line= ($-\log_{10}(1e-4)$ for reference. Circled dot indicates data-point/SNP above red line of Genome-Wide significance.

Jawbone circumference, Head length and Height at withers*

Circumference measurements, just above the eyes of the horse, was used as data for this phenotype. The significant marker (EMP= 0.03497) is located on chromosome 8 0.6 Mb from the *ZNF521* gene (Table 3). This gene encodes a stem cell-associated transcription co-factor (Spina et al., 2013).

The length of the head was significantly associated (EMP= 0.03596), also with a marker on chromosome 8, (Table 3). The marker was found on the same location and distance from the *ZNF521* gene as explained above.

Height at withers showed a borderline significant Genome-wide association* (EMP= 0.05794*) and the marker is located on chromosome 8 (Table 2 & 3). The marker is located close to the same *ZNF521* gene, as mentioned above). Another gene close to the marker is the *SS18* gene. This gene encodes a transcriptional co-activator that has been suggested to be involved in the development of a type of cancer called “synovial sarcoma” (Storlazzi et al., 2003).

Sub study 2

T-test CHROMOSOME 4_{head} & CHROMOSOME 1_{body length}

All horses were genotyped, in total ten t-tests were done for group 1 and 2, with BVs retrieved for the trait head and direct values for the trait body length. Out of these ten, six t-tests were done for head morphology (Chromosome 4). For group 1 (only new horses) three t-tests were performed and three for group 1 and 2 combined.

The remaining four t-tests out of the total ten were done for body length (Chromosome 1) with direct values. Out of these four, three t-tests was for group 1 (only new) and only one t-test was for when combining group 1 and 2. Only the one t-test when combining group 1 and 2 for body length because of the complete lack of the genotype GG within group 2 (horses from previous study), therefore only a t-test between GA/AA could be done. These tests were divided by the chromosomes as above that were used for this sub study (2), Chromosome 4 (head) and 1 (body length).

CHROMOSOME 4_{head}

Group A₁

No significant differences within the pairwise comparison of BVs on genotypes CC, TT or CT were observed. The allele CC had an influence on bringing the means down slightly but when allele CT is present means are slightly elevated. CT has slight impact on elevating means, *see table A1*.

Table A1. Pairwise comparison; for Head Morphology on group 1						
Trait(group 2)	TT(n=5)	CT(n=46)	CC(n=112)	P(TT/CT)	P(TT/CC)	P(CT/CC)
Head morphology	^a 102(SE)	^a 103.787(SE)	^a 102.327(SE)	^b 0.685(103.615)	^b 0.940(102.313)	^b 0.240(102.764)
Average of BVs for the trait head morphology in Icelandic horses with genotype CC TT CT						
a mean per genotype						
b Pairwise comparison were made using Student's t-test (means between to genotypes within brackets).						
Group 1 new horses genotypes for present study						

Group A₂ combination of group 1 (new) and group 2 (previous)
 No significant differences within the pairwise comparison of BVs on genotypes CC, TT or CT were observed. Homozygous genotypes TT and CC have an influence on bringing the means down. The genotype CT has influence on elevating means slightly, *see table A2*.

Table A2. Pairwise comparison; for Head Morphology on group 1 + 2 combined test						
Trait(group 1 + 2)	TT(n=9)	CT(n=93)	CC(n=264)	P(TT/CT)	P(TT/CC)	P(CT/CC)
Head morphology	^a 99(SE)	^a 101.366(SE)	^a 101.083(SE)	^b 0.377(101,160)	^b 0.428(101,015)	^b 0.696(101,157)
Average of BVs for the trait head morphology in Icelandic horses with genotype CC TT CT						
a mean per genotype						
b Pairwise comparison were made using Student's t-test (means between to genotypes within brackets).						
Group 1 new horses genotypes for present study						
Group 2 horses retrieved from previous study by Shrestha et al., (unpublished)						

CHROMOSOME 1_{body length}

Group B₁

One significant difference within the pairwise comparison of BVs on genotypes GG, AA or GA was observed ($p=0.007$: mean=143.213; *Table B₁*). Once again homozygous genotype GG had an influence on bringing the mean down and the genotype GA had a slight influence here to on elevating the means where it is involved (*Table B₁*).

Table B ₁ . Pairwise comparison; for Body length on group 1						
Trait(group 2)	AA(n=60)	GA(n=12)	GG(n=20)	P(AA/GA)	P(AA/GG)	P(GA/GG)
Body Length	^a 142.017(SE)	^a 139.0(SE)	^a 143.80(SE)	^b 0.470(143.514)	^b 0.007(143.213)	^b 0.074(143.875)
Average of BVs for the trait body length in Icelandic horses with genotype GG AA GA						
a mean per genotype						
b Pairwise comparison were made using Student's t-test (means between to genotypes within brackets).						
Group 1 new horses genotypes for present study						

Group B₂

No significant differences here within pairwise comparison with BVs on genotypes GG, AA or GA were observed (*Table B₂*). The genotype GG did not exist within horses from group 1 (*Table B₂*), therefore no pairwise comparison was possible for this t-test (*Table B₂*).

Table B ₂ . Pairwise comparison; for Body length on group 1 + 2 combined test						
Trait(group 1 + 2)	AA(n=117)	GA(n=19)	GG(n=20+0)	P(AA/GA)	P(AA/GG)	P(GA/GG)
Body Length	^a 144.030(SE	^a 148.632(SE	x	^b 0.30(144.673)	x	x
Average of BVs for the trait body length in Icelandic horses with genotype GG AA GA						
a mean per genotype						
b Pairwise comparison were made using Student's t-test (means between to genotypes within brackets).						
Group 1 new horses genotypes for present study						
Group 2 horses retrieved from previous study by Shrestha et al., (unpublished)						
x No GG existed within group 1 (previous study by Shrestha et al., unpublished). Therefore no combined t-test could be done						

Discussion

The answering frequency for the questionnaire was 30.9 %, which is lower than expected and the explanation for such low interest in answering the questionnaire could be due to the fact that the questionnaire was pretty extensive. Another possibility could be that it was sent out at a cold and dark time of the year, where you would expect the horse owners to reject such tasks as measuring in a cold stable or outside. This reason is more unlikely though, a more likely reason is the fact that these horse owners had participated in a numerous of previous studies, therefore they possibly lost interest or didn't have time. As mentioned the horses in this study had been used for previous studies and genotypes already existed. These genotypes were used in a GWAS together with the direct values retrieved from the questionnaire made for this present study. Reminders were sent out twice to the horse owners to encourage more responses. Most reviews of the questionnaire as a whole has been positive and the questions were thought to be easy to understand and fill in. The list of horse owners available from previous studies and thus leaving existing genotypes were at first 178 but 29 horse owners moved and did not register a new forwarded address, leaving the old address as the presumed correct address. This lowered the expected answering frequency that was planned for in the beginning of this study.

The PLINK association test for this study, gave, after permutation, 5 significant p-values, (see appendix 3 for compilation of significant associations in Manhattan plots with concurring qq-plots). Possible candidate genes for the length of the horse has been found and identified on loci on chromosomes 3 and 9 (Signer-Hasler et al., 2012; Tetens et al., 2013). The study by Signer-Hasler et al. (2012) identified locus for height at withers on chromosome 3 ("eca3"). The candidate gene on eca3 was associated nearby the gene *LCORL/NAPG* and eca9 was associated nearby the gene *ZFAT* (Signer-Hasler et al., 2012; Tetens et al., 2013). There have been suggestions that in cattle, a variant in the *NCAPG* gene could be a potential causative variant for various growth-related traits (Setugi et al., 2009). These loci have also been found to influence height in humans (Gudbjartsson et al. 2008; Weedon et al. 2008; Soranzo et al. 2009; Lango Allen et al. 2010). This can further be controlled and showed with continued future studies similar to this study, but with larger amount of tested individuals. In the present study, a borderline significant association was found between a SNP marker and the phenotype height at withers, although not the same chromosome ("eca3") as previously mentioned by Signer-Hasler et al. (2012). The marker found in the present study was located on chromosome 8. Heritability for height at withers was shown to be 0.62 in a study of the Pantaneiro horses by

Miserani et al. (2002) although the heritability was shown to be 0.57 in another study by (Gharahveysi et al. (2008).

The phenotype that would be similar to “body size” that Signer-Hasler et al. (2012) used would be the phenotype “body length” within present study. Body length did not generate significant associations in present study ($EMP2 = 0.08492$), but was localized to chromosome 8 as well, as three out of the five significant and borderline significant phenotypes were localized in this present study. The three other phenotypes that in fact generated significant and borderline significant associations were: Jawbone circumference, Head length and (Height at withers* = borderline significant association). Although height at withers* was the phenotype that generated borderline significant association. The previous study, by Shrestha et al. (unpublished), found borderline significant associations for body length on chromosome 1. The reason for the lacking significant association for body length in this study is possibly due to the low amount of samples. Other reasons why no significant association was found on body length as well as other measurements such as ear length and distance between ears could be that some measures had no earlier documentation with reference to heritability and might not be related to any or very small genetic variation. As mentioned earlier, studies had been done on body length and height at withers where heritability was found to be 0.57 and 0.27, respectively on 2500 Iranian Arabian horses (Gharahveysi et al., 2008). As well as the study on 2000 Pantaneiro horses by Miserani et al. (2002) where heritability for body length and height at withers was found to be 0.72 and 0.61, respectively.

Four out of the five significant associations found in present study's association analysis were connected to head morphology, where two out of the four were located on chromosome 8. It seems chromosome 8 has some connectivity to morphology in general. Also focus lied on the lower part of the head with exception to the trait head length. With regard to one part of the aim of present study, which was to possibly see which of the measurements that mattered more or less to the complex breeding value that head morphology is, it seems the lower part could play a slightly more important part than other parts of the head within this subject. Regarding the other phenotype with significant association, head length, has been seen as an important trait for head assessments, connectivity to body size as well as its connectivity to correctness of gaits (Signer-Hasler et al., 2012). The heritability for head length has been shown to be moderately heritable as for example in Pantaneiro horses with $h^2 = 0.55$ (Miserani et al., 2002) as well as the moderate heritability of head in Icelandic horses ($h^2 = 0.33$) (Albertsdottir et al., 2008). Possibly also the significant association of head length matters to the complex breeding value of head morphology.

The reason for the mishap with the TaqMan assay is still to remain a mystery, the assay was tested by other people than me as a student. They tested the TaqMan assay for chromosome 20 independently from each other, their result was as for this study, and it was faulty and could not amplify the DNA sequences. Contact was taken with the company that the TaqMan assays were ordered from and their opinion was that they made a mistake constructing the assay. The company sent a new assay, this was also faulty. With further communicating with the company a problem with amplification or probe system was mentioned as reason for the mishap, though there was no certainty with a clear conclusion. The conclusion of my research group was that the company had some malfunction with constructing this assay.

Although there was enough material left to be able to finish this study with the rest of the chromosomes (4; head and 1; body length). However the results were not significantly associated and proven with regard to the found chromosomal regions by Shrestha et al., (unpublished). But the follow-up on the study Shrestha et al., (unpublished) that was stated as aim of study was completed however using a limited set of individuals and thus, further studies with larger data material would be required to pursue, as mentioned earlier, to obtain conclusive evidence for genetic association to these traits.

The t-tests of horses (chromosomes 4: head morphology and 1: body length) generated one statistically significant difference of means ($P < 0.05$) out of a total of 10 resulted p-values. One reason for the lacking of additional significant p-values could be due to the small samples size in present study, group 1 with the new horses ($n_{\text{tot}} = 256$; $n_{\text{chr 1}} = 93$; $n_{\text{chr 4}} = 163$). Also, for complex traits like body length and head morphology, several genes play a role. Furthermore, each gene by itself only show a minor effect on these traits and consequently may be difficult to detect using small sample sizes.

When combined t-tests were done using 366 horses with chromosome 4_{head}, no p-values for this subgroup A₂ was statistically significant ($P < 0.05$). No statistically significant p-values were observed for the combined t-test using 157 horses with chromosome 1_{body length}. The thought was that possibly the combined, larger population could have been able to lift the power somewhat, but this was not the case here. There were no genotypes GG for Chr 1_{body length} within group 2 when retrieved from previous study Shrestha et al., (unpublished). Therefore no t-tests where this genotype was included could be performed for subgroup B₂. The genotype GG seemed to have an impact on bringing the means down through all groups and subgroups and this could be seen in group 1 in both the present study and in Shrestha et al., (unpublished) (group 1). Although the 256 horses used in the present study could not provide more than one low enough p-value as for the limit of $\alpha = 0.05$, a slight change could be seen within means when GG was involved. For example t-test results for subgroup B₂, where the result could not present a statistically significant p-value, this possibly due to the lacking of GG.

The regions on chromosomes 4 (head) and 1 (body length) used for this current study and previous study by Shrestha et al. (unpublished) were located at positions where genes nearby did not have any apparent connection to head morphology, body length nor conformation.

Since this study's plan was reliant on the previous study by Shrestha et al., (unpublished) and this previous study did not bring up genes that was connected to conformation within region on affected chromosomes (4 and 1), neither was there plans made for this within current study. Nonetheless genes of other character laid nearby and one of them were a gene of name PRKAG2 (protein kinase, AMP-activated, noncatalytic, gamma-2) (Sanz et al., 2008) which was in region on chromosome 4. The SNP for region on chromosome 4 was located within this gene. The gene encodes a functional mammalian AMPK (5-AMP-activated protein kinase) heterotrimer (Dranchak et al., 2006). The AMPK is a heterotrimeric complex of three subunits that connect to each other, in this case a unit of three enzymes (Sanz et al., 2008). The mammalian activated protein kinase (AMPK) is an enzyme which is important for the cellular energy status and is expressed in many tissues, for example liver and skeletal muscles (Kahn et al., 2005). Associations for a heterozygous

mutation within this gene have been seen for the human disease where glycogen storage of the heart is deficient (Mizuta et al., 1984; Eyshi et al., 1985).

The other SNP in region on chromosome 1 was at position 184233kb and a gene nearby within this chromosomal region is for example the ATL1 gene at position: 184,377,908bp - 184,440,859bp (Ensemble website, 2016). And it codes for atlastin-1, a dynamin-related GTPase, which is a space protein, it is required for the upkeep of mitochondrial fusion (Wong et al, 2000). This protein plays a role in formation of the tubular endoplasmic reticulum (ER) network and in axon elongation in neurons (Zhu et al., 2006). For current study time was not enough for further search of genes nearby SNP location, this would have been of interest though.

Association analysis with regard to head morphology and body length generated significant associations to markers, although not to markers and positions that concur with previous studies' as Shrestha et al., (unpublished), Signer-Hasler et al., (2012) that found connectivity to conformation. The chip Illumina SNP50, seemingly gave reliability with present study's population of Icelandic horses and produced statistically significant associations to marker for the phenotypes, head morphology and body length.

Error sources

Questionnaire

The questionnaire that was used for this study was well designed but the timing/time of year for sending it out could have been another. The low amount of answers that was received can be explained with this fact above. Also it was an extensive questionnaire with many questions, although very thoughtfully put, a shorter one could have made it easier for the horse owners to answer and returned faster and in larger amounts.

Future studies

It could be interesting to conduct future studies on Icelandic horses, where another posting of the same questionnaire is done, at a better time of year. Perhaps the summer is a good time, to see if the rate of answers will increase and also if measurements or such will differ from the results in this study. Also it has been suggested that along with a questionnaire, a researcher would visit horse owners and personally perform the measurements asked for in the questionnaire (G. Andersson, personal communication, May 21, 2016). With a larger data set more factors may be found. And at a later time, it would be very interesting to see what these associations on chromosomes 1, 4 and 20 (borderline and significant) could lead to. Numerous of studies have been conducted, and conformation with its different parts seem to be very important for the performance in Icelandic horses. Since head morphology and body length has been seen in numerous of studies to have a possible connection to Icelandic horse's locomotion such as in the study by von Lengerken & Werner, 1969; see Weeren & Crevier-denoix, 2006, interest for the future would be to pursue more studies on this subject.

Conclusions

There are good possibilities for rendering knowledge and possibly then improving the trait body length with its high heritability of 0.72 (Miserani et al., 2002) given that

both head morphology and body length has been thought to be connected to Icelandic horse's locomotion. There lies importance and probable interest in different industries, such as the monetary influenced horse racing industry. Although heritability does not stand in as great favour for head morphology ($h^2=0.33$) as does for body length (0.72) (Miserani et al., 2002; Albertsdottir et al., 2002) possibilities can increase with larger sample sizes in future studies.

Acknowledgments

To my advisor, Gabriella Lindgren and assistant supervisors Merina Shrestha and Kim Jäderkvist Fegraeus for all the time you spent on many valuable discussions and patience to answer many and more questions. As well as to my examiner Göran Andersson for useful tips and valuable help and support when needed. All horse owners that participated in the questionnaire. Thanks also to my partner, Thomas, my children, Emma and Leo, my mother Anitha and siblings Virginia, Katarina and Mikael for support and valuable comments.

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11. APPENDIX 1 Questionnaire

GÄLLER HÄSTEN:

NAMN:
FEIF NR.

Enkät: "Man & svans" samt exteriör hos islandshästar

Hästens

namn _____

World Fengur registrerings-ID _____

Namn på hästens far _____

Registrerings-ID hästens far _____

Namn på hästens mor _____

Registrerings-ID hästens mor _____

Vänligen svara på frågorna nedan.

Nedan följer ett par frågor som rör sjukdom eller åkomma som kan ha inverkan på hästens hårtillväxt (hur man och svans ser ut). Svaren på nedanstående två frågor är mycket viktiga för att kunna bedöma kvaliteten på man och svans i efterföljande frågor.

1) Har din häst visat några tecken på sommareksem (kliar sig under säsong, håravfall, hudförtjockning, skorpbildning och/eller fjällning/flagning)? Sommareksem är säsongsbundet och visas i huvudsak under våren/sommaren och försvinner i slutet av hösten/vintern.

- ☐ JA
- ☐ NEJ

Kommentarer: _____

2) Har hästen någon annan sjukdom/åkomma eller allergi som gör att den kliar sin man och/eller svans?

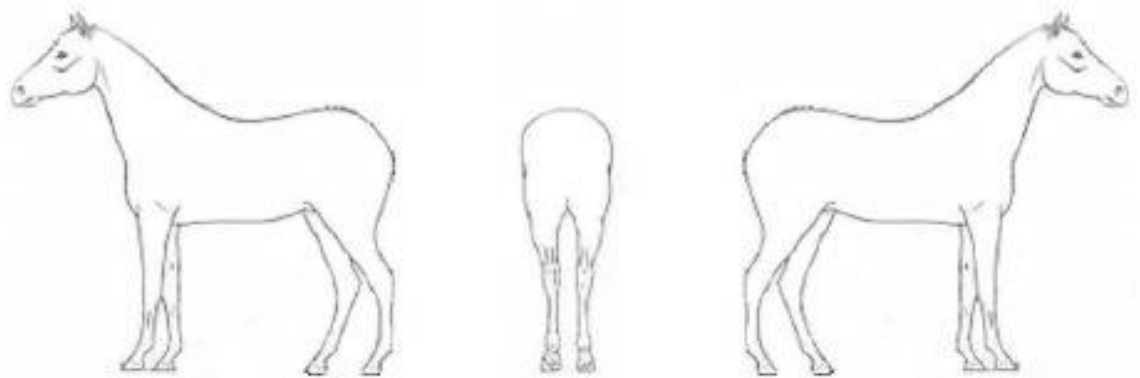
- ☐ JA
- ☐ NEJ

OM JA på ovanstående fråga, vilken typ av sjukdom/åkomma:

Man & svans

3) Vänligen rita på bilderna nedan hur pannluggen, man, svans och i förekommande fall, hår på benen ser ut på din häst (ungefär hur lång och tjock). Använd sidovyer av hästarna nedan för att markera om manen faller på vänster eller höger sida av halsen, eller om det faller på båda sidor naturligt.

Om möjligt, skulle vi uppskatta om Ni kan skicka (via SMS eller e-post) bilder på din häst från de tre vyerna nedan. (Se information om hur du skickar oss bilderna i slutet av detta frågeformulär).



4a) Hur lång är manen som längst på din häst? cm. (Vänligen dra håret rakt vid mätning)

.....

Mätutrustning som använts:

b) Ange nedan tjockleken på din hästs man

(1 = mycket tunn man 5 = måttligt tjock, 9 = extremt tjock man):

(Var god använd poängsystemet nedan):

1	2	3	4	5	6	7
8	9					

c) **Hårkvaliteten på hästens man är:** (1 = burrigt; 5 = enstaka hårstrån är vågiga; 9 = raka hårstrån) Se bilder nedan för vidare förklaring)
(Var god använd poängsystemet nedan):

1	2	3	4	5	6	7
	8	9				



**Burrit
utseende**
Referens till Creative
Commons.
[A brown Icelandic
horse](#) av Lisenhejhej
CC BY NC SA



Raka hårstrån.
Källa Katja Nilsson

d) **Klipper eller rycker du din hästs man regelbundet?**

- ☐ JA
- ☐ NEJ

e) **Hur lång är pannluggen på din häst?**cm. (Vänligen dra håret rakt vid mätning)

f) **Hur tjock är din hästs pannlugg?**

(1 = mycket tunn pannlugg, 5 = måttligt tjock, 9 = extremt tjock pannlugg):
(Var god använd poängsystemet nedan):

1	2	3	4	5	6	7
	8	9				

g) **Klipper eller rycker du regelbundet din hästs pannlugg?**

h) **Kan du ange nedan tjockleken på din hästs svans:** (1 = mycket tunn svans, 5 = måttligt tjock, 9 = extremt tjock svans):

(Var god använd poängsystemet nedan):

1	2	3	4	5	6	7
	8	9				

i) **Klipper du regelbundet din hästs svans?**.....

Huvudets exteriör

5a) Jämför med bilderna och markera vilken typ av huvudform din häst har i skalan

nedan: (1 = konvex profil, 4 = rak profil, 7 = konkav profil):

1 2 3 4 5 6 7 Vet ej



Konv



Rakt



Konk

b) Hur skulle du bäst beskriva din hästs huvud och käkar.

Köttigt huvud med tjock käke

Mindre köttigt huvud med tunnare käke

Huvud med synlig benstomme, lätta käkar

Kommentar _____

Vi vill gärna att du skickar oss bilder på din hästs huvud om det är möjligt. En bild framifrån och en med hästens profil. (Se information om hur du skickar oss bilderna i slutet av detta frågeformulär).

Se bilder nedan under bokstäverna.

M – Längden på örat (från framsidan av örats bas till örats topp) cm

Mätverktyg som använts (måttstock, måttband etc.)?

N – Avstånd mellan de inre, lägre ögonvinklarna cm

Mätverktyg som använts?

O – Näsborrarnas avstånd (från den ena övre näsborrens kant till den andra) cm

Mätverktyg som använts?

P – Avståndet mellan öronen (det kortaste avståndet närmast huvudet) cm

Mätverktyg som använts?

Q – Avståndet mellan vänster öga till mitten (från den vänstra nedre ögonvrån till mitten av hästens ansikte, dvs. hästens näsrygg) cm

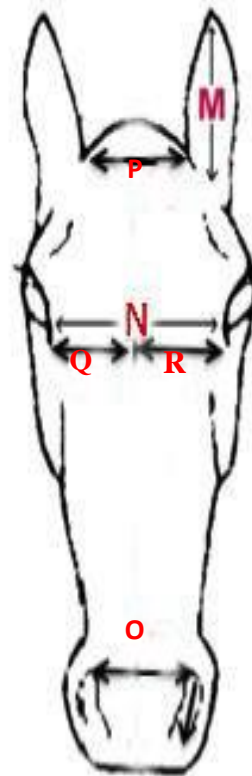
Mätverktyg som använts.....

R- Avståndet höger öga till mitten (från den högra nedre ögonvrån till mitten av hästens ansikte, dvs. hästens näsrygg) cm

Mätverktyg som använts.....

S – Näsborrarnas längd (från näsborrens övre kant ner till den nedre näsborrens kant) cm

Mätverktyg som använts.....



A – Var god mät din hästs omkrets precis ovan näsborrarna, (här kan måttband användas med fördel, se bild nedan).

..... cm.

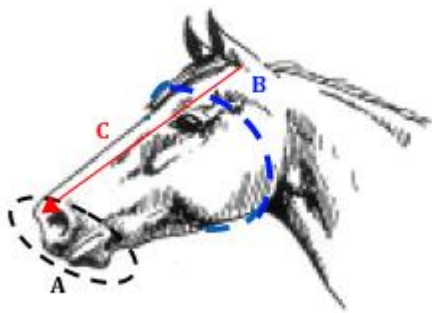
Kommentarer: _____

B – Käkbenets omkrets: var god mät omkretsen precis ovanför din hästs öga och hela vägen runt (här kan måttband användas med fördel, se bild nedan).

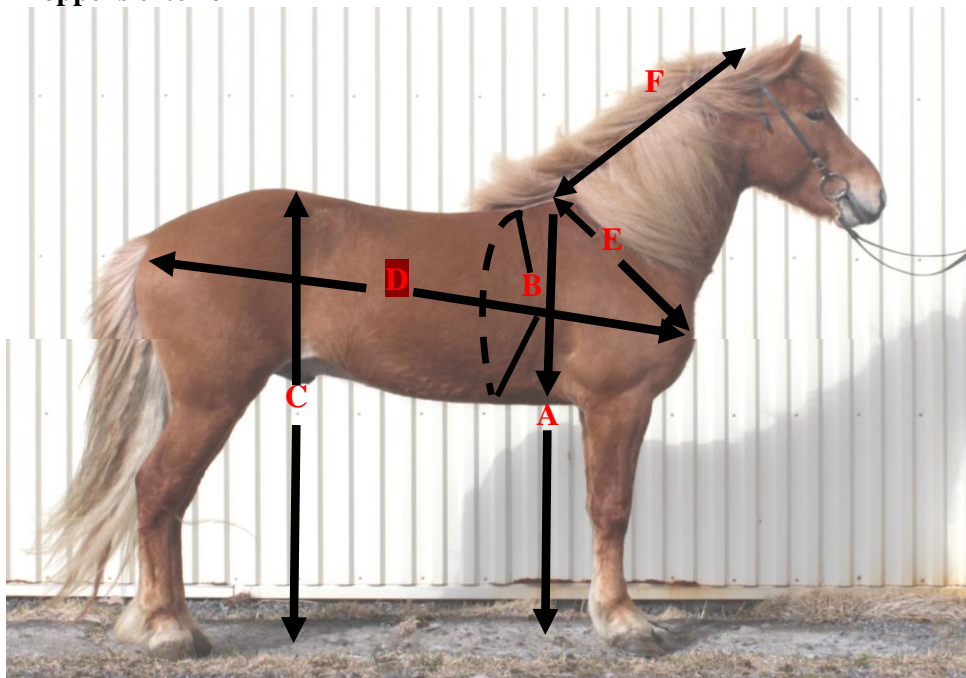
..... cm.

Kommentarer: _____

C – Vänligen mät längden på din hästs huvud från sidan. (Börja från framsidan av örats rot/bas och mät fram till näsborrens främsta kant.)
(Se till att mätverktyget du använder är så rakt som möjligt, se bild nedan).



Kroppens exteriör



Ovan ser du en bild av en häst som står ”korrekt”.

(Det är viktigt att hästen står så korrekt som möjligt där hästen står på ett jämnt icke sluttande underlag. *Med hovarna (vertikalt) under hästen, ståendes jämnt med alla hovar inte mer än en hofs längd mellan bak- eller framben, så att hästen inte står för brett, speciellt i bak).*

A - Mankhöjd cm (Mät mankhöjd med tumstock och ett vattenpass eller något annat rakt föremål (pinne eller stång)). Ange vilka mätinstrument som användes).

A1. Är mankhöjden mätt med eller utan skor?

o med skor (Tjocklek: _____ cm.)

o utan sko

Vilken mätutrustning användes ovan? _____

B – Bröstmått cm (Mät bröstet precis bakom manken och där sadelgjordens rem brukar vara). (Kontrollera att bandet är rakt och sträcka när intervallet mäts)

Vilken mätutrustning användes ovan? _____

C – Korshöjd cm (Mät korshöjden vid den högsta punkten med en tumstock och ett vattenpass eller något annat rakt föremål (pinne eller stång)).

Vilken mätutrustning användes ovan? _____

D – Längden på hästen cm (Mät längden på vänster sida från bogbladskanten till sittbenet. Mät med en tumstock eller något annat rakt föremål (pinne eller stång)).

Vilken mätutrustning användes ovan? _____

E - Längd på skulderblad cm (Mät från bogbladskanten till den högsta punkten på manken)

Vilken mätutrustning användes ovan? _____

F – Längden på nacken cm (Mät längden av halsen i en rak linje från baksidan av öronen till manken där det finns ett litet håll). (Huvudet och nacken ska vara i ett normalt upprätt läge, se bild med pilar ovan).

Vilken mätutrustning användes ovan? _____

7) Hur skulle du beskriva ryggen utseende på din häst?

(1 = Svankrygg; 3 = normal; 6 = karprygg, se förklarande bilder nedan)

1 2 3 4 5 6 7 Vet
ej



Svankrygg

Med referens till Creative Commons
Sway back av Lotse CC BY NC SA)



Normal rygg

Med referens till Creative Commons
Julgamento Árabe REFON José Reynaldo da
Fonseca CC BY NC SA)



Karprygg

Med referens till Creative Commons
Horse av leant CC BY NC SA)

Tack så mycket för att du/Ni har deltagit i den här undersökningen. Om du/Ni har några andra synpunkter på de frågor som ställs, fyll gärna i rutan nedan. Vi skulle också vilja veta om det var lätt att förstå frågorna och att fylla i svaren.

Information om ägare:

Ägarens namn.....

Adress

Postnummer

Ort

Telefonnummer

Mobiltelefon:

E-post

Information om hur du skickar oss enkäten och bilder på frågor ovan:

E-post: vefe0001@stud.slu.se

Mobiltelefon: [0704309512](tel:0704309512). (Använd detta nummer om du skickar bilder från telefonen).

Post:

Veronica Felt

Institutionen för husdjursgenetik

Sveriges lantbruksuniversitet

Box 7023,

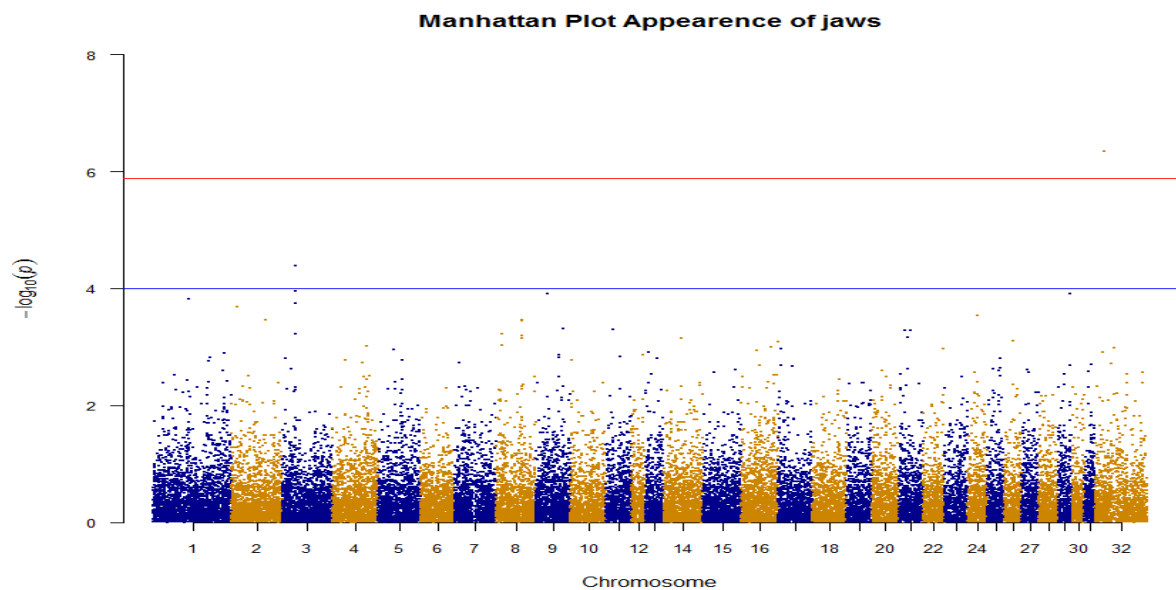
750 07 Uppsala

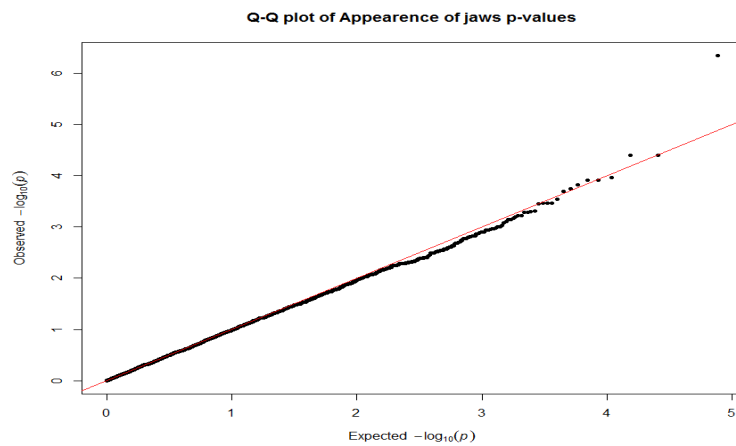
12. APPENDIX 3. Table of significant phenotypes after association test and permutation.

Phenotype	Question#	P-value	EMP2	CHR	SNP	BP
Head Jaws	5b)	0,000000458	0,01698	X	BIEC2-1113629	20743698
Muzzle circumference, just above nostrils	A)	1,15E-13	0,03896	30	BIEC2-828159	26321284
Jawbone circumference, just above eyes	B)	5,771E-13	0,03497	8	BIEC2-1049443	47054647
Head length	C)	1,744E-10	0,03596	8	BIEC2-1049443	47054647
Height at withers	A)	5,42E-13	0,05794	8	BIEC2-1049443	47054647
Body length	D)	4,613E-09	0,08492	8	BIEC2-1049443	47054647

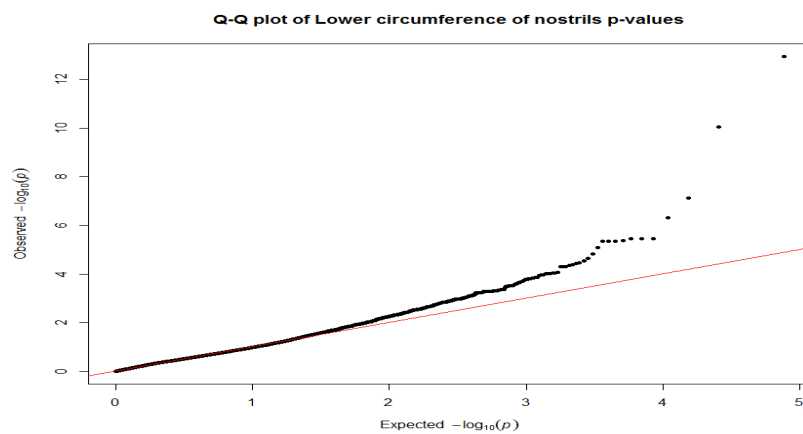
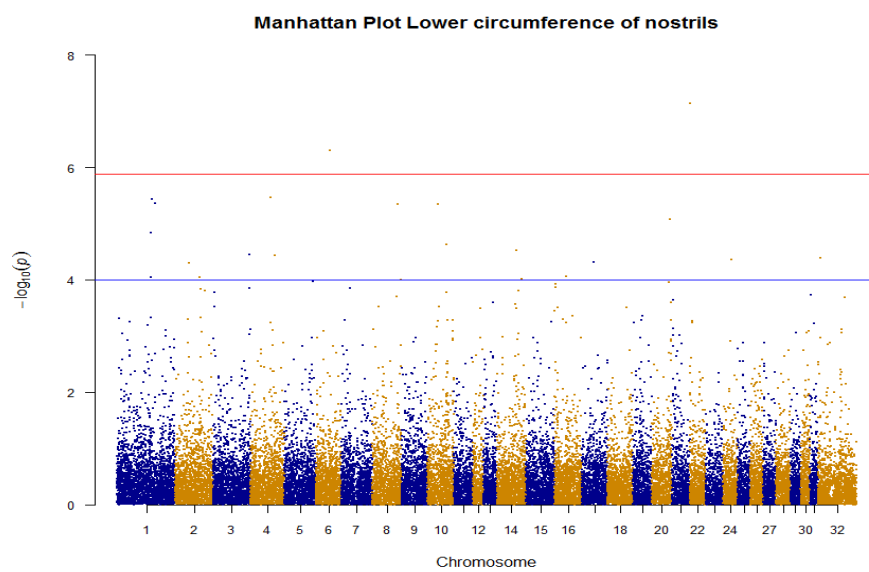
13. APPENDIX 4. Manhattan plot and qq-plot for each phenotype with significant association.

13.1 Appearance of jaws

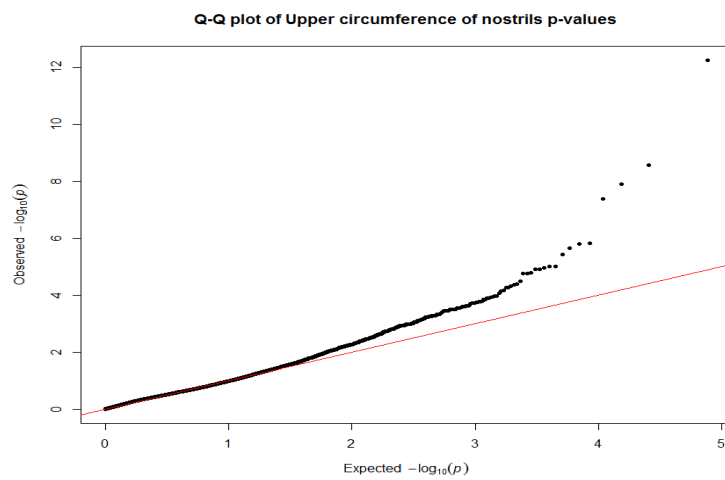
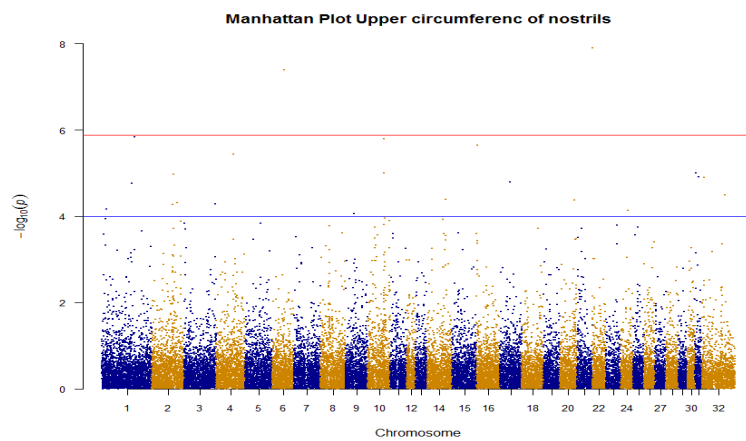




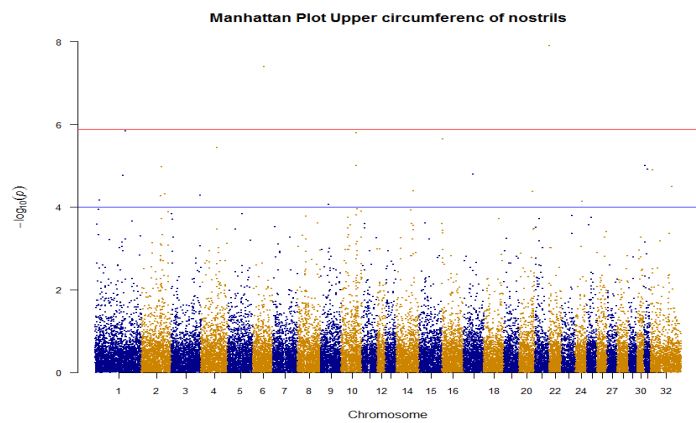
13.2 Muzzle circumference, just above nostrils

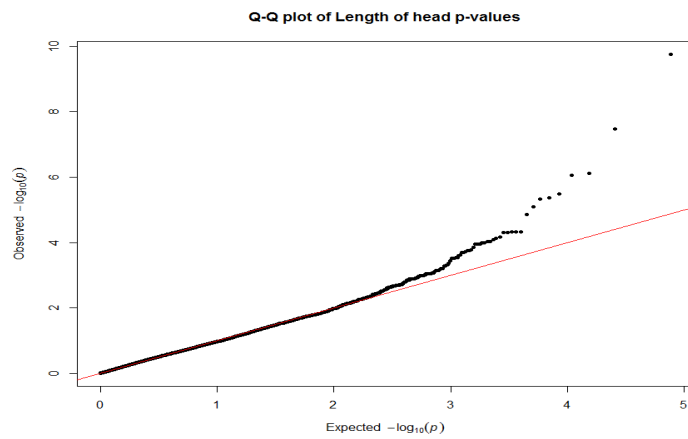


13.3 Jawbone circumference, just above eyes

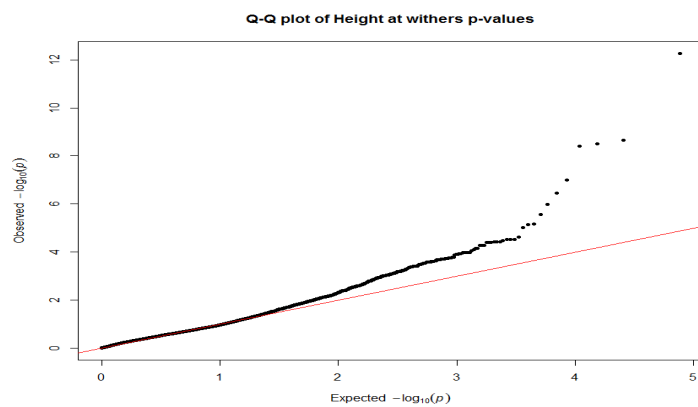
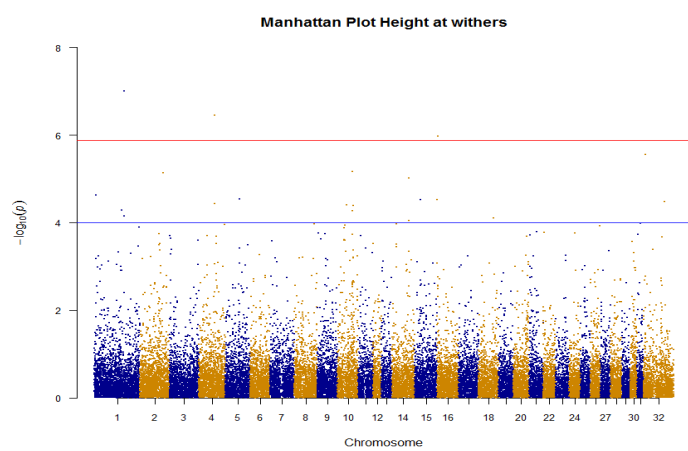


13.4 Head length

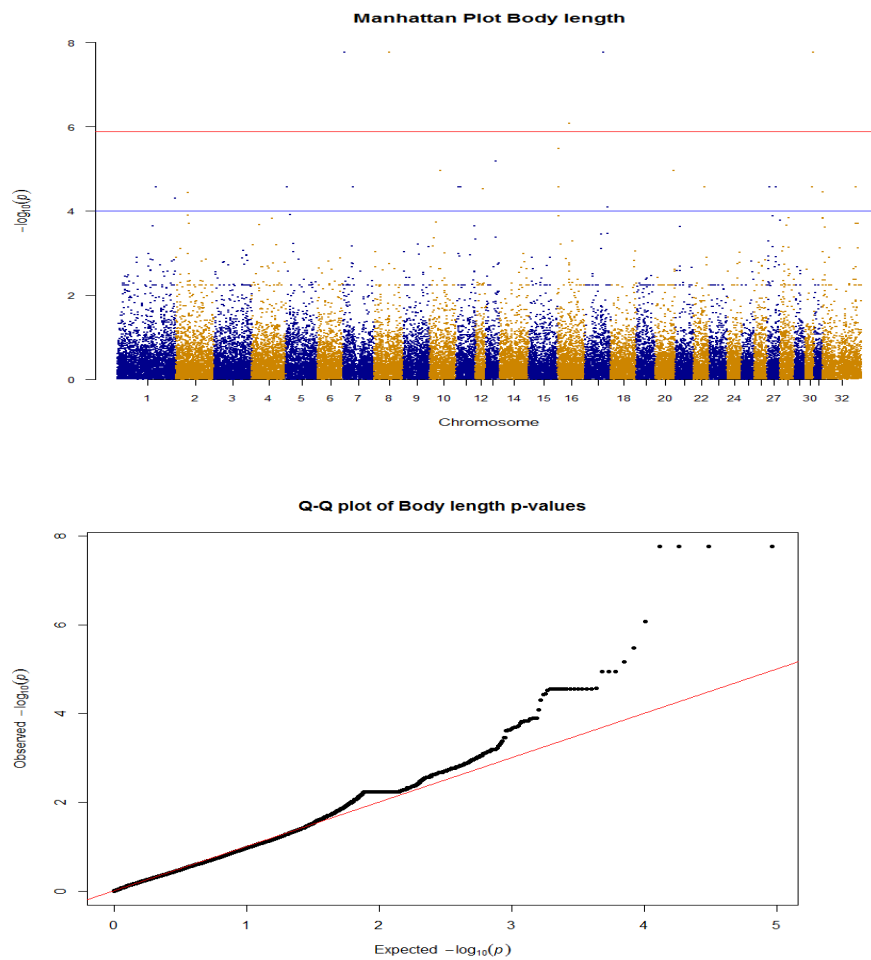




13.5 Height at withers



13.6 Body length BORDERLINE (EMP= 0. 08492)



14. APPENDIX 5. Compilation of association results before permutation in PLINK

Association test results; top 10 significant results on each phenotype (phenotype is linked to question number) that was tested

Question number 5a); Head shape:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
4	BIEC2-851229	16952566	45	- 1.474	0.305	0.352	- 4.833	1.748e-05
19	BIEC2-430681	17882739	45	- 1.909	0.3482	0.4116	- 5.484	2.046e-06
4	BIEC2-877863	99398440	44	- 1.404	0.3129	0.324	- 4.487	5.528e-05
13	BIEC2-230125	31034068	45	- 1.882	0.4316	0.3066	- 4.36	7.969e-05
X	BIEC2-1133337	74815307	45	- 1.89	0.3745	0.3721	- 5.048	8.643e-06
X	BIEC2-1133198	74633586	45	- 1.879	0.4382	0.2996	- 4.288	9.989e-05
9	BIEC2-1085703	32421165	45	- 1.221	0.2868	0.2963	- 4.255	0.0001109
1	BIEC2-23264	54364688	45	1.114	0.2642	0.2926	4.218	0.0001247
19	BIEC2-430400	16232843	45	- 1.435	0.3452	0.2866	- 4.157	0.0001509
19	BIEC2-430402	16232906	45	- 1.435	0.3452	0.2866	- 4.157	0.0001509

Question number 5b); Appearance of jaws bony or fleshy):

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
X	BIEC2-1113629	20743698	45	-0,7746	0,1306	0,4501	-5,933	4,58E-07
3	BIEC2-775579	30225193	46	0,8648	0,1897	0,3209	4,56	4,06E-05
3	BIEC2-775582	30226811	46	0,8648	0,1897	0,3209	4,56	4,06E-05
3	BIEC2-775536	30127979	46	0,75	0,1767	0,2906	4,245	0,000111
9	BIEC2-1083113	28609308	46	-1,6	0,3797	0,2875	-4,214	0,000123
29	BIEC2-762192	29582783	46	-0,8872	0,2107	0,2873	-4,211	0,000124
1	BIEC2-36363	83773384	46	0,9283	0,2238	0,281	4,147	0,000151
3	BIEC2-775492	29902119	46	0,7163	0,175	0,2759	4,094	0,000179
2	BIEC2-457677	14327003	46	-1,382	0,3411	0,2715	-4,05	0,000205
24	BIEC2-639630	22812253	46	-1,111	0,2818	0,2609	-3,941	0,000287

Question number M); Ear length:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
X	BIEC2-1133337	74815307	46	- 7.454	1.194	0.4696	- 6.242	1.489e-07
1	BIEC2-7000	14357360	46	- 9.073	1.708	0.3908	- 5.312	3.417e-06
26	BIEC2-693115	32111072	46	- 4.471	0.8824	0.3685	- 5.067	7.728e-06
21	BIEC2-555396	19956261	46	- 6.722	1.386	0.3484	- 4.85	1.578e-05
12	BIEC2-166382	1065764	46	- 4.685	1.007	0.3299	- 4.654	2.998e-05
18	BIEC2-418849	75112145	46	- 4.478	0.9687	0.3269	- 4.622	3.318e-05
16	BIEC2-326801	567491	46	- 5.079	1.104	0.325	- 4.602	3.541e-05
10	BIEC2-113474	32447110	46	- 5.388	1.182	0.3207	- 4.557	4.096e-05
10	BIEC2-112926	31665967	46	- 7.017	1.574	0.3113	- 4.46	5.611e-05
10	BIEC2-112959	31734972	46	- 7.017	1.574	0.3113	- 4.46	5.611e-05

Question number N); Distance between lower corners of eyes:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
X	BIEC2-1133337	74815307	46	- 7,62	1,359	0,4168	- 5,607	1,27E-06
10	BIEC2-111274	25665951	46	- 5,969	1,125	0,3903	- 5,307	3,48E-06
10	BIEC2-111276	25669787	46	- 5,969	1,125	0,3903	- 5,307	3,48E-06
18	BIEC2-418727	75038326	46	- 6,449	1,216	0,3901	- 5,305	3,50E-06
11	BIEC2-141313	16463280	46	- 6,063	1,26	0,3448	- 4,812	1,79E-05
12	BIEC2-166382	1065764	46	- 5,123	1,088	0,335	- 4,708	2,52E-05
26	BIEC2-693115	32111072	46	- 4,621	0,9832	0,3342	- 4,699	2,59E-05
10	BIEC2-113474	32447110	46	- 5,967	1,27	0,334	- 4,697	2,61E-05
X	BIEC2-1141598	85676771	46	- 7,082	1,53	0,3274	- 4,628	3,26E-05
4	BIEC2-854315	22287877	46	- 8,922	1,957	0,3209	- 4,56	4,07E-05

Question number O); Distance between nostrils:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
7	BIEC2-988617	27468171	46	- 4.434	0.9114	0.3498	- 4.866	1.501e-05
7	BIEC2-988655	27496195	46	- 4.434	0.9114	0.3498	- 4.866	1.501e-05
10	BIEC2-112926	31665967	46	- 4.434	0.9114	0.3498	- 4.866	1.501e-05

10	BIEC2-112959	31734972	46	- 4.434	0.9114	0.3498	- 4.866	1.501e-05
10	BIEC2-112960	31735239	46	- 4.434	0.9114	0.3498	- 4.866	1.501e-05
10	BIEC2-113648	32798631	46	- 4.434	0.9114	0.3498	- 4.866	1.501e-05
13	BIEC2-230164	31063498	46	- 4.35	0.9206	0.3367	- 4.725	2.374e-05
11	BIEC2-141313	16463280	46	- 3.251	0.7007	0.3285	- 4.64	3.135e-05
13	BIEC2-230125	31034068	46	- 4.802	0.7233	0.5005	- 6.639	3.87e-08
X	BIEC2-1133337	74815307	46	- 4.325	0.7283	0.4449	- 5.938	4.157e-07

Question number **P**); Distance between ears:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
13	BIEC2-230125	31034068	46	- 7.698	1.598	0.3452	- 4.816	1.766e-05
10	BIEC2-112926	31665967	46	- 8.427	1.774	0.3391	- 4.751	2.182e-05
10	BIEC2-112959	31734972	46	- 8.427	1.774	0.3391	- 4.751	2.182e-05
10	BIEC2-112960	31735239	46	- 8.427	1.774	0.3391	- 4.751	2.182e-05
10	BIEC2-113648	32798631	46	- 8.427	1.774	0.3391	- 4.751	2.182e-05
X	BIEC2-1133337	74815307	46	- 7.248	1.538	0.3354	- 4.712	2.479e-05
19	BIEC2-426879	8166783	46	- 6.471	1.387	0.3311	- 4.667	2.874e-05
19	BIEC2-426883	8167201	46	- 6.471	1.387	0.3311	- 4.667	2.874e-05
X	BIEC2-1141598	85676771	46	- 7.364	1.637	0.315	- 4.498	4.964e-05
10	BIEC2-113474	32447110	46	- 6.022	1.378	0.3026	- 4.369	7.493e-05

Question number **Q**); Distance between left eye to middle of face:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
4	BIEC2-868392	63246545	46	- 3.29	0.5208	0.4757	- 6,318	1.149e-07
4	BIEC2-874167	92982509	46	- 3.154	0.6388	0.3565	- 4.937	1.186e-05
1	BIEC2-28685	68949965	46	- 4.898	1.001	0.3524	- 4.894	1.369e-05
4	BIEC2-851229	16952566	46	- 2.928	0.602	0.3496	- 4.863	1.512e-05
9	BIEC2-1102799	67016929	46	- 3.649	0.7524	0.3484	- 4.85	1.578e-05
26	BIEC2-693115	32111072	46	- 2.467	0.5099	0.3473	- 4.839	1.639e-05
16	BIEC2-350264	53092742	46	- 4.483	0.9297	0.3458	- 4.822	1.73e-05
1	BIEC2-7000	14357360	46	- 5.234	0.9613	0.4025	- 5.445	2.193e-06
14	BIEC2-263538	69321795	46	- 4.387	0.9401	0.3311	- 4.667	2.869e-05
14	BIEC2-263552	69441855	46	- 4.387	0.9401	0.3311	- 4.667	2.869e-05

Question number **R**); Distance between right eye and middle of face:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
X	BIEC2-1133337	74815307	46	- 3,883	0,591	0,4953	- 6,571	0,00000004884
19	BIEC2-426879	8166783	46	- 3,265	0,5625	0,4337	- 5,805	0,0000006514
19	BIEC2-426883	8167201	46	- 3,265	0,5625	0,4337	- 5,805	0,0000006514
12	BIEC2-166382	1065764	46	- 2,649	0,4792	0,41	- 5,529	0,000001651
11	BIEC2-141313	16463280	46	- 3,023	0,5673	0,3922	- 5,329	0,000003232
26	BIEC2-693115	32111072	46	- 2,302	0,4437	0,3794	- 5,187	0,000005191
10	BIEC2-93366	2104609	46	- 3,763	0,727	0,3784	- 5,175	0,000005393
10	BIEC2-113474	32447110	46	- 2,878	0,5841	0,3556	- 4,927	0,00001225
7	BIEC2-988617	27468171	46	- 3,759	0,7771	0,3472	- 4,838	0,00001646
7	BIEC2-988655	27496195	46	- 3,759	0,7771	0,3472	- 4,838	0,00001646

Question number **S**); Length of nostril from top of nostril to bottom of nostril:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	BIEC2-52626	121557963	46	- 3,976	0,5427	0,5495	- 7,326	3,81E-09
8	BIEC2-1049443	47054647	46	- 3,503	0,4994	0,528	- 7,016	1,08E-08
6	BIEC2-951747	44568405	46	- 4,066	0,6242	0,4909	- 6,513	5,93E-08
22	BIEC2-574487	414888	46	- 3,841	0,6558	0,4382	- 5,858	5,45E-07
18	BIEC2-416479	60395417	46	- 1,985	0,3629	0,4048	- 5,471	2,01E-06
4	BIEC2-868678	64768396	46	- 3,401	0,6252	0,4021	- 5,439	2,23E-06
30	BIEC2-828159	26321284	46	- 3,617	0,6841	0,3885	- 5,287	3,72E-06
2	BIEC2-500156	95180555	46	- 3,095	0,5975	0,3788	- 5,179	5,32E-06
4	BIEC2-868850	65701999	46	- 2,207	0,4507	0,3527	- 4,896	1,36E-05
4	BIEC2-868855	65706054	46	- 2,207	0,4507	0,3527	- 4,896	1,36E-05

Question number **A**); Muzzle circumference:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
30	BIEC2-828159	26321284	46	- 34,17	3,23	0,7178	- 10,58	1,15E-13
8	BIEC2-1049443	47054647	46	- 26,37	3,116	0,6195	- 8,464	8,80E-11
22	BIEC2-574487	414888	46	- 28,11	4,36	0,4858	- 6,447	7,42E-08
6	BIEC2-951747	44568405	46	- 26,76	4,548	0,4403	- 5,884	5,00E-07
4	BIEC2-868678	64768396	46	- 23,29	4,387	0,3905	- 5,309	3,45E-06
1	BIEC2-46165	109791349	46	- 15,78	2,981	0,3889	- 5,292	3,66E-06
1	BIEC2-46175	109792388	46	- 15,78	2,981	0,3889	- 5,292	3,66E-06
1	BIEC2-52626	121557963	46	- 23,1	4,41	0,3841	- 5,238	4,38E-06
10	BIEC2-113741	33005513	46	- 24,97	4,775	0,3833	- 5,229	4,51E-06
8	BIEC2-1064361	83242286	46	- 19,58	3,747	0,3831	- 5,227	4,54E-06

Question number B); Jawbone circumference:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
8	BIEC2-1049443	47054647	46	- 51,66	5,142	0,6965	- 10,05	5,77E-13
30	BIEC2-828159	26321284	46	- 55,55	7,487	0,5558	- 7,42	2,78E-09
22	BIEC2-574487	414888	46	- 53,98	7,744	0,5248	- 6,971	1,26E-08
6	BIEC2-951747	44568405	46	- 52,64	7,952	0,499	- 6,62	4,14E-08
1	BIEC2-52626	121557963	46	- 44,25	7,955	0,4129	- 5,563	1,48E-06
10	BIEC2-127732	60511069	46	- 30,06	5,432	0,4104	- 5,534	1,63E-06
16	BIEC2-328129	3572529	46	- 22,68	4,177	0,4012	- 5,43	2,30E-06
4	BIEC2-868678	64768396	46	- 42,91	8,12	0,3883	- 5,284	3,75E-06
31	BIEC2-831352	2385259	46	- 22,66	4,542	0,3614	- 4,99	9,97E-06
10	BIEC2-127850	60897624	46	- 26,88	5,39	0,3611	- 4,987	1,01E-05

Question number C); Head length:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
8	BIEC2-1049443	47054647	46	- 32,4	3,925	0,6076	- 8,254	1,74E-10
6	BIEC2-951747	44568405	46	- 35,5	5,315	0,5034	- 6,678	3,39E-08
20	BIEC2-541524	56574929	46	- 32,8	5,695	0,4299	- 5,76	7,59E-07
4	BIEC2-867698	59657965	46	- 30,19	5,279	0,4264	- 5,719	8,72E-07
7	BIEC2-988275	27002834	44	- 18,16	3,39	0,406	- 5,358	3,31E-06
30	BIEC2-828159	26321284	46	- 31,01	5,919	0,3841	- 5,239	4,37E-06
14	BIEC2-260501	61417422	46	- 19,06	3,652	0,3822	- 5,218	4,68E-06
14	BIEC2-263037	68631278	46	- 15,96	3,154	0,3678	- 5,059	7,93E-06
5	BIEC2-889623	5316581	46	- 24,65	5,045	0,3519	- 4,887	1,40E-05
16	BIEC2-329542	10590554	46	- 21,26	4,703	0,3171	- 4,52	4,62E-05

Question number A); Height at withers:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
8	BIEC2-1049443	47054647	46	- 77,6	7,707	0,6973	- 10,07	5,42E-13
22	BIEC2-574487	414888	46	- 83,7	11,19	0,56	- 7,483	2,25E-09
30	BIEC2-828159	26321284	46	- 83,25	11,26	0,554	- 7,393	3,05E-09
6	BIEC2-951747	44568405	46	- 82,91	11,32	0,5495	- 7,326	3,81E-09
1	BIEC2-52626	121557963	46	- 71,57	11,24	0,4794	- 6,365	9,81E-08
4	BIEC2-868678	64768396	46	- 69,27	11,57	0,449	- 5,988	3,51E-07
16	BIEC2-328129	3572529	46	- 34,89	6,162	0,4215	- 5,662	1,06E-06
X	BIEC2-1109435	9211395	46	- 47,43	8,822	0,3964	- 5,376	2,76E-06
10	BIEC2-127732	60511069	46	- 42,94	8,416	0,3717	- 5,102	6,88E-06
2	BIEC2-500156	95180555	46	- 58,94	11,6	0,3698	- 5,082	7,36E-06

Question number D); Body length:

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
8	BIEC2-1049443	47054647	46	- 100,3	13,8	0,5457	- 7,269	4,61E-09
20	BIEC2-541524	56574929	46	- 111,7	18	0,4668	- 6,207	1,67E-07
30	BIEC2-828159	26321284	46	- 111,7	18	0,4668	- 6,207	1,67E-07

1	BIEC2-88728	181447348	46	- 77,38	14,32	0,3988	- 5,402	2,53E-06
16	BIEC2-327028	1388768	46	- 50,55	9,422	0,3955	- 5,366	2,86E-06
4	BIEC2-868678	64768396	46	- 93,61	17,88	0,3838	- 5,235	4,42E-06
27	BIEC2-722672	39530677	46	- 93,22	17,93	0,3807	- 5,2	4,96E-06
16	BIEC2-341307	37591812	45	- 91,99	18,21	0,3723	- 5,051	8,58E-06
1	BIEC2-46165	109791349	46	- 58,57	12,69	0,3261	- 4,615	3,40E-05
1	BIEC2-46175	109792388	46	- 58,57	12,69	0,3261	- 4,615	3,40E-05

15. APPENDIX 6. MDS-plot of population stratification test done in QC for association test in PLINK.

