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Master Thesis

Genome-Wide Association Study for Fertility Related Traits in Austrian Fleckvieh Cattle

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Contents

Summary

Fertility related traits are important traits for dairy farmers. For example, a more precise prediction of gestation length and as a consequence of the calving date could aid in a better adapted feeding strategy for pregnant cows. Gestation length is correlated with other calving traits like calving ease and stillbirth, which can invoke high costs if problems occur.
Therefore the purpose of this study was to find regions in the genome responsible for direct/maternal gestation length, direct/maternal calving ease, direct/maternal stillbirth as well for fertility disorders like retained placenta, inflammation of the uterus and puerperal disease. All data has been provided by ZuchtData Datenverarbeitungs GmbH and consisted of estimated breeding values for each trait as well as genotype data of 6730 Austrian and German Fleckvieh bulls.

The study identified a region on chromosome 21 that seems to be responsible for calving ease (direct and maternal), stillbirth (direct) and gestation length (direct and maternal). The gene responisible for Prader-Willi-Syndrome is situated in this genomic region. Prader-Willi-Syndrome is a disorder causing restricted fetal movements and a higher percentage of assisted births´.

For stillbirth (direct) and calving ease (direct/maternal), a region on chromosome 14 was found. Within this area the PLAG1 and several other genes are located. Those are responsible for cattle height and weight, which is highly correlated with calving ease and stillbirth.

Separate Analyses related to stillbirth were performed, selecting data sets with short and long gestation lengths, at least one standard deviation below and above the mean, assuming that stillbirth may be caused by different genes in case of short versus long pregnancies. However this analysis brought no significant results.

For fertility disorders the number of animals was limited in comparison to the other traits. Only one SNP was found to be associated, but no genes were located nearby.

Zusammenfassung

Fruchtbarkeit und deren angelehnte Merkmale wie Kalbeverlauf, Totgeburtenrate, Trächtigkeitsdauer aber auch frühe Fruchtbarkeitsstörungen stellen sehr wichtige Merkmale für Rinderzüchter und Milchbauern dar. Probleme in diesem Bereich können sehr schnell zu wirtschaftlichen Verlusten führen.
Eine präzisere Vorhersage der Trächtigkeitsdauer könnte außerdem auch das Betriebsmanagement erleichtern, da dadurch die Fütterung der Tiere besser auf deren Bedarf abgestimmt werden könnte.
Daher war es das Ziel dieser Arbeit relevante Regionen bzw. Gene im Rindergenom zu finden, die für Trächtigkeitsdauer (direkt/maternal), Kalbeverlauf (direkt/maternal), Totgeburtenrate (direkt/maternal) und frühe Fruchtbarkeitsstörungen (direkt), wie Gebärmutterverhalten, Uterusentzündungen und puerperale Erkrankungen verantwortlich sind.

Alle Daten wurden von der ZuchtData Datenverarbeitungs- GmbH bereitgestellt. Diese bestanden aus geschätzten Zuchtwerten für jedes Merkmal sowie aus genotypischen Daten für 6730 österreichisch-deutsche Fleckviehstiere.

Die Studie identifizierte eine Region am Chromosom 21, die gleich für mehrere Merkmale verantwortlich zu sein scheint, und zwar für Kalbeverlauf (direkt/maternal), Totgeburtenrate (direkt) sowie auch für direkte und maternale Trächtigkeitsdauer. In eben dieser Region befinden sich auch Gene die verantwortlich sind für das Prader-Willi- Syndrom, eine Krankheit die verminderte fetale Bewegungen und einen höheren Prozentsatz an Kaiserschnitten beim Menschen hervorruft.

Auch am Chromosom 14 wurde einige Gene gefunden die für mehrere Merkmale (Kalbeverlauf und Totgeburtenrate) verantwortlich sind. Einige dieser Gene wie zum Beispiel PLAG1 sind verantwortlich für Größe und Gewicht von Rindern aber auch Menschen. Auch diese Merkmale sind stark korreliert mit Kalbeverlauf und Totgeburtenrate.

Die Daten der Trächtigkeitsdauer wurden zusätzlich aufgeteilt. Jene Daten die eine Standardabweichung über bzw. unter dem Durchschnitt für Trächtigkeitsdauer lagen wurden für eine separate Analyse herangezogen. Dabei wurde angenommen, dass Totgeburten bei kurzer bzw. langer Trächtigkeitsdauer von unterschiedlichen Genen beeinflusst werden. Allerdings brachte diese Analyse keine signifikanten Ergebnisse zu Tage.

Für die Analyse der frühen Fruchtbarkeitsstörungen standen im Vergleich zu den anderen Merkmalen weniger Daten zur Verfügung. Es wurde nur ein assoziierter Marker gefunden, in dessen Nähe allerdings keine relevanten Gene vorhanden sind.

1 Introduction

Time around parturition is of high risk for both, mother and offspring leading to high veterinary costs for dairy cattle farmers when complications occur. Therefore dairy managers should focus on prevention of risk factors associated with problems around the calving event like calving difficulty, stillbirth, retained placenta or other metabolic diseases (Ghavi Hossein-Zadeh, 2013).
As very short gestations enlarge the risk of stillbirth and gestations longer than normal lead to very big calves, with higher calving difficulty, those extremes should be avoided.
Gestation length (GL) itself is influenced by several environmental and genetic effects; therefore it differs between breeds, sexes of the calves, etc. The knowledge about these effects can aid in a more precise prediction of the calving date and in reducing metabolic diseases because of a more appropriate feeding strategy for pregnant cows. For this purpose herd managers could use EBVs or genomic information of sires for GL to aid in predicting the calving date, because the use of a sire can increase or decrease the gestation length. Since it is a highly heritable trait it is open to rapid change under selection (Norman et al., 2011, 2009).

Since genomic information via SNP chip data is available, the prediction of inheritance of different traits has become easier and a few studies have already focused on finding significant regions for gestation length in the cattle genome and reported different significant regions for various breeds (Maltecca et al., 2011, 2009; Schrooten et al., 2000).
Schrooten et al (2000) found the most significant region for gestation length on chromosome 4 between markers TGLA159 and TGLA420, but no identification for other QTLs in the Dutch Holstein Frisian population.
Maltecca et al (2009, 2011) also investigated Holstein Frisian, Brown Swiss and a Jersey x Holstein crossbred reported different findings. While the first two breeds show small peaks on chromosome 4 as well, the crossbred did not.
However, the most significant SNP for Holstein Frisian was reported on BTA 18 within the siliac acid binding Ig-like lectin 5 (SIGLEC5) gene, which is speculated to result in a leptin deficiency and leading to delayed parturition.

Another factor during the calving event, to which a farmer should pay attention, is calving ease.
This trait is influenced by the dam as the ease of giving birth (maternal effect) and by the offspring of a bull as the ease of being born (Cue and Hayes, 1985).
When calves do have problems during the process of parturition they experience higher physiological stress which could lead to a reduced passive immunity and higher mortality rates (Barrier et al., 2013). Additionally, Eaglen et al. (2011) showed that such calves have a lower milk yield in their own first lactation compared to calves which are born without assistance.
But not only the calf suffers from a difficult parturition. Dams show a higher risk for retained placenta (Coleman et al., 1985), which itself is correlated with a greater incidence of endometritis, an inflammation of the uterus that again enlarges the number of services for conception (Gilbert et al., 2005). Additionally it´s incidence is correlated with longer gestations than normal (Han and Kim, 2005).
Sattlecker (2014) who investigated genetic parameters of calving traits in Fleckvieh cattle encourages those statements, as in this breed early fertility disorders occur

more often after difficult calvings, while the correlation of the traits was not significant. The proportion of such disorders is also higher for cows who recently experienced stillbirth, although no significant correlation was found as well (Sattlecker, 2014).
For a genetic improvement of those traits genomic information would be beneficial. Olsen et al. (2011) focused on finding important regions for retained placenta and fertility treatments, which include among other traits inflammation of the uterus. They come to the conclusion that markers on BTA 5 and 9 are in association with retained placenta. In addition they found important regions for fertility treatments on chromosomes 1 and 8.

Furthermore, difficult calvings are associated with a higher risk of a stillborn calf. Meyer et al (2001) showed that the odds of stillbirth are 6.76 to 11.36 (for primi- and multiparous cows) times higher than the odds of a live calf when the calving ease score was greater than 3, so the calf needed assistance. Among other factors like parity, season or sex of the calves, gestation length exerts an influence on stillbirth rate. Especially in cases where calving difficulty (a greater score then 3) and short gestations (- 15 days compared to the average) coincide, stillbirth rate is increasing (Meyer et al., 2000). Sattlecker (2014) confirmed these findings, the correlation between direct calving ease and direct stillbirth (0,661 ±0,140) was strong for Fleckvieh.
Gestation length has an effect on stillbirth rate, the correlation was significant (0,421 ±0,137). Gestation length is also significantly correlated with calving ease too, with longer gestation lengths causing more calving difficulties (Sattlecker, 2014).

Difficult calvings lead to economic losses because of reduced milk yield, higher veterinary and replacement costs for lost calves and cows (Meijering, 1984), so their occurrence should be reduced.

Please note that the traits investigated in this study are affected by direct and maternal effects. According to Fürst and Fürst-Waltl (2006), maternal effects are negatively correlated with the direct effects in most cases (Fürst and Fürst-Waltl, 2006).
So far, a few researchers have focused on finding genomic regions related to direct and maternal calving ease and stillbirth. While Pausch et al. (2011)found significant QTLs on chromosomes 14 and 21 for Fleckvieh, Olsen et al (2010) reports a significant region on Chromosome 6 for Norwegian Red affecting both stillbirth and dystocia.
On the other hand, Cole et al. (2009) mentioned Chromosome 18 as important region for both traits in Holstein Frisian, whereby the Siglec-5 gene was important too, as described for gestation length by Maltecca et al (2011).

As calving ease and stillbirth are very lowly heritable traits and direct and maternal effects are negatively correlated, a change through selection processes is difficult (Cue and Hayes, 1985).
Therefore the use of correlated traits, like gestation length (Eaglen et al., 2012; Hansen et al., 2004) and the knowledge about significant genes could aid in this.

Therefore the objective of this study was to find significant regions throughout the genome for gestation length, calving ease, stillbirth and early fertility disorders (retained placenta, inflammation of the uterus and puerperal diseases). As those

traits are correlated with each other, it was also a challenge to find whether they are affected by the same genes or genomic regions.
Since stillbirth rates tend to be higher for calves with gestation length below the average (Meyer et al., 2000), it was a question too, whether genes for stillbirth differ if the gestation length is shorter or longer than the mean.

2 Material and Methods

2.1 Animals and phenotypes

For this genome wide association study 6730 genotyped sires of the Germany-Austrian Fleckvieh cattle population have been used, which where genotyped with the Illumina 50k SNP Chip.
Phenotypes in form of estimated breeding values for direct and maternal gestation length (GL), calving ease (CE), stillbirth (SB) and direct early fertility disorders (FD) have been provided by ZuchtData EDV-Dienstleistungen GmbH (Vienna). They are estimated routinely with a BLUP animal model, whereby the fixed effects herd*year, region*year*calving month, sex, lactation*calving-age-class, permanent environment effect, calf (paternal effect) are considered.

⇒ Gestation length is counted as days from insemination to parturition.
⇒ Calving ease is scored in five categories from unassisted birth to emobryotomy, although the last two categories (caesarean section and embryotomy) are merged for the breeding value estimation.
⇒ Stillbirth is recorded as categorical trait, where deaths till 48 hours after birth are counted.
⇒ Early fertility disorders contain inflammation of the uterus, retained placenta and puerperal diseases till 30 days after calving (Fuerst et al., 2013).

Since the use of EBVs in association studies can result in several problems, like a larger increase in the type I error and reduced power (Ekine et al., 2014), we decided to use deregressed breeding values instead, which were calculated after Garrick et al. (2009).
The deregression process removes the parent average effect for each animal, which is necessary because of shrinking individual information towards parent average by using BLUP evaluation (Garrick et al., 2009).

After the deregression those animals with reliabilities lower than 0.3 have been removed from the dataset. Therefore, different numbers of animals were available for each trait:

- 6564 for both calving ease and stillbirth direct
- 6563 for calving ease maternal
- 6561 for stillbirth maternal
- 2546 for gestation length direct
- 2437 for maternal gestation length and
- 1943 for fertility disorders

The situation for stillbirth with lower and longer gestation lengths was different. In this case the already deregressed datasets for gestation length and stillbirth have been merged. Afterwards the standard deviation for gestation length had been calculated. Then the stillbirth data for animals with more than one standard deviation above and below the average were used for separate analyses.

Only a small number of animals were available for the analysis:

- 525 for direct stillbirth with a higher gestation than the mean (SBdGLh)

- 418 for direct stillbirth with a lower gestation length (SBdGLl)
- 371 for maternal stillbirth with a higher gestation (SBmGLh
- 362 for maternal stillbirth with a lower gestation length (SBmGLl)

Another step before the quality control was a check for normal distribution of the deregressed estimated breeding values. The results of this are shown in Figure 1 for calving ease direct. The figures for the other traits are in the appendix.
For fertility disorders the reliabilities were very low.

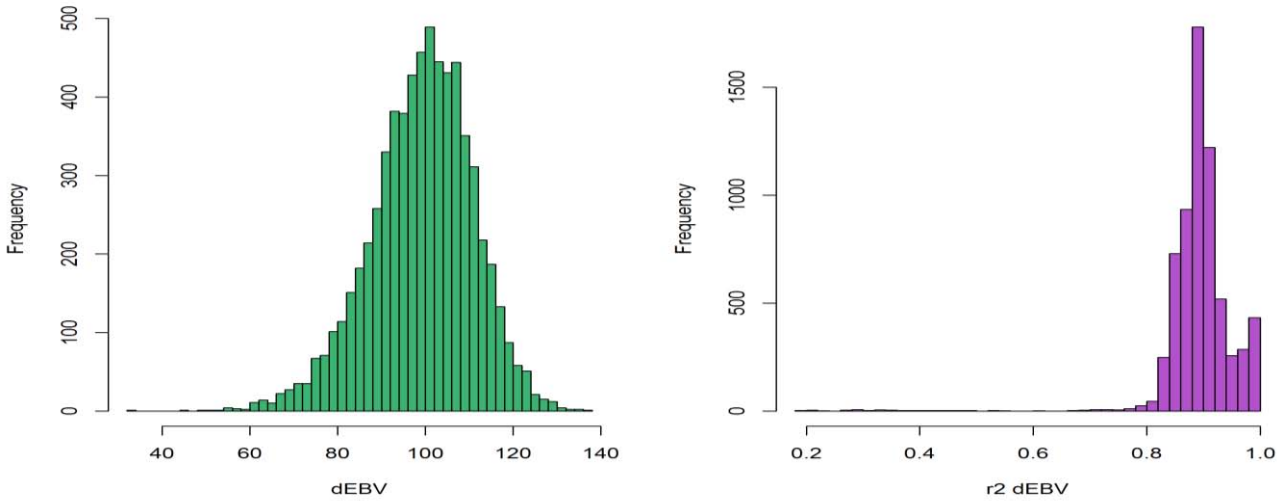

Figure 1: Distribution and reliabilities (r^2) of deregressed Breeding values for direct calving ease

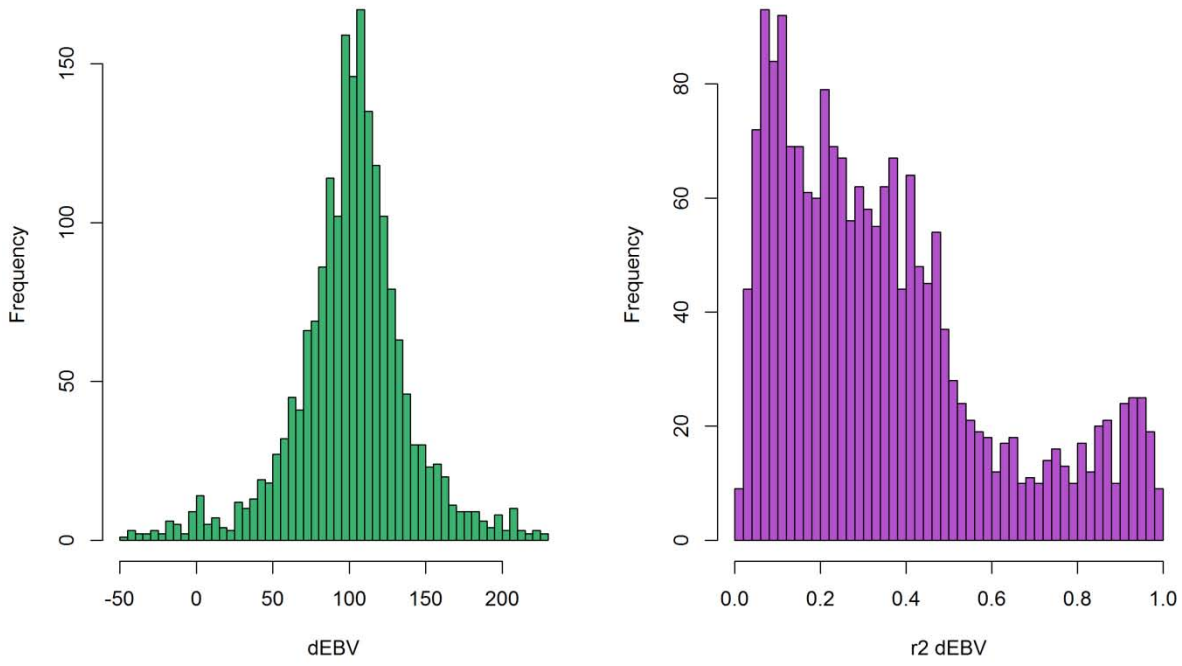

Figure 2: Distribution and reliabilities (r^2) of deregressed breeding values for fertility disorders

2.2 Genotypes and Quality control

The genotypic data was provided by ZuchtData and contained 50006 markers.

The quality check was done using the Software PLINK (Purcell et al., 2007) after following criteria:

- SNP call rate of 95%
- Individual call rate of 90%
- Minor allele frequency of 0.02
- Hardy- Weinberg-Equilibrium of $1x10^{-5}$

Since the datasets for the traits were unequal we decided to use the maximal number of animals available for each trait. Therefore quality control was done separately for all five traits.

After quality control no animals have been removed for missing phenotypes. For all normal traits except fertility disorders and gestation length 41 animals have been removed for low genotyping. In terms of fertility disorders only 3 and gestation length only 5 animals were removed for this purpose. The specific stillbirth traits only showed 1 individual was removed for missing phenotypes.

The test for Hardy Weinberg equilibrium caused different numbers of excluded SNPs for most of the traits. The specific stillbirth traits were slightly different, the exclusion varied only from 113 to 165 markers.
The missingness and frequency tests for SNPs resulted in equal numbers for calving ease direct and maternal as well for stillbirth direct and maternal with 969 SNPs failing missingness test and 8803 SNPs failing the frequency test.
For fertility disorders the numbers were: 1465 SNPs failed missingness test and 8804 SNPs failed frequency test.
The numbers of animals and SNPs after quality control for each trait are shown in Table 1.

Trait	Animals before QC	Animals after QC	SNPs after QC
GL direct	2546	2541	39 872
GL maternal	2437	2432	39 833
CE direct	6564	6523	40 060
CE maternal	6563	6522	40 059
SB direct	6564	6523	40 060
SB maternal	6561	6520	40 058
FD	1943	1940	39 832
SBdGLhigh	427	427	40 009
SBdGLlow	418	417	39 756
SBmGLhigh	371	370	39 673
SBmGLlow	362	362	40 064

Table 1: Number of animals before and after quality control and number of SNPs after QC

2.3 Statistical Analysis

The first step was a single marker regression without correction. For this a linear model was fitted which included the genotype and the phenotype.
However, population substructure was expected, and a correction was done following the approach of Utsunomiya et al. (2013). The procedure was as follows:

(i) Eigenvectors were calculated from the pair-wise genomic IBD coefficients estimated from the SNP markers using the Multidimensional Scaling approach using the software PLINK (Purcell et al., 2007).
(ii) Afterwards a Pearson correlation test with phenotypes and eigenvalues was done in R. The most correlated eigenvalues were then used in a multiple linear regression.
(iii) Because of uncertainty of the estimation a weighted analysis was determined in R as well using Garrick weights as weighting factor:

$$y_i = u + \sum_{j=1}^{1:N} V_{ij} b_j + e_i$$

where y_i is the EBV of sire i, u is the overall mean, V_{ij} is value i (corresponding to sire i) in the eigenvector j calculated in the IBD estimation, b_j is the estimated effect of eigenvector j, and e_i is the residual effect for sire i.

(iv) After that the residuals of the weighted analysis of the eigenvalues were obtained and used in a single marker regression as follows:

$$y_i^* = u + Xb_j + e_i$$

Where y_i^* is the estimated breeding value of sire i, u is the overall mean, X is the genotype of sire i, b_j is the marker regression coefficient of SNP j (i.e. the allele substitution effect of the SNP) and e_i is the residual effect of sire i.

(v) Markers which passed the Bonferroni correction calculated as $\alpha/Nsnps = 0.05/39{,}804 = 1.256 \times 10^{-6}$ were considered as significant.
(vi) Additionally the False Discovery Rate (FDR) was calculated as well using the *fdrtool*in R and those SNPs with q-values greater than 0.05 were considered significant.
(vii) After this discovery of the 20 most significant SNPs from the Bonferroni threshold plus those significant within the False Discovery Rate (q-value approach) where used for the gene searching for each trait.

The Ensemble Biomart database was used for the searching of the genes within the important region for each SNP (+/- 500kb). Afterwards the BioGPS annotation portal was used to identify the function of each gene.

For each SNP delivering relevant genes the proportion of additive genetic variance has been calculated, following the approach of Utsunomiya et al. (2013):

$$\%\pi_{\sigma}^{2} = \frac{2pq\beta_{g}^{2}}{\sigma^{2}_{a}} * 100$$

where p and q are the allele frequencies, β is taken from the output after the weighted analysis and σ^{2}_{a} is the additive genetic variance of the trait.

3 Results and Discussion

3.1 Gestation length

3.1.1 Direct gestation length

The first run of the genome wide association was one without correction. However, this revealed a big deviation between expected and observed -log p-values.

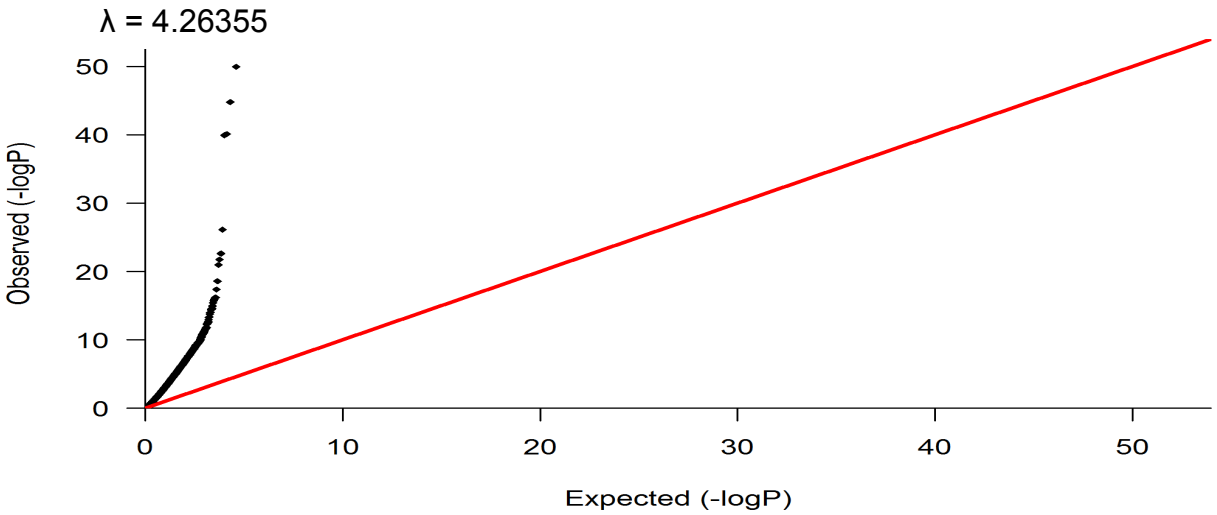

Figure 3: Q-Q- plot for direct gestation length without correction

With a look at the manhattan plot one could conclude that nearly every chromosome shows important SNPs, while the highest peak is on chromosome 21. Because of those results it was necessary to correct for population structure.

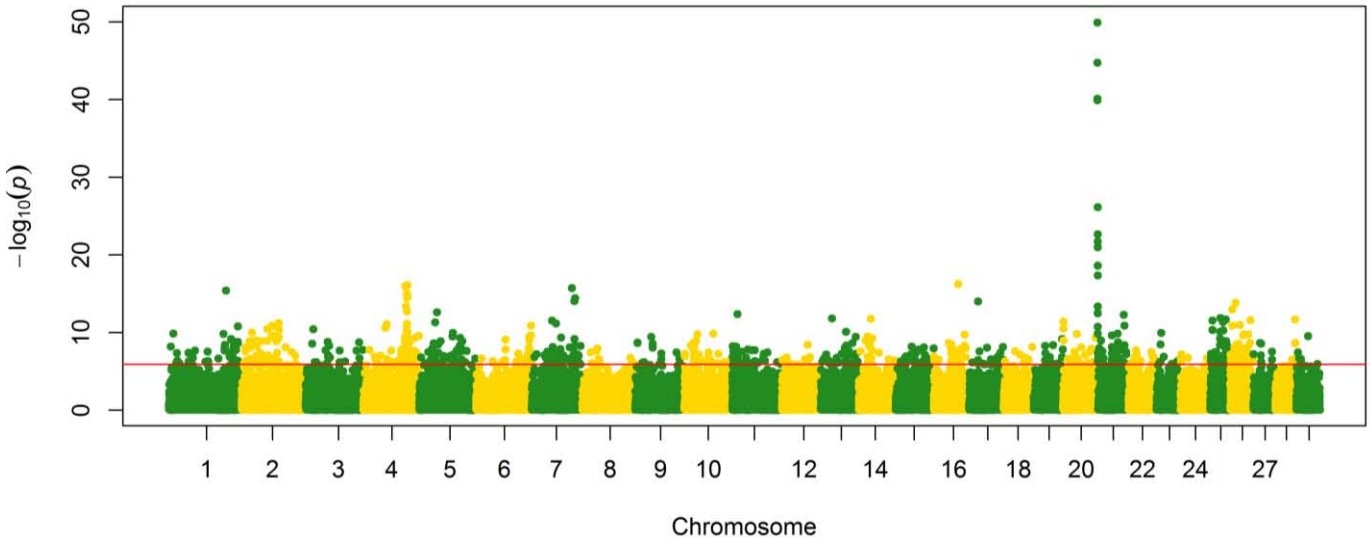

Figure 4: Significance levels (minus-log-P-values) for direct gestation length without correction

The eigenvectors were calculated and weighted after Garrick weights and the most correlated ones where then used for the analysis.

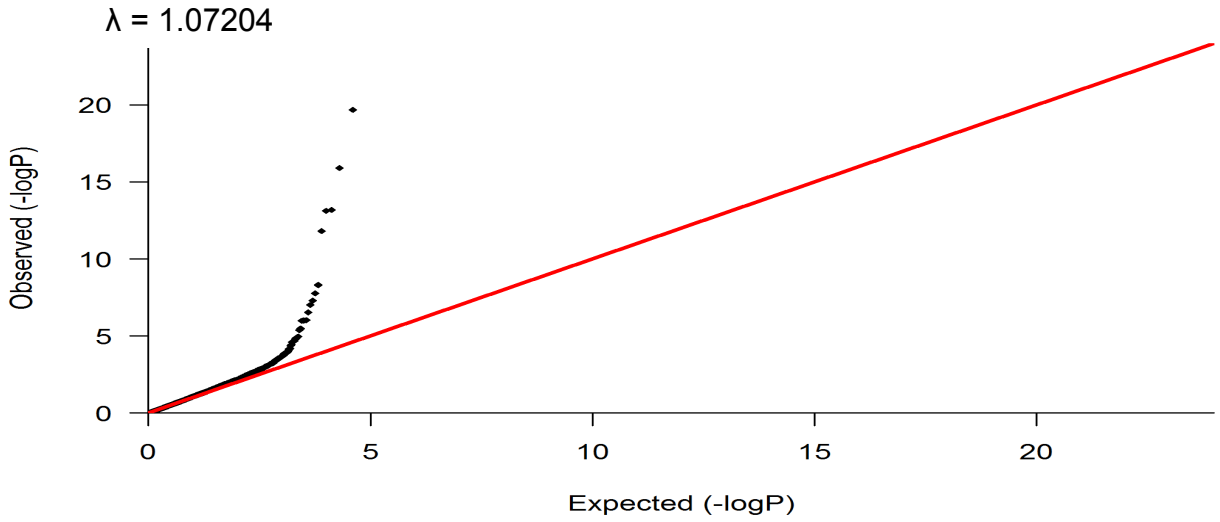

Figure 5: QQ-plot for direct gestation length after correction

Afterwards it was clear that chromosome 21 is the most important one for the direct gestation length, and chromosome 4 also shows a peak.

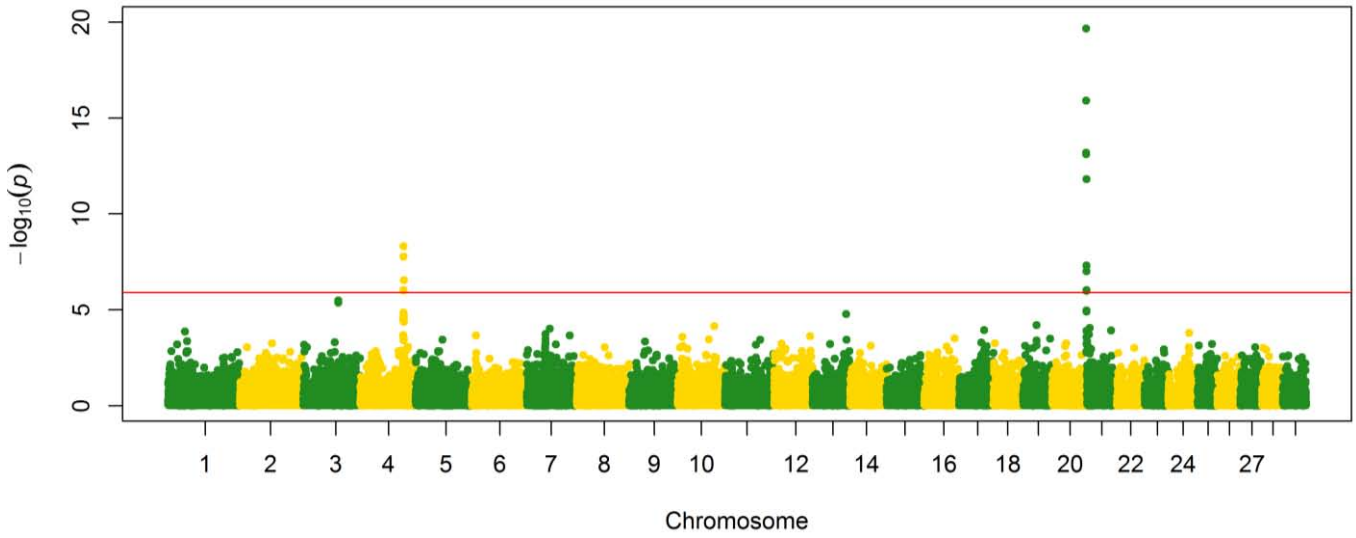

Figure 6: Significance levels (minus-log-P-values) for direct gestation length after correction

With the Bonferroni threshold, as indicated in Figure 6, 14 SNPs came up as significant, whereby with the q-value approach 23 SNPs deemed significant even if only 3 of them are located near significant genes for the trait, namely PDE4B, CD40 and MMP9.
The most significant SNP was ARS-BFGL-NGS-53975 on chromosome 21.However in an area of 500 000 base pairs to the left and to the right of this SNP no significant genes for gestation length have been found until now. Yet, near this position lies the region for Prader-Willi-Sydnrome which is a multisystem disorder that enlarges the risks for assisted birth´ (Cassidy et al., 2012). In a direct sense this is not connected to gestation length. Since it was a purpose of the study to find out the common genes

for various traits, this might be important because this region was also found to be associated with calving ease and stillbirth.

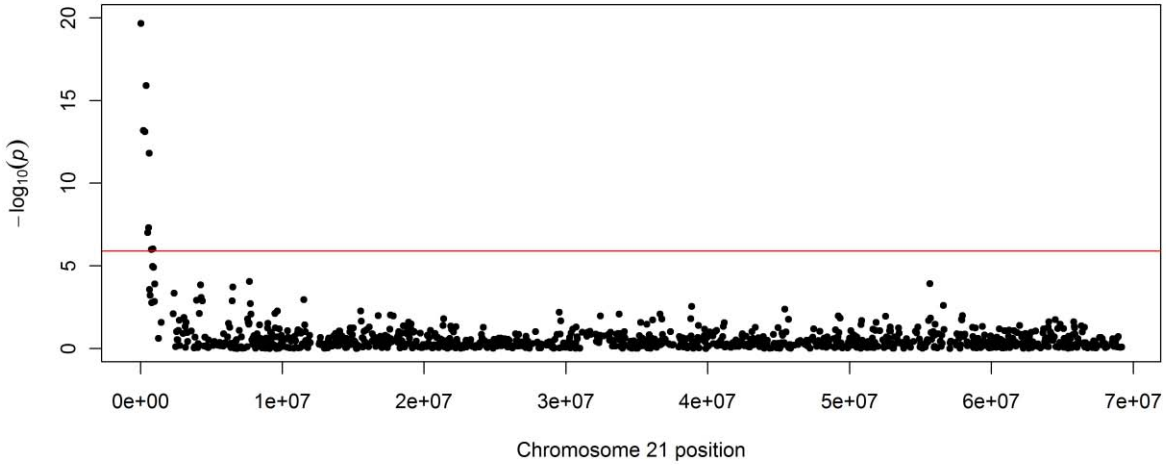

Figure 7: Chromosome 21 (direct gestation length)

It is interesting that within the region covered by the 11significant SNPs on chromosome 21 only three genes had been detected, with no direct funcitional evidence related to the trait. Only by looking at the correlated traits like calving ease those genes seem to be important.
Chromosome 4 also shows a peak above the Bonferroni line, but does not contain any significant genes either. Maltecca et al (2011) found some significant regions for gestation length on this chromosome as well, but these were on different positions.

Like mentioned earlier only a few genes had been detected which are important for gestation length through this analysis. One of them was PDE4B which is inside one mega base of two SNPs, namely BTB-01071906 and BTA-107777-no-rs on chromosome 3. This gene takes part in inflammation, likely for those in the uterus during pregnancy which can cause preterm delivery (Schmitz et al., 2007) and affect gestation length negatively in this way.
Another important SNP that is situated on chromosome 13 is ARS-BFGL-NGS-112051, in which area two genes are situated. CD40 is one of several genes that are up-regulated by the nuclearfactor-κB during the onset of labour (Khanjani et al., 2011). In this sense this gene plays an indirect role for gestation length.

Another important gene around one mega base of ARS-BFGL-NGS-112051 is MMP9. This is involved in the degradation of membranes and extracellular matrix components. Furthermore increased levels can lead to preterm birth because of premature rupture of membranes. In any case this gene shows a fetal increase during the time of parturition (Crider et al., 2005).

3.1.2 Maternal gestation length

For this part of the trait a big deviation between observed and expected p- value before the correction appeared, similar to that seen for the direct effect.

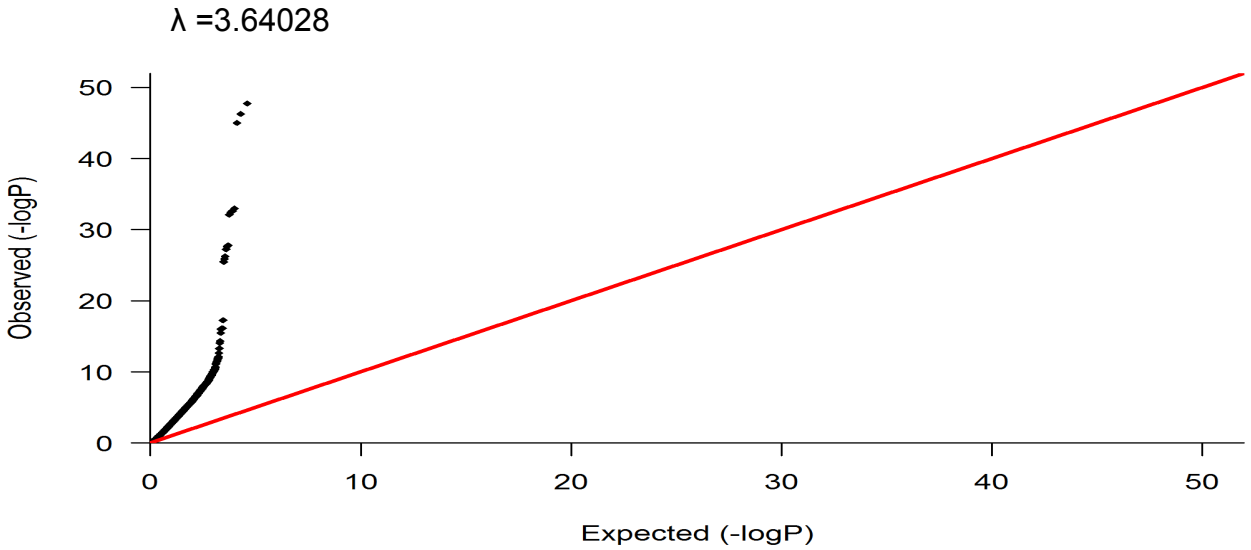

Figure 8: QQ-plot for maternal gestation length without correction

Additionally the Manhattan plot showed as well a peak on chromosome 21.

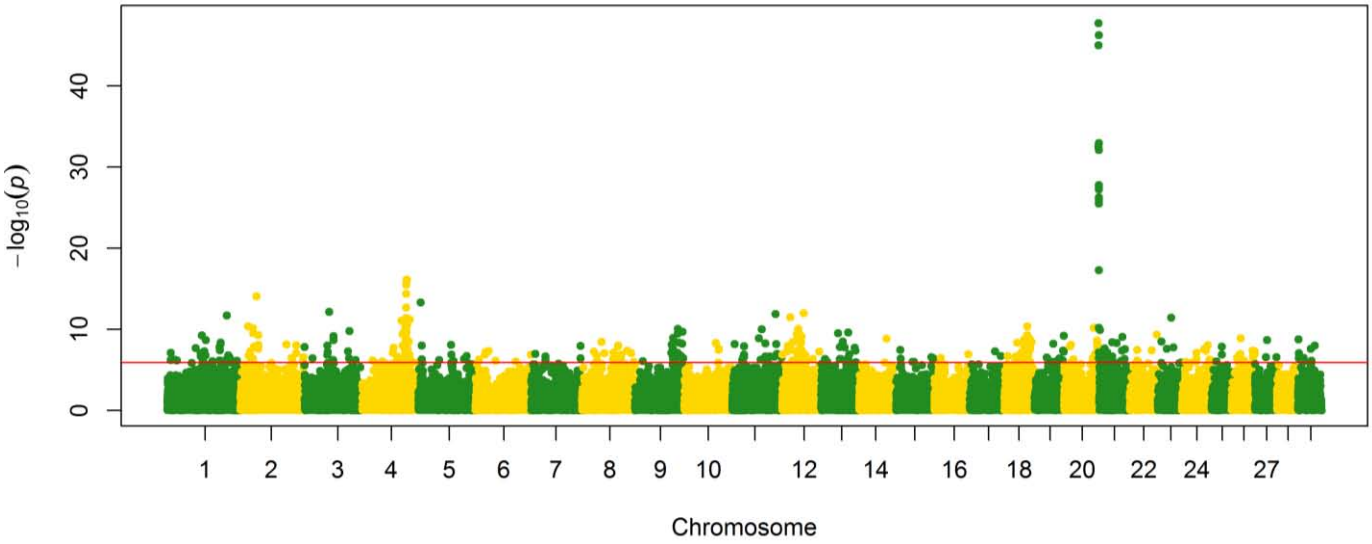

Figure 9: Significance levels (minus-log-P-values) for maternal gestation length without correction

The correction procedure was performed and delivered similar results to those for the direct part of the trait. At can be seen in Figure 10, the deviation between observed and expected p-values has been reduced.

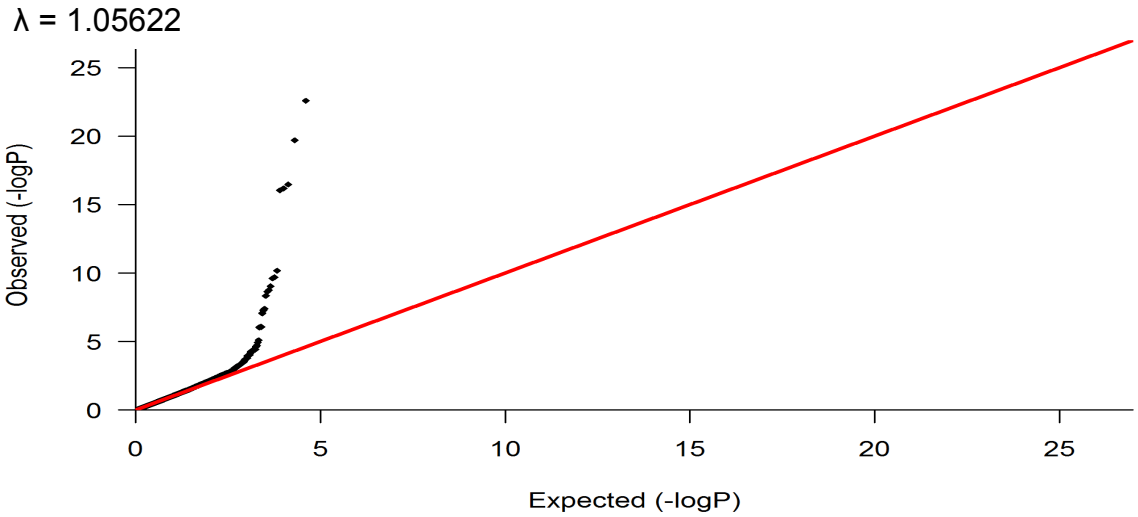

Figure 10: QQ-Plot for maternal gestation length after correction

The peak on chromosome 21 remained was seen as well, while the peak on chromosome 4 nearly disappeared. This time only 18 SNPs were discovered above the Bonferroni threshold, whereby 15 of them were situated on chromosome 21, and the rest on chromosome 11. On chromosome 21, 11 of those markers were the same as those for the direct part of the trait.
However, for all those markers no significant genes could be found, but here as well the correlated traits should not be disregarded.

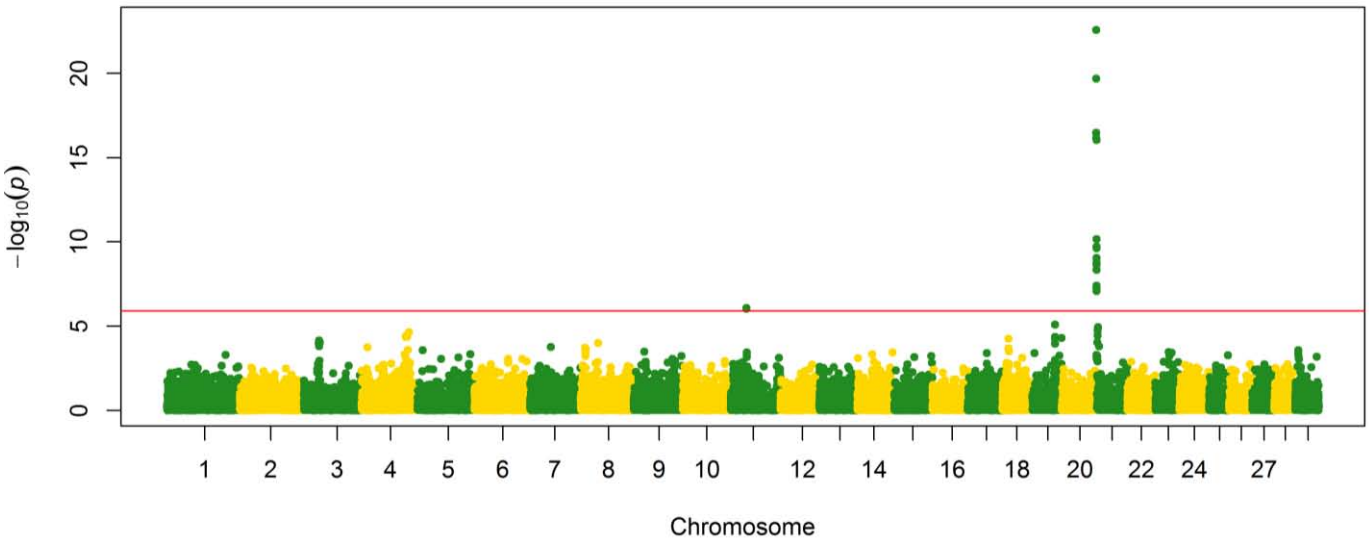

Figure 11: Significance levels (minus-log-P-values) for maternal gestation length after correction

Applying the false discovery rate, four additional markers were identified on chromosomes 4, 19 and 21.

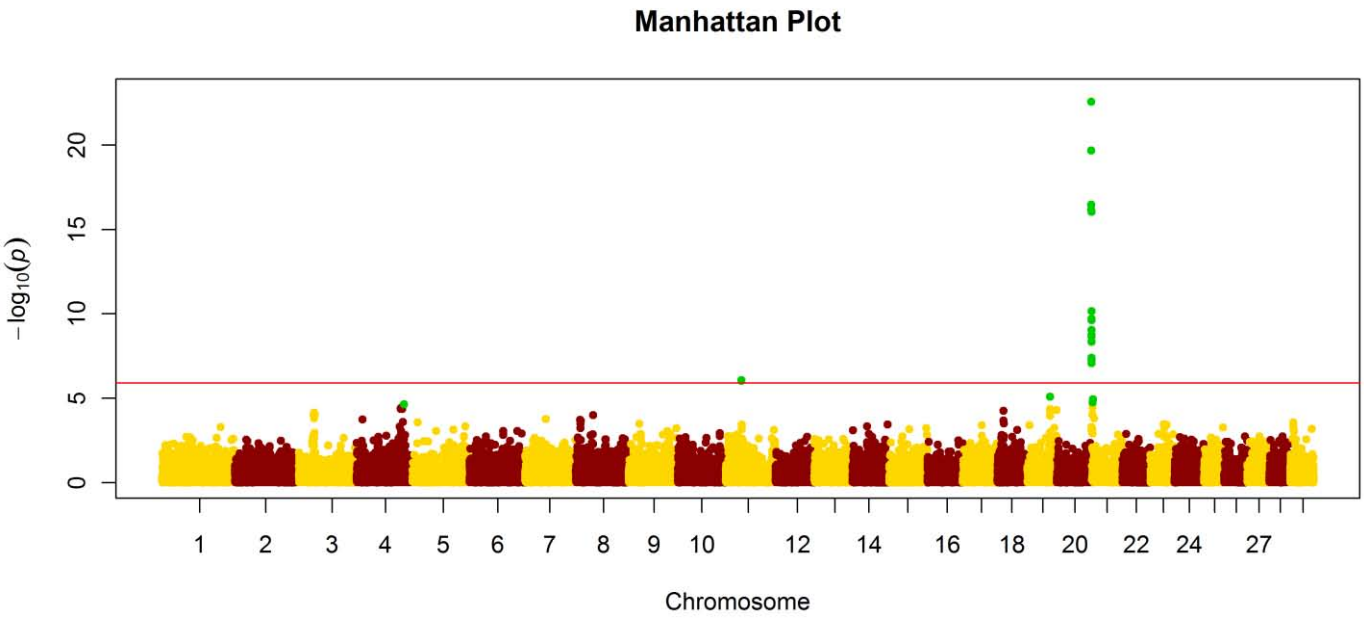

Figure 12: Significance levels (minus-log-P-values) for maternal gestation length (q-value approach)

A gene search evidenced only one SNP linked to important genes for the maternal gestation length.
This was Hapmap49721-BTA-71904 on chromosome 4 which is situated in a surrounding area of two important genes, namely MKRN1 and MRPS33, even if they are on different directions of the marker. The first one is on a base position from 104,662,859 to 104,680,302 and is up-regulated in cord blood during labor (Peng et al., 2011). The other one is situated from 105,101,880 to 10,5110,529 base pairs and is down-regulated in the placenta during term in comparison to mid-gestation (Winn et al., 2007). Indeed it has no direct effect on the gestation length because it is necessary for protein biosynthesis. However, one could conclude that an overexpression prevents the onset of labor.

After this analysis we can support Hansen et al (2004) who stated that both calf and cow are affecting parturition genetically.

Especially on chromosome 21 there is a significant association because there are markers for both parts of the trait, but no previous described genes have been found yet. Additionally the two correlated traits stillbirth and calving ease showed peaks at the same region too. This is not surprising since those traits show genetic correlation even if it is only weak (Hansen et al., 2004).

3.2 Calving ease

3.2.1 Direct calving ease

The first run of the genome wide association study was like for gestation length a single marker regression without correction. A lot of SNPs were above the Bonferroni correction as well, but two peaks were already there on chromosome 14 and once more 21.

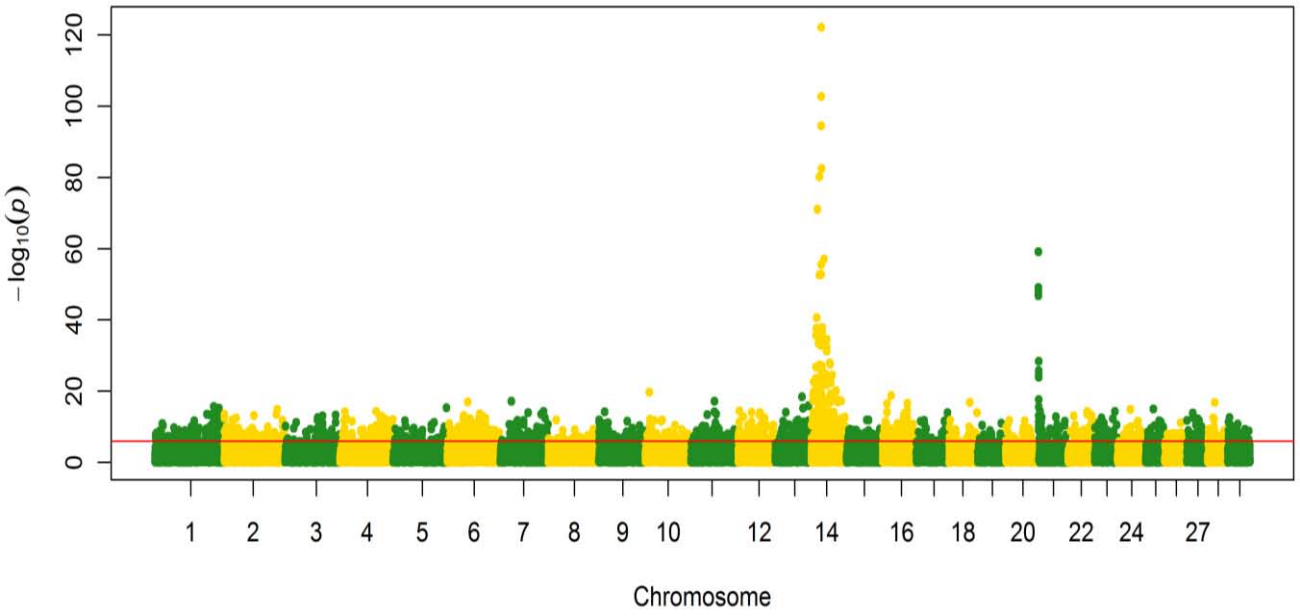

Figure 13: Significance levels (minus-log-P-values) for direct calving ease before correction

However, on the QQ-plot it can easily be seen on the big deviation of the observed and expected negative log of the p- values, that the population substructure was not considered.

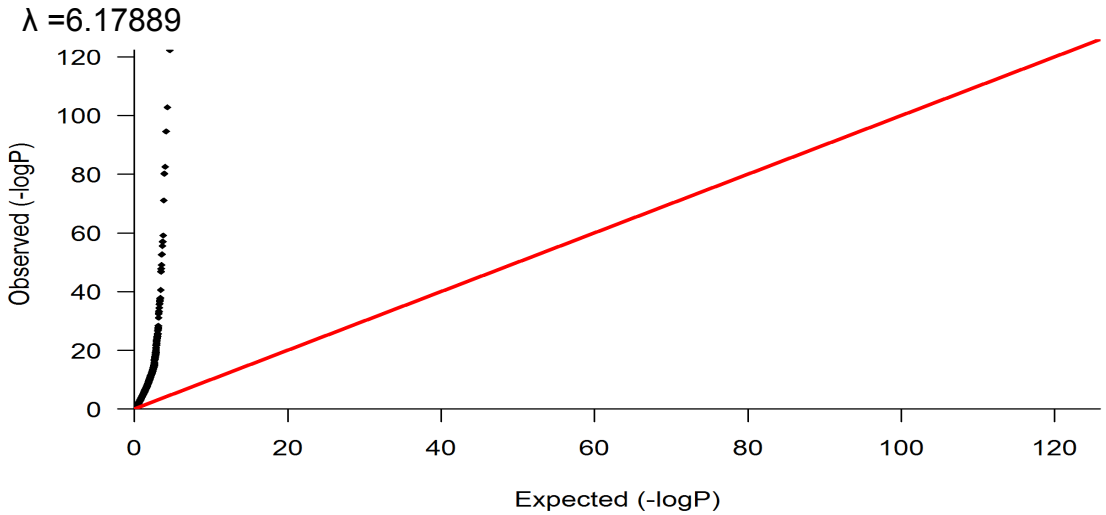

Figure 14: QQ- Plot direct calving ease before correction

Because of this big deviation, a correction for population structure was implemented through a calculation of eigenvectors again. They were then used for a correlation test, followed by a weighting procedure for the most correlated ones.
After all those steps the big deviation could be reduced (λ= 1.25492) but the peaks on chromosome 14 and 21 remained.
By using the Bonferroni threshold 37 markers came up significant. Only one marker was belonging to another chromosome, in particular it was chromosome 25.

With the q-value threshold of the false discovery rate, 84 SNPs from 16 different chromosomes were found as significant.

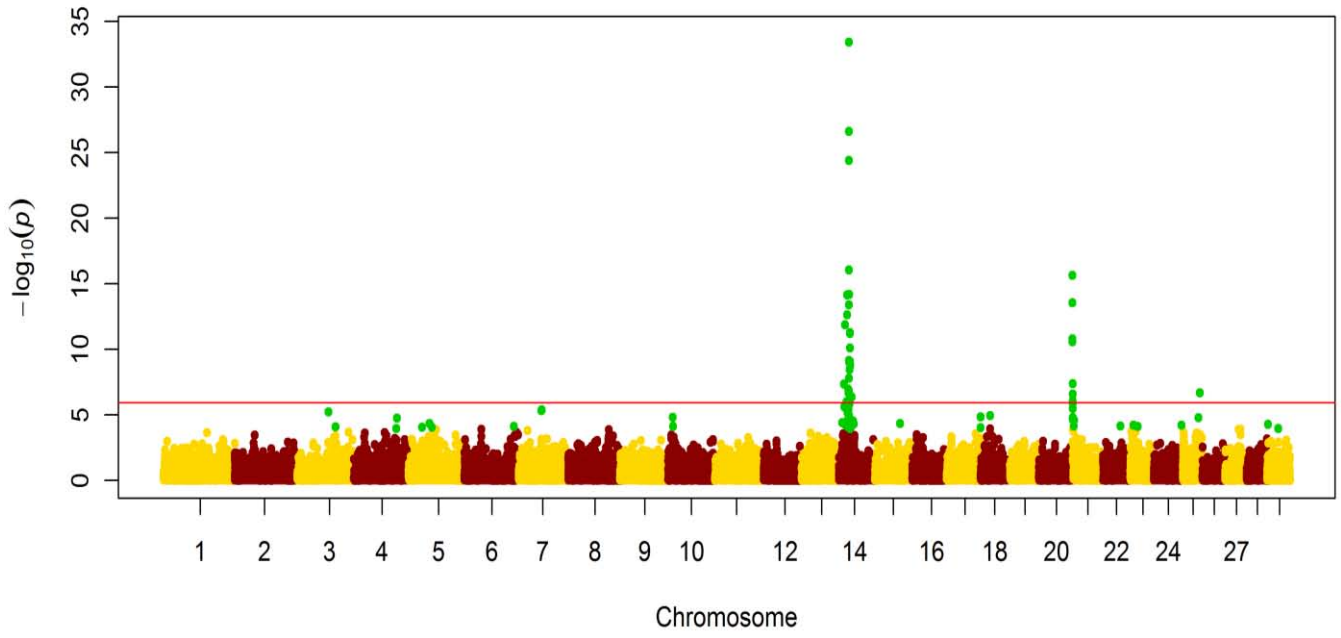

Figure 15 : Direct calving ease after weighted analysis
green dots indicate SNPs passing the q-value threshold

The region with the highest peak was on chromosome 14 within a base-pair position from 7,163,830 to 33,563,197. Around this area 28 (46 for q-value threshold) markers deemed significant and with around 114 genes in the region, but only a few of them showed a connection with calving ease.

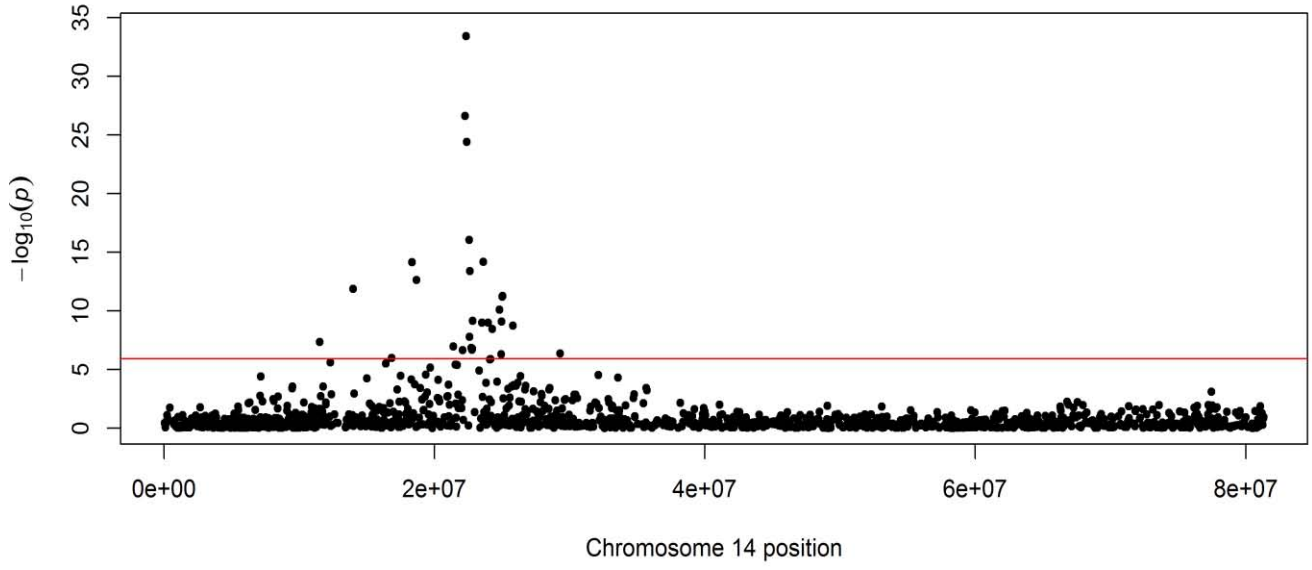

Figure 16: Chromosome 14 (Calving ease direct)

The most significant marker was BTA-91250-no-rs on base position 22,346,858, which explains 20.09 % of the additive genetic variance. This and all other markers with have significant associations can be seen in Table 2. Interestingly most of the markers on chromosome 21 were the same as for gestation length, so it seems like mentioned earlier that at least at this chromosome the traits are inherited together.

Around 1 MB of the most important position three genes were detected, but only one of those had been described earlier in cattle, namely SNTG1.

Mészáros et al. (2014) described SNTG1 as one of the most important genes for longevity in Fleckvieh cattle as well as the DERL1 gene, which has also been detected by this search.
Fürst and Fürst-Waltl(2006) also stated a small but positive correlation between those traits for Fleckvieh cattle. One reason for this could be the lower growth and health performance of dystocial calves (Barrier et al., 2013), as well as a lower milk yield (Eaglen et al., 2011) which could lead to an earlier culling.
The same applies for the CSPP1 gene, which has been described to play an important role in productive life of Holstein cattle (Cochran et al., 2013).

For chromosome 14, more than 10 other genes were detected to be connected with direct calving ease.

So CHCHD7, MOS, LYN, PLAG1, PENK, RPS20, SDR16C5, XKR4 and ENSBTAG00000039031were mentioned by Utsunomiya et al (2013) as influencing genes for cattle height and birth weight. Additionally some of them plus TGS1 have as well been described by Pausch et al (2011) in their study about calving ease in German Fleckvieh cattle. Since birth weight and calving ease are correlated with each other (Jamrozik and Miller, 2014), this detection is not surprising.

However the CRH gene has been described to play an important role during physiological parturition. If its serum values are high, pregnant women are more prone to a preterm birth (Petraglia et al., 2010). As this ends with lower birth weight (Wulf, 1997), one could conclude that the delivery is therefore also easier.

CHR	SNP	BP	A1	P	BETA	MAF	Variance
14	BTA-91250-no-rs	22346858	A	3,91E-31	-3,4390	0,0970	-20,09
14	ARS-BFGL-NGS-104268	22260373	A	2,51E-24	-2,8770	0,1111	-18,94
14	BTB-01417924	22382727	G	4,25E-22	-2,6310	0,1229	-18,91
14	Hapmap59686-rs29020689	22563086	A	9,28E-14	-1,8810	0,1574	-16,63
21	ARS-BFGL-NGS-108925 ✳	390041	A	2,36E-13	1,4590	0,3180	21,09
14	ARS-BFGL-NGS-28867	18338297	G	7,38E-12	-2,2170	0,0958	-12,81
21	ARS-BFGL-NGS-53975 ✳	28424	G	2,85E-11	1,3950	0,2820	18,83
14	ARS-BFGL-NGS-112623	18683217	A	2,40E-10	-1,5590	0,1876	-15,84
14	Hapmap27935-BTC-065354	25031801	A	5,53E-09	-1,4890	0,1780	-14,52
14	Hapmap23524-BTC-065402	25006866	A	6,37E-09	-1,4840	0,1781	-14,48
21	ARS-BFGL-NGS-114372 ✳	312143	C	1,69E-08	1,3070	0,2401	15,90
21	Hapmap52072-rs29018920 ✳	205438	A	2,79E-08	1,2840	0,2416	15,68
14	Hapmap23172-BTC-011263	24817953	A	8,31E-08	-1,3780	0,1891	-14,09
14	Hapmap31672-BTC-065429	24968796	A	8,36E-07	-1,4520	0,1466	-12,11
14	Hapmap39876-BTA-97370	23975651	C	1,03E-06	-1,1570	0,2518	-14,53
14	Hapmap24928-BTC-010710	25801743	A	1,87E-06	-1,1740	0,2385	-14,21
14	BTB-01143648	24287029	A	3,77E-06	-2,6400	0,0359	-6,09
21	ARS-BFGL-NGS-10260 ✳	616016	G	4,42E-05	0,9347	0,3905	14,83
14	ARS-BFGL-NGS-75663	21389408	A	1,15E-04	-0,8757	0,4771	-14,56
25	Hapmap40770-BTA-60247	38713017	A	2,20E-04	1,1210	0,1851	11,27
14	ARS-BFGL-BAC-8052	22096519	A	2,25E-04	-3,0780	0,0202	-4,06
21	ARS-BFGL-NGS-60326 ✳	761674	G	2,64E-04	0,9222	0,3278	13,55
14	UA-IFASA-7902	24933302	A	5,09E-04	-0,9988	0,2294	-11,77
21	ARS-BFGL-NGS-92774 ✳	866252	G	8,82E-04	0,8594	0,3474	12,99
21	ARS-BFGL-NGS-31507 ✳	518992	A	1,15E-03	1,0220	0,1908	10,52
21	ARS-BFGL-NGS-70221	677218	G	1,33E-03	0,8088	0,4960	13,48
14	Hapmap31956-BTC-054628	24153510	G	1,35E-03	-0,9187	0,2605	-11,80
14	ARS-BFGL-NGS-8308	24100495	C	1,48E-03	-0,7942	0,4807	-13,22
21	Hapmap47036-BTA-52529	789899	G	3,52E-03	0,7760	0,4886	12,93
14	UA-IFASA-6711	21540829	G	3,95E-03	-0,7789	0,4158	-12,61
14	UA-IFASA-7382	21668492	G	4,29E-03	-1,1610	0,1246	-8,44
14	UA-IFASA-6634	19683618	A	7,10E-03	-1,5120	0,0643	-6,07
10	ARS-BFGL-NGS-99907	10871878	A	1,64E-02	0,9924	0,1570	8,76
21	ARS-BFGL-NGS-37987 ✳	913751	G	1,74E-02	0,7567	0,3312	11,17
21	ARS-BFGL-NGS-73082 ✳	568738	A	2,34E-02	0,7611	0,3078	10,81
14	ARS-BFGL-NGS-28234	19361956	A	2,79E-02	-1,3600	0,0705	-5,94
14	Hapmap40715-BTA-34541	32119820	A	3,05E-02	-1,3610	0,0717	-6,04
14	UA-IFASA-6489	17495634	A	3,59E-02	-0,7198	0,3619	-11,08
14	UA-IFASA-7599	33563197	A	5,03E-02	-0,8276	0,2093	-9,13
23	ARS-BFGL-NGS-101416	6872244	G	6,52E-02	-0,6667	0,4278	-10,88
14	Hapmap47925-BTA-36060	18288065	A	7,20E-02	-0,8196	0,2095	-9,05
23	Hapmap46306-BTA-55660	16894989	T	7,92E-02	0,6529	0,4702	10,84
10	ARS-BFGL-NGS-105394	11991392	G	8,17E-02	0,7017	0,3297	10,34
5	Hapmap39353-BTA-73120	30500363	C	8,86E-02	0,8522	0,1750	8,20
14	ARS-BFGL-NGS-35159	24641762	C	1,12E-01	-0,7068	0,2846	-9,59

Table 2: Associated markers for calving ease direct ordered after p-values

✳ same markers as for gestation length

The peak on chromosome 21 was at the beginning between the markers ARS-BFGL-NGS-53975 at base-pair position of 28,424 and ARS-BFGL-NGS-92774 on base position 866,252 containing altogether 8 SNPs with 3 connected genes.
The q value threshold identified more SNPs up to a base position of 4,359,465, although there were no more relevant genes within this region.

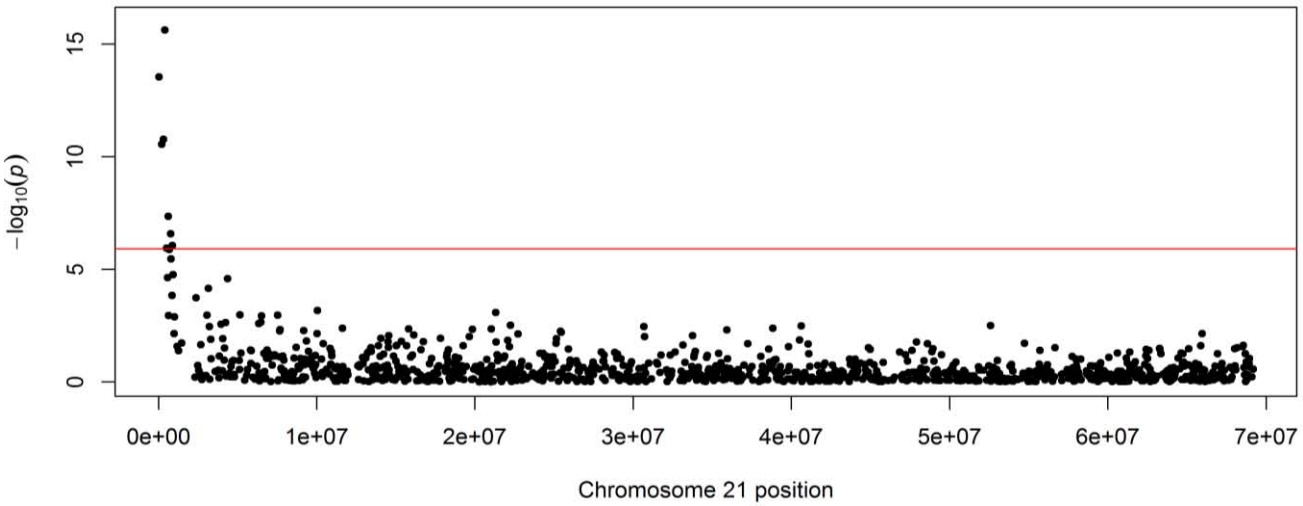

Figure 17: Chromosome 21 (direct calving ease)

The three genes contained SNRPN, MAGEL2 and NDN which are all inside the region for Prader-Willi-Syndrome in humans. This disease causes decreased fetal movement, lower birth weights, abnormal fetal position at delivery and an increased incidence of assisted deliveries and caesarean sections (Cassidy et al., 2012) which has as well been detected by Pausch et al (2011) for calving ease in Fleckvieh cattle.

The only SNP above the Bonferroni line on another chromosome than 14 and 21 was Hapmap40770-BTA-60247 on chromosome 25 on a base position of 38,713,017kb. Nearby this SNP lays the CYTH3 gene which shows a decrease in expression during complicated pregnancies (Kanamarlapudi et al., 2012).

Through the q-value threshold 16 other chromosomes showed significant markers, but only four of them included genes associated to calving ease. These are AQP5, CUL7, PAPD4, PTGER2 and SLC39A7.

AQP5 lies on chromosome 5 in cattle and has been described to be down-regulated during parturition in mice (Helguera et al., 2009). If this regulation does not work well, the process of delivery could be affected.

Hansen et al (2004) describes a strong correlation between calf size and calving ease. Therefore the CUL7 gene on chromosome 24 could have influence in the realization of the trait, because it is associated with the 3-M- Syndrome, a disorder that causes lower birth weights and statures in human (Meazza et al., 2013).

Similar to this PAPD4 on chromosome 10 (cattle), is associated with long bone length in mice, which causes a bigger stature (Kenney-Hunt et al., 2006). This could influence the calving ease in the other direction, making it more difficult.

The PTGER2 on chromosome 10 is like the CRH associated with preterm birth (Ryckman et al., 2010), which results in smaller infants too.

We may conclude that direct calving ease is influenced by many genes, but most of them are on chromosomes 14 and 21 and are mostly correlated with height and weight in cattle as well as in human.
Additionally, the two traits calving ease and gestation length seem to be partly inherited together at least for those genes on chromosome 21, which is not surprising given the strong genetic correlation of those traits (0,508 ±0,095) (Sattlecker, 2014).

3.2.2 Maternal calving ease

For the maternal part of the trait, nearly the same big deviation (λ = 11.3122) between observed and estimated p- values has been observed for the uncorrected analysis.

However the distribution on the Manhattan plot was different to the direct trait, because no real peaks appeared and a lot of SNPs were above the Bonferroni line.

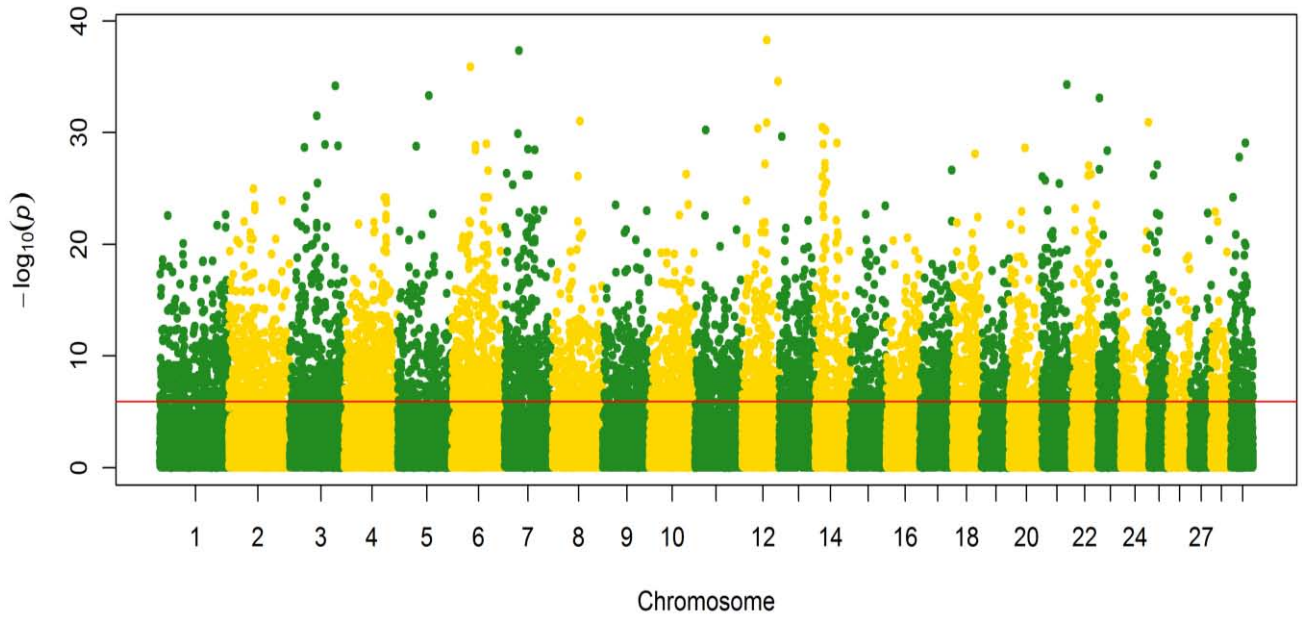

Figure 18: Significance levels (minus-log-P-values) for maternal calving ease without correction

With the weighted analysis the deviation is reduced. Through the correction process a lot of significant markers disappeared and two peaks stayed.

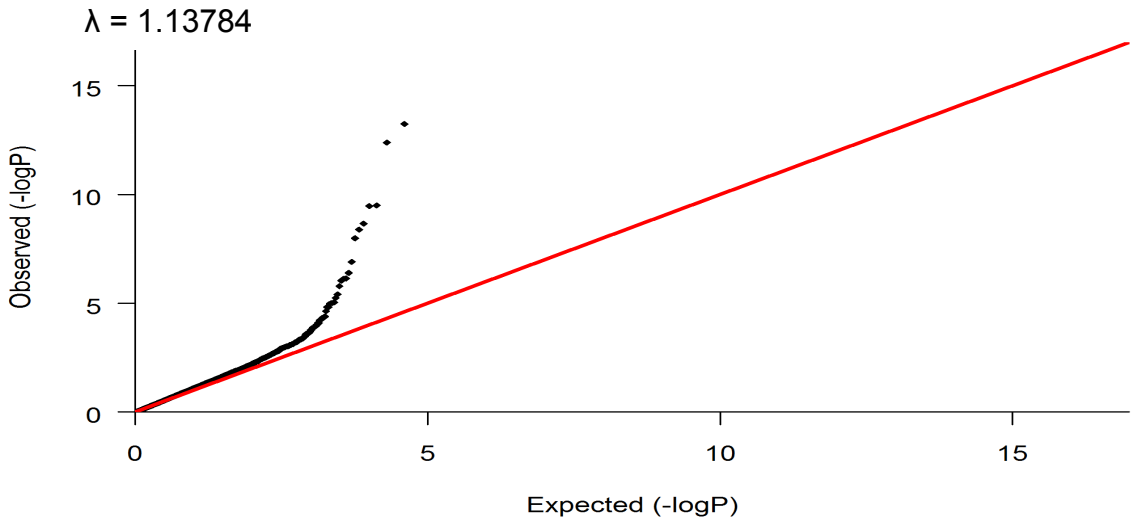

Figure 19: QQ - Plot after for maternal calving ease correction

Like in the direct trait those peaks were on chromosomes 14 and 21, but in contrast the highest p-value was for a marker on chromosome 21(Table 3).
In this trait the Bonferroni and q-value approaches brought different numbers of significant markers too. As can be seen in Figure 20 only 12 markers were above the Bonferroni line. However, within the q-value threshold 22 SNPs were detected.

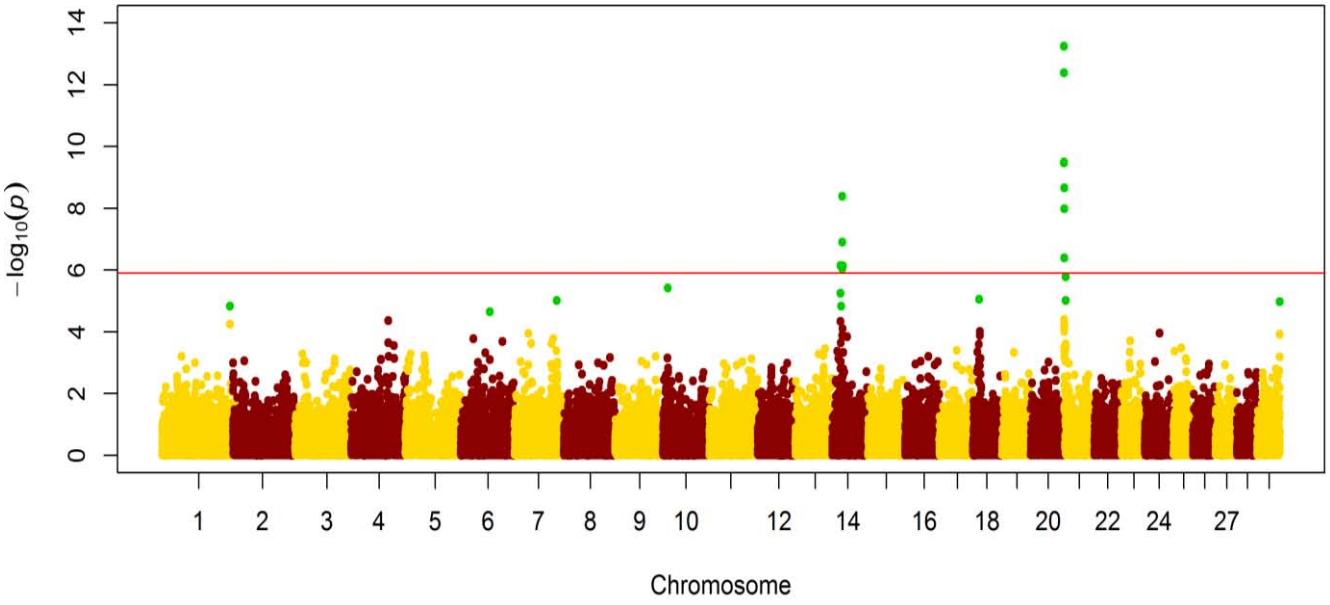

Figure 20: Significance levels (minus-log-P-values) for maternal calving ease after weighted analysis

The most significant SNP is ARS-BFGL-NGS-108925 on base position 390,041 kb and explains an additive genetic variance of 28.05%. This marker and several more are the same ones as those significant for direct calving ease as well as direct gestation length.

Only three markers on chromosome 21 were different to direct calving ease, namely BTA-54983-no-rs, BTA-52244-no-rs, ARS-BFGL-NGS-115386, although in their surrounding area no relevant genes have been found.

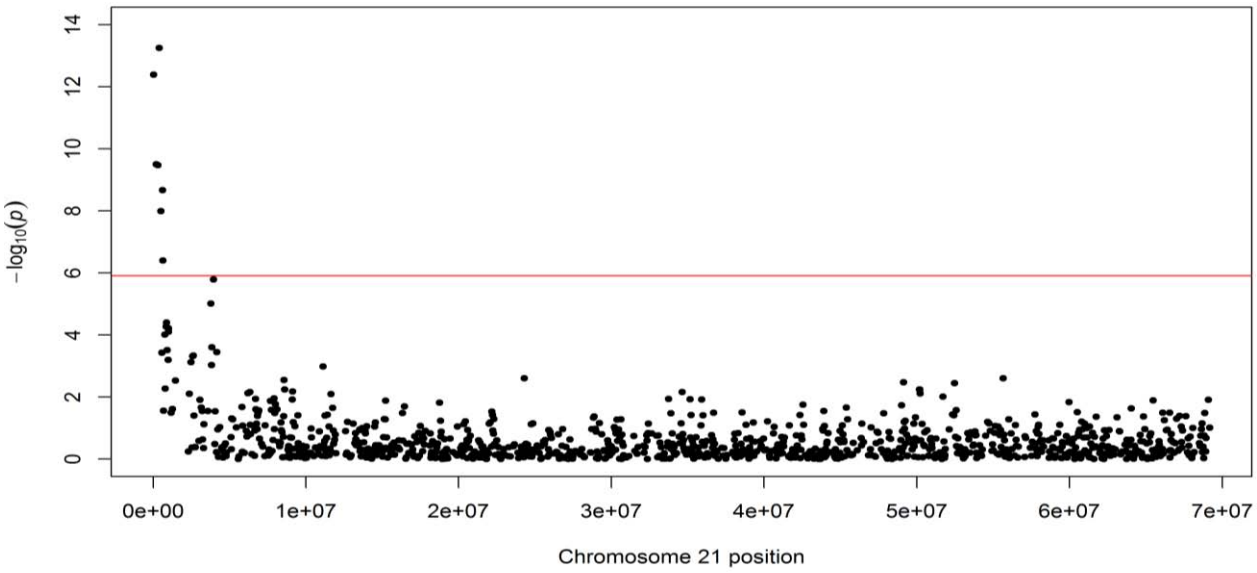

Figure 21: Chromosome 21 (maternal calving ease)

The same applies for chromosome 14, where all detected markers (7) were the same as during the search for direct calving ease. Therefore maternal calving ease is mostly affected by the same genes as the direct calving ease in Fleckvieh cattle.

Cole et al. (2011, 2009) came to similar results: at least one significant SNP on BTA 18 in Holstein cattle is affecting both, direct and maternal calving ease.
In contrast to these findings Olsen et al. (2010) found no QTLs which influence both components of the trait.
So it seems that calving ease is differently inherited in various breeds.

The correlation of those traits has been identified to differ too. So described Cue and Hayes (1985) as well as Eaglen et al (2012) a negative correlation, which would suggest that a calf which is born easier tends to have more complicated births itself and vice versa. Therefore one could conclude that different genes are responsible for the inheritance of these traits. On the other hand Hansen et al (2004) found a positive correlation of these traits.

Only six markers were significant on other chromosomes than 14 and 21, whereby only Hapmap42446-BTA-118372 on chromosome 18 brought an important gene for parturition to light. ANKRD11is associated with an increase in birth weight in humans, and as well with the KBG syndrome, which causes a smaller stature (Engel et al., 2014).
All important markers for this trait are indicated in Table 3.

CHR	SNP	BP	A1	P	BETA	MAF	Variance
21	ARS-BFGL-NGS-108925	390041	A	5,71E-11	-1,2930	0,3181	-28,05
21	ARS-BFGL-NGS-53975	28424	G	4,09E-10	-1,2870	0,2821	-26,06
21	Hapmap52072-rs29018920	205438	A	3,20E-07	-1,1720	0,2416	-21,47
21	ARS-BFGL-NGS-114372	312143	C	3,37E-07	-1,1780	0,2402	-21,50
21	ARS-BFGL-NGS-10260	616016	G	2,17E-06	-0,9880	0,3905	-23,52
14	BTA-91250-no-rs	22346858	A	4,10E-06	1,6130	0,0971	14,13
21	ARS-BFGL-NGS-31507	518992	A	1,03E-05	-1,1630	0,1908	-17,96
14	ARS-BFGL-NGS-104268	22260373	A	1,25E-04	1,3630	0,1111	13,46
21	BTA-54983-no-rs	637540	C	3,99E-04	-1,4330	0,0892	-11,65
14	ARS-BFGL-NGS-28867	18338297	G	7,25E-04	1,3670	0,0958	11,85
14	BTB-01143648	24287029	A	7,47E-04	2,1450	0,0359	7,42
14	BTB-01417924	22382727	G	9,05E-04	1,2110	0,1229	13,05
10	ARS-BFGL-NGS-105394	11991392	G	3,90E-03	-0,7952	0,3298	-17,58
14	ARS-BFGL-NGS-112623	18683217	A	5,61E-03	0,9360	0,1876	14,27
18	Hapmap42446-BTA-118372	14224859	A	8,91E-03	-1,0770	0,1265	-11,90

Table 3: Important Markers for maternal calving ease

3.3 Fertility disorders

For this trait only direct information was available. Like in all other traits the first run of the genome wide association study was done without a correction process. There was a deviation between observed and estimated -log p-values, even if not so big like for the traits presented before.

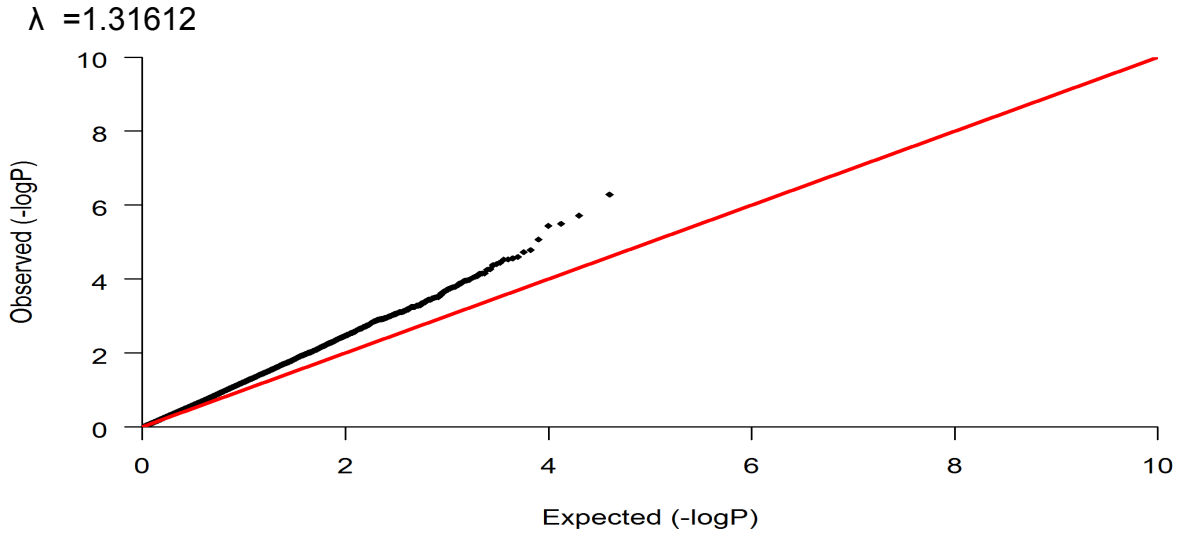

Figure 22: QQ-plot for fertility disorders without correction

The Manhattan plot (Figure 23) shows that the analysis without correction indicates only one marker above the Bonferroni threshold. This is potentially due to the lower number of animals in the study, or because of the composition of the trait that consists of three different diseases. A few other SNPs are near the significance threshold and on some chromosomes like chromosome 10 it seems that there could be a peak. However because of the deviation of the p-values a correction procedure was implemented anyway.

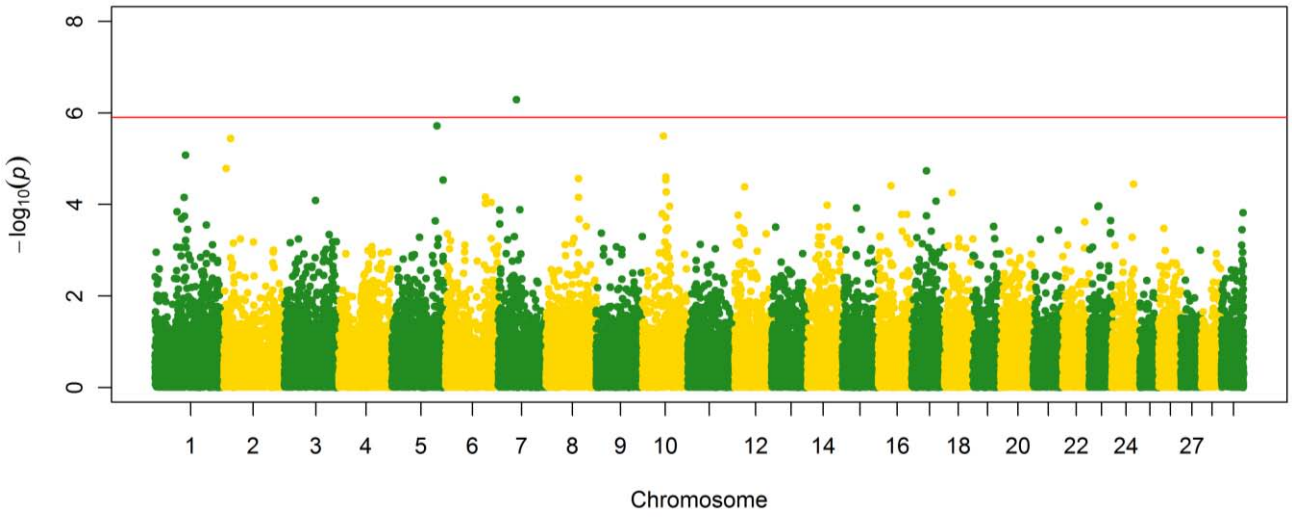

Figure 23: Significance levels (minus-log-P-values) for fertility disorders (without correction)

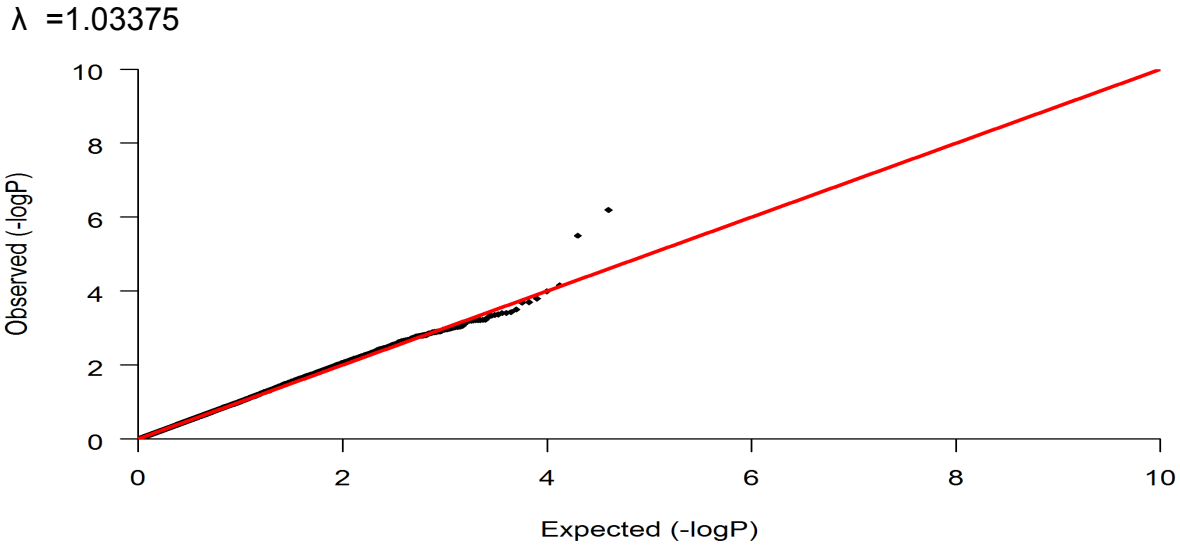

Figure 24: QQ-plot for fertility disorders after correction

The SNP above the significance threshold stayed significant while the peaks on the other chromosomes were less pronounced.

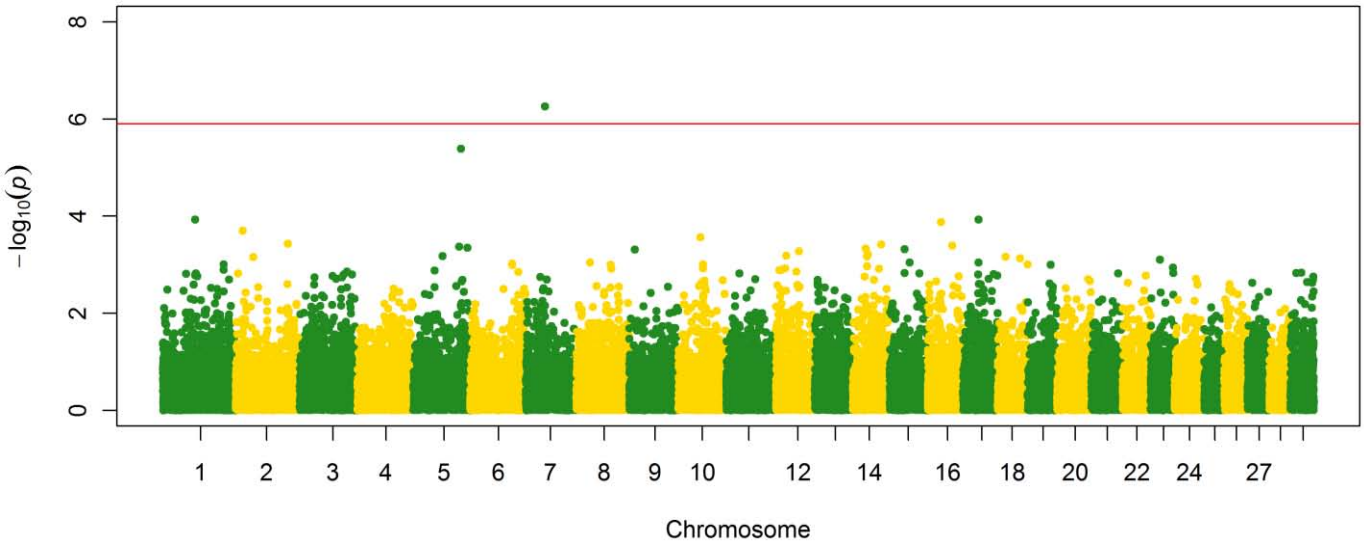

Figure 25: Significance levels (minus-log-P-values) for fertility disorders after correction

Search of genes did not indicate genes related to retained placenta, inflammation of the uterus nor puerperal disease near the significant SNP ARS-BFGL-NGS-79312 on chromosome 7 on base position of 42,695,382.Only one gene, OR2W3, could be interpreted as having a quasi related effect, because it is causing male infertility (Plaseski and Noveski, 2012).

Another few markers have been detected on chromosome 5, on which Olsen et al (2011) already found at least one relevant SNP for retained placenta. However their SNP was far away from those in this search, so no genes have been detected in a direct context.
The highest marker on this chromosome was Hapmap47511-BTA-114200 (on base-pair position of 105,350,648 base pairs) which includes the NTF3 gene. This one is down-regulated in the ectopic endometrium of women who suffer from endometriosis (Khan et al., 2012), which is defined by the presence of functional endometrial tissue outside the uterine cavity, usually located in the pelvis but can also be present in other regions (Bungum et al., 2014). So, in a direct manner this gene has nothing to do with endometritis or inflammation of the uterus.

Furthermore, a few more genes have been discovered in this search, which are associated with endometriosis. Those were MUC4 on chromosome 1 near Hapmap41227-BTA-32644 that shows an association with endometriosis development as well as with human infertility (C. Y. Chang et al., 2011), as well as ACTR6 on chromosome 5 (base position: 64,666,892) (McKean, 2006).
In summary no genes with a direct relevance for the three traits retained placenta, inflammation of the uterus and puerperal disease could be found. One reason for that could be the lower number of animals used in this study. More investigation with more data should be done in the future because those traits are very important for dairy cows, because of an increase of time open (Gilbert et al., 2005). An approach for this could be a splitting of data, as was done in this thesis for stillbirth in combination with gestation length because gestation length also affects the occurrence of retained placenta (Han and Kim, 2005; Sattlecker, 2014).

3.4 Stillbirth

3.4.1 Direct stillbirth

Direct stillbirth showed nearly the same results as direct calving ease. So the Manhattan plot without correction for population structure (Figure 26) showed a lot of markers above the Bonferroni threshold, even though two peaks were already coming up on chromosomes 14 and 21.

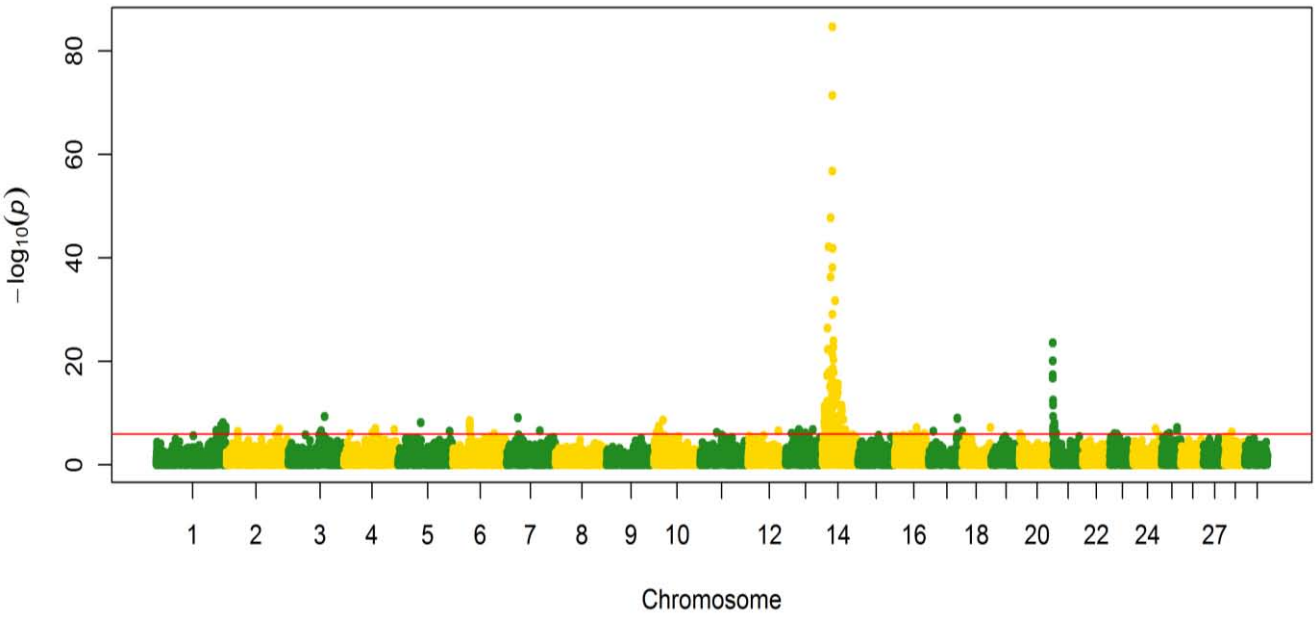

Figure 26: Significance levels (minus-log-P-values) for stillbirth direct without correction for population structure

Besides the deviation of the observed and expected p-value was very high too, although the genomic inflation factor (output after the weighted analysis in Plink) was lower than in calving ease (2.47132 compared to 6.17889).

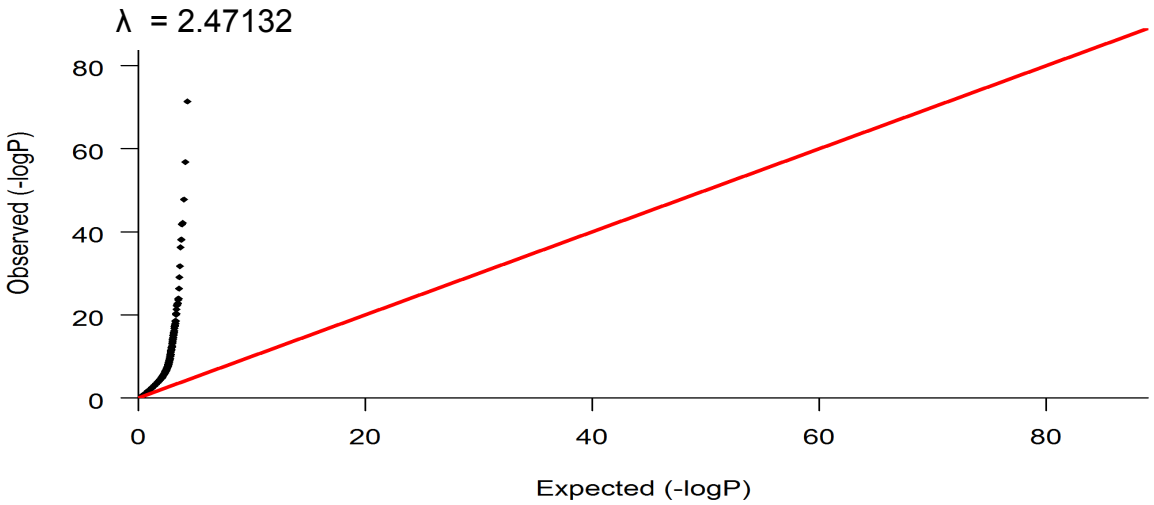

Figure 27: Q-Q Plot for direct stillbirth

Since this too much of a deviation the correction was performed. The peaks on the two chromosomes remained, with 39 markers above the Bonferroni line.

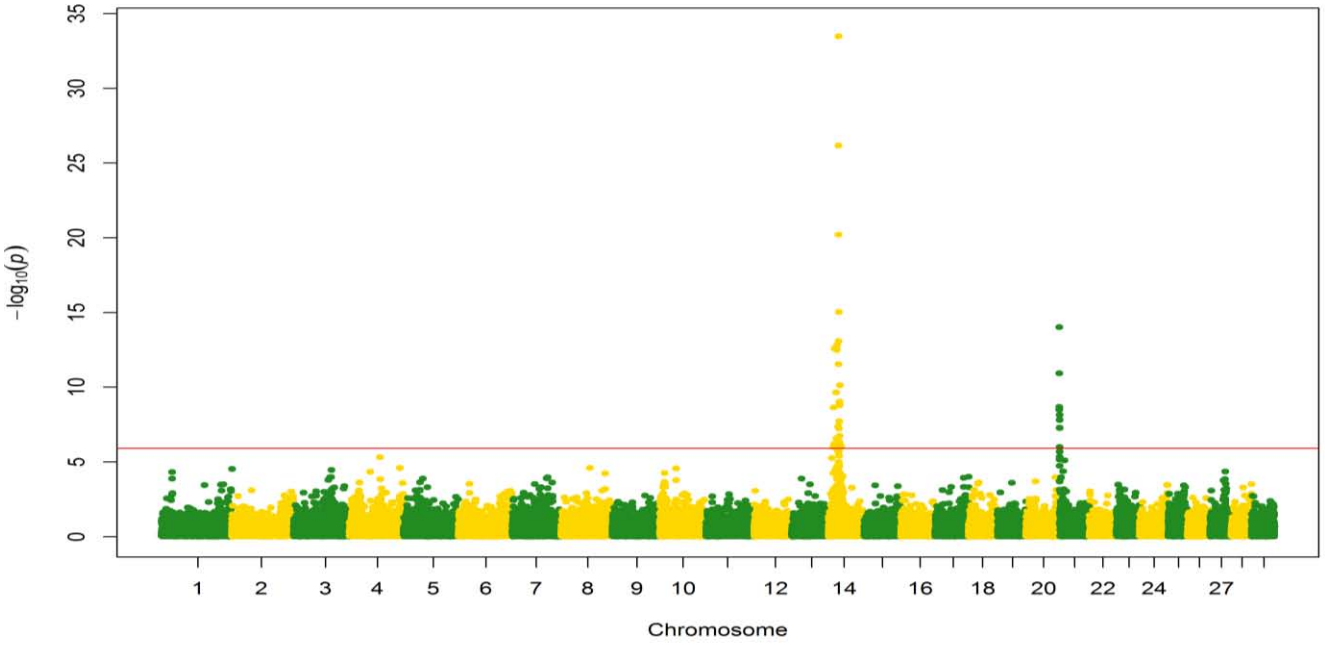

Figure 28: Significance levels (minus-log-P-values) for direct stillbirth after correction

Additionally, the q- values have been calculated and identified 79 significant markers from which 57 were discovered during the direct calving ease search too. For the gene search all of those SNPs have been used, those containing important gene regions are shown in Table 4.

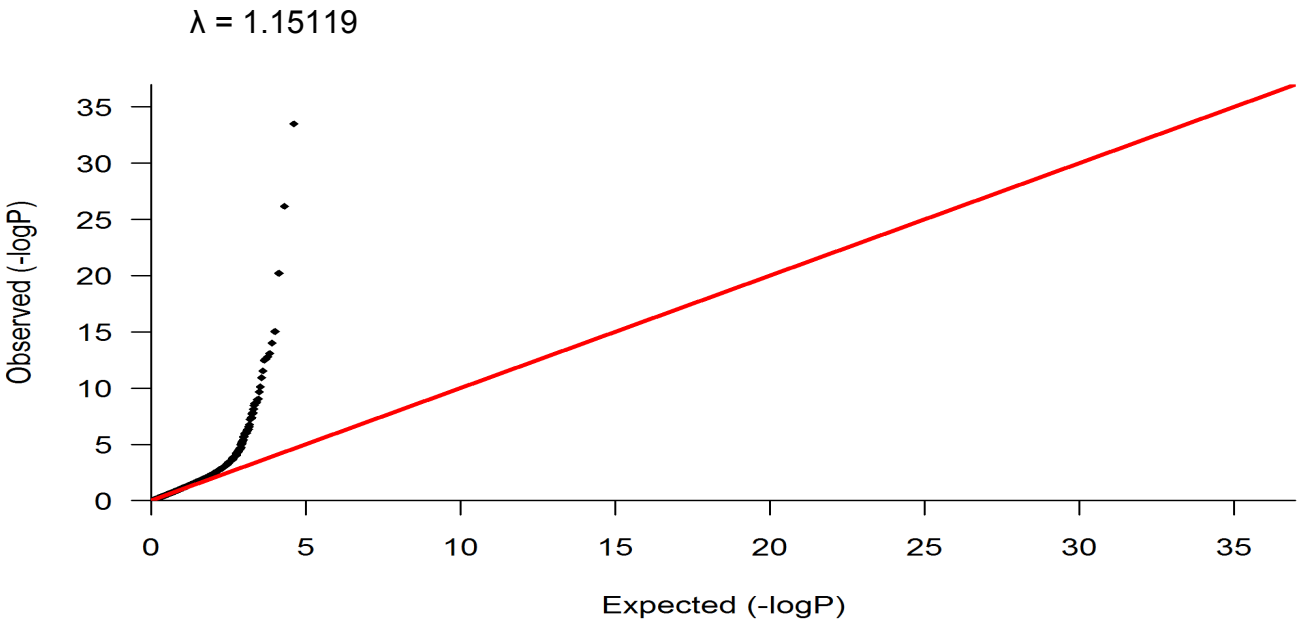

Figure 29: QQ-Plot for direct stillbirth after correction

On chromosome 14 only 6 markers were different to direct calving ease, although no significant genes have been found near their genomic region.

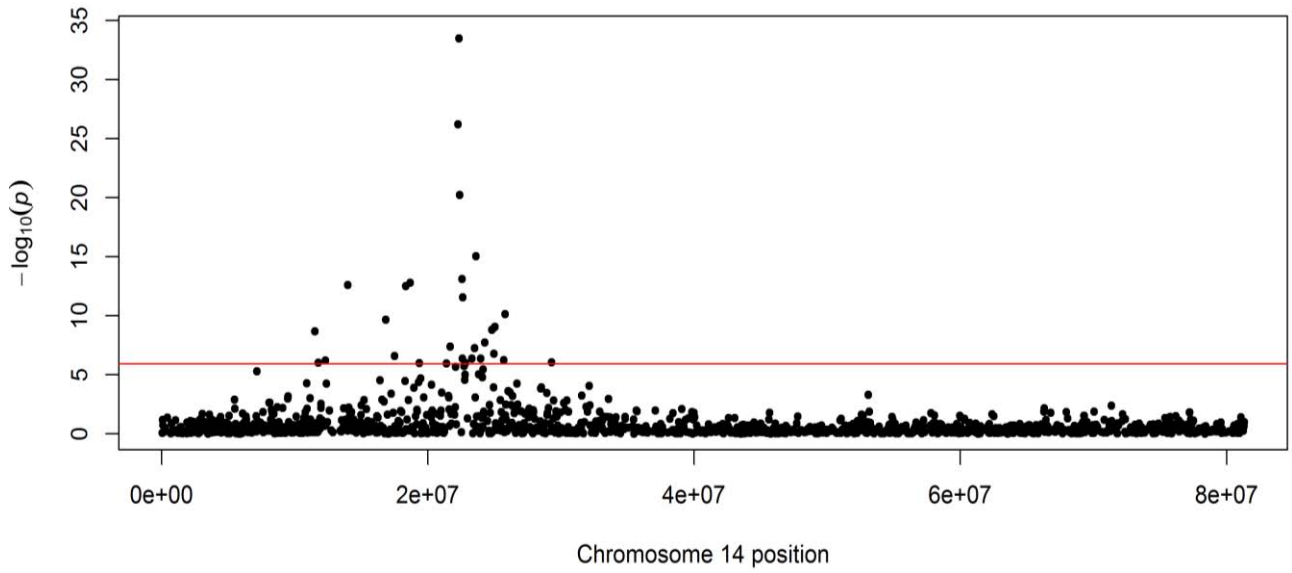

Figure 30: Chromosome 14 (Direct Stillbirth)

So, stillbirth and calving difficulty are inherited together and the same genes are responsible for them, at least on chromosome 14.
Hansen et al (2004) estimated genetic correlation between calving difficulty, stillbirth and calf size which showed that larger calves are more prone to difficult birth and have a higher risk of being stillborn.
Furthermore Olsen et al (2010) reported at least one QTL on chromosome 6 in the Norwegian Red breed to affect both direct calving difficulty and stillbirth.

CHR	SNP	BP	A1	P	BETA	MAF	Variance	Trait
14	BTA-91250-no-rs	22346858	A	3,31E-31	-4,1840	0,0970	-66,66	⁎+ x
14	BTB-01417924	22382727	G	6,21E-18	-2,9010	0,1229	-56,86	⁎+ x
14	Hapmap46735-BTA-86653	23614077	G	9,26E-13	-2,1200	0,1772	-56,20	x
14	Hapmap59686-rs29020689	22563086	A	8,43E-11	-2,0520	0,1574	-49,48	⁎
14	ARS-BFGL-NGS-112623	18683217	A	1,65E-10	-1,9070	0,1876	-52,84	⁎+
14	ARS-BFGL-NGS-28867	18338297	G	3,27E-10	-2,5220	0,0958	-39,73	⁎+ x
14	BTB-01532239	22634364	A	2,91E-09	-1,5190	0,3151	-59,60	x
14	Hapmap24928-BTC-010710	25801743	A	7,46E-08	-1,5440	0,2385	-50,99	⁎
14	Hapmap27935-BTC-065354	25031801	A	9,26E-07	-1,6080	0,1780	-42,78	⁎
14	Hapmap23524-BTC-065402	25006866	A	1,02E-06	-1,6030	0,1781	-42,66	⁎
14	Hapmap23172-BTC-011263	24817953	A	1,66E-06	-1,5540	0,1891	-43,33	⁎
14	BTA-35971-no-rs	11504490	A	2,24E-06	-4,1760	0,0215	-15,94	
14	BTB-01143648	24287029	A	1,91E-05	-3,0590	0,0359	-19,24	⁎+
14	UA-IFASA-7382	21668492	G	4,23E-05	-1,6810	0,1246	-33,34	⁎
14	Hapmap31672-BTC-065429	24968796	A	1,79E-04	-1,5000	0,1466	-34,12	⁎
14	UA-IFASA-6489	17495634	A	2,75E-04	-1,0870	0,3619	-45,64	⁎
14	Hapmap39876-BTA-97370	23975651	C	4,40E-04	-1,1630	0,2518	-39,84	⁎
14	ARS-BFGL-NGS-102351	22603705	G	4,44E-04	-1,1820	0,2428	-39,51	
14	Hapmap41234-BTA-34285	23320028	A	4,60E-04	-2,3960	0,0480	-19,91	
14	ARS-BFGL-NGS-116462	25707512	A	6,21E-04	-1,0220	0,4076	-44,87	
14	ARS-BFGL-NGS-76907	11771541	A	1,02E-03	-1,8550	0,0786	-24,44	
14	BTB-00557532	22838802	G	1,07E-03	1,0390	0,3374	42,23	
14	ARS-BFGL-NGS-28234	19361956	A	1,09E-03	-1,9220	0,0705	-22,91	⁎
14	ARS-BFGL-NGS-75663	21389408	A	1,19E-03	-0,9750	0,4771	-44,23	⁎
14	BTB-01530788	22720374	G	1,98E-03	1,0170	0,3399	41,49	
14	Hapmap31956-BTC-054628	24153510	G	3,81E-03	-1,0670	0,2605	-37,37	⁎
4	BTB-00195350	69828237	C	4,80E-03	1,2450	0,1629	30,87	
14	BTB-00557585	22803367	G	1,05E-02	0,9436	0,3350	38,22	
14	ARS-BFGL-NGS-8308	24100495	C	1,77E-02	-0,8606	0,4807	-39,06	⁎
14	ARS-BFGL-NGS-102610	19469546	A	2,06E-02	-0,9345	0,3157	-36,71	
10	ARS-BFGL-NGS-107699	36924887	G	2,71E-02	0,9203	0,2980	35,00	
14	BTB-01530836	22768981	A	2,89E-02	0,8941	0,3379	36,37	
14	Hapmap47925-BTA-36060	18288065	A	3,44E-02	-1,0390	0,2095	-31,29	⁎
14	ARS-BFGL-NGS-19602	19306045	G	4,56E-02	-0,8451	0,4848	-38,38	
10	ARS-BFGL-NGS-99907	10871878	A	5,74E-02	1,1260	0,1570	27,10	
14	UA-IFASA-7696	12380364	A	5,97E-02	-1,4580	0,0828	-20,13	
14	ARS-BFGL-BAC-1180	20282089	G	7,55E-02	1,0710	0,1676	27,17	
14	Hapmap40715-BTA-34541	32119820	A	9,11E-02	-1,5520	0,0717	-18,78	⁎
3	ARS-BFGL-NGS-114340	83693384	G	1,09E-01	-0,8370	0,3173	-32,97	

Table 4: List of important SNPs for direct stillbirth
⁎ Direct calving ease, +maternal calving ease, x direct stillbirth (high GL)

By the way CHCHD7, LYN, PLAG1, PENK, RPS20, SDR16C5, XKR4 and ENSBTAG00000039031 which were detected by Utsunomiya et al (2013) in terms of calf size came up as significant genes for stillbirth too.
However the most significant SNP in our analysis for stillbirth was BTA-91250-no-rs on base position 22,346,858 on chromosome 14 which was the same as in direct calving ease. So the SNTG1 gene is responsible for stillbirth too as well as the DERL1. Both genes are known to be associated with longevity in Fleckvieh cattle (Mészáros et al., 2014), even though its connection to stillbirth needs to be scrutinized.

Near the ARS-BFGL-NGS-75663 marker on base position 21,389,408 kb the SNAI2 gene, which is associated with congenital heart defects in human. These are the foremost cause of birth defect-associated infant mortality (Osoegawa and Iovannisci, 2014). Furthermore, this gene is down regulated in placental material of women with recurrent miscarriages, which is defined as three or more pregnancy losses before 20- 22 weeks of pregnancy (Rull et al., 2013).

Furthermore CRH was detected to be associated with stillbirth. This gene itself has been found to be involved in preterm birth (Petraglia et al., 2010), which enlarges the risk for infant mortality by factor 10 (Wulf, 1997).

The peak on chromosome 21 was as well nearly the same as for direct calving ease.

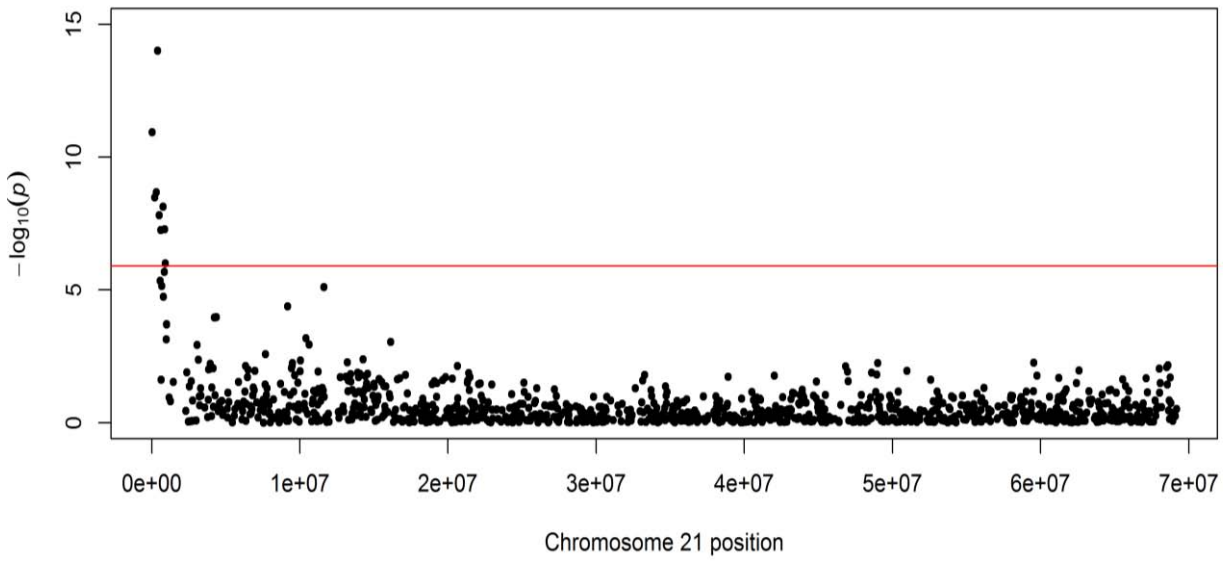

Figure 31: Chromosome 21 (Stillbirth direct)

In this case 16 markers had been selected, from which 14 were the same as for direct calving ease. The highest SNP was on base position 390,041kb (ARS-BFGL-NGS-108925), although in the vicinity of one megabase no gene was discovered to be connected to stillbirth.

The genes were not the same as those found for calving ease. Indeed MAGEL2, NDN and SNRPN are inside that area as well, because they are the same markers.

As mentioned before, those genes are responsible for Prader-Willi-Syndrome in human, but this disorder does not cause stillbirth (Cassidy et al., 2012).
However this region came up during the searches for five traits in this thesis, namely calving ease direct and maternal, gestation length direct and maternal and stillbirth direct. So in part those traits are inherited together. Since Sattlecker (2014) describes high genetic correlation between those, it was not surprising to find a common gene.

Applying the q value threshold 5 other chromosomes were found with connected genes, namely chromosomes 3, 4, 8, 10 and 27.

Near ARS-BFGL-NGS-114340, which explains 32.97% of the additive genetic variance on chromosome 3 is the USP1 gene located. A deletion in this gene leads to perinatal lethality in mice. Nearly 80% of the mice lacking this gene die within 1-2 days after birth (Kim et al., 2009).

Fetal growth restriction, a failure of the fetus to reach its genetically predetermined growth potential can lead to stillbirth if it´s onset is late in pregnancy. There are a lot of factors influencing this restriction and genes are involved. Two of them have been found during this search, in particular NFE2L3 on chromosome 4 and NUSAP1 on chromosome 10 (Sabri, 2013).

Another gene on chromosome 10 is the PAPD4 gene on base position 10,549,964 in the area of ARS-BFGL-NGS-99907which explains 27.10% of the additive genetic variance. The gene itself is associated with bone length in mice (Kenney-Hunt et al., 2006). Since calves bigger than the average show a much higher stillbirth rate (Hansen et al., 2004), this gene could influence stillbirth indirectly.

3.4.2 Maternal stillbirth

After the first run of the genome wide association study for maternal stillbirth the Manhattan plot did not show real peaks even if 38 markers were present above the Bonferroni line.

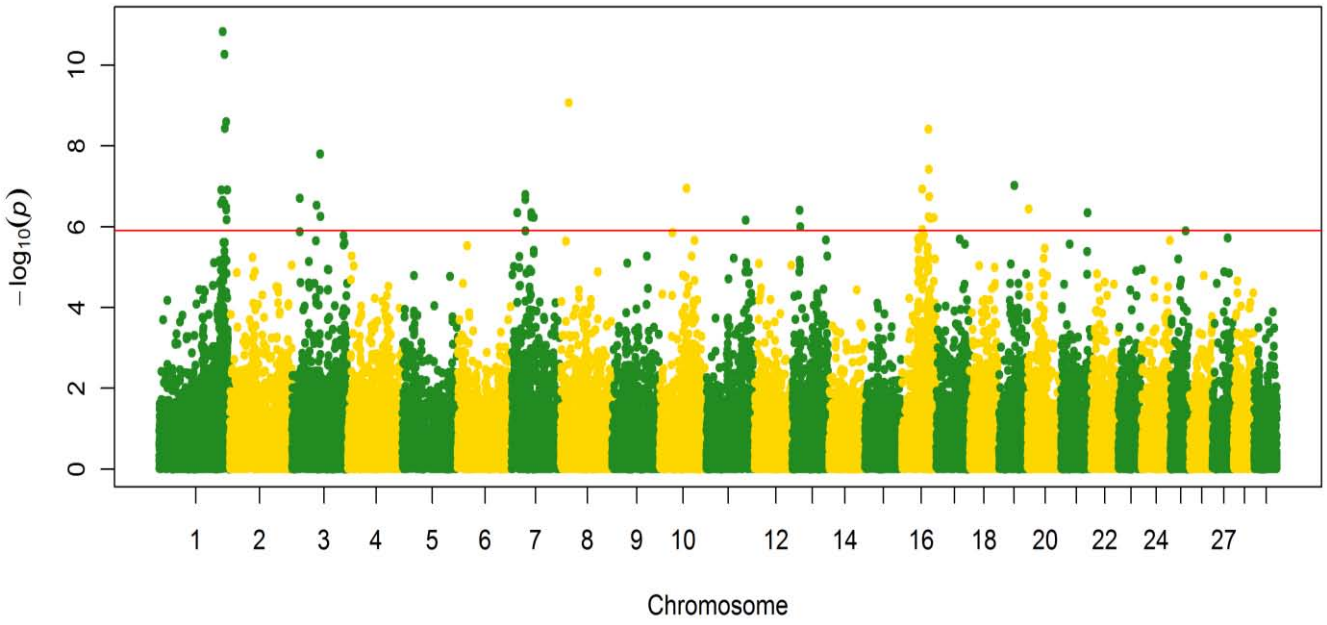

Figure 32: Significance levels (minus-log-P-values) for maternal stillbirth without correction

However the deviation was enormous, like always in the analysis before the correction processes.

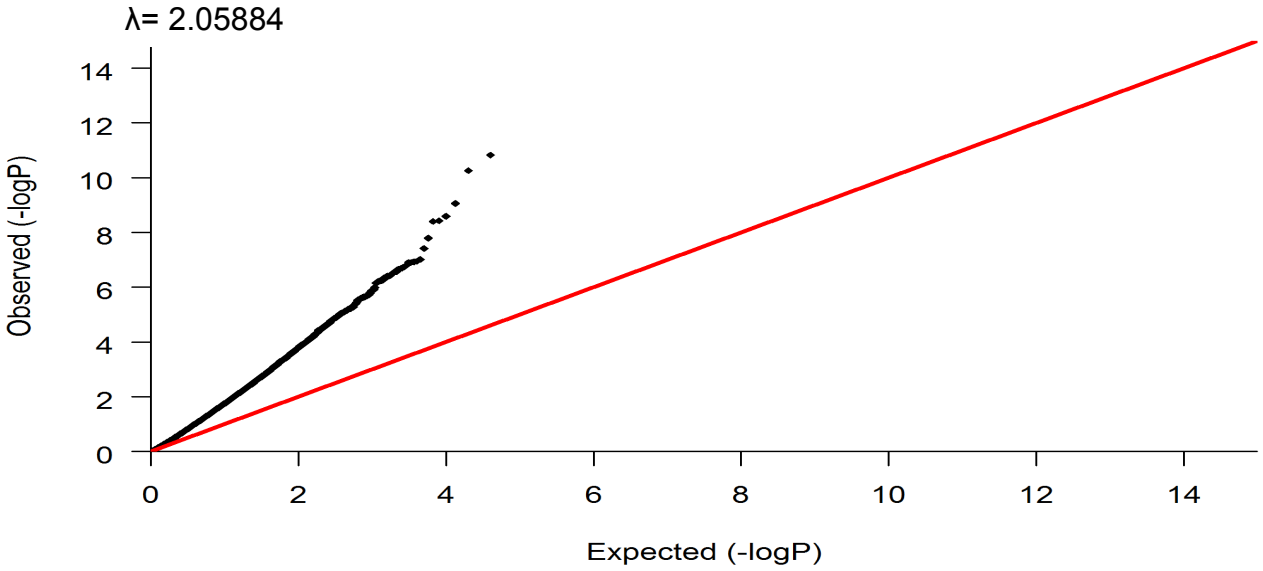

Figure 33: QQ-Plot for maternal stillbirth without correction

The correlation test for the eigenvectors and a weighted analysis of the most correlated ones has been performed. No significant markers were showing up after correction.

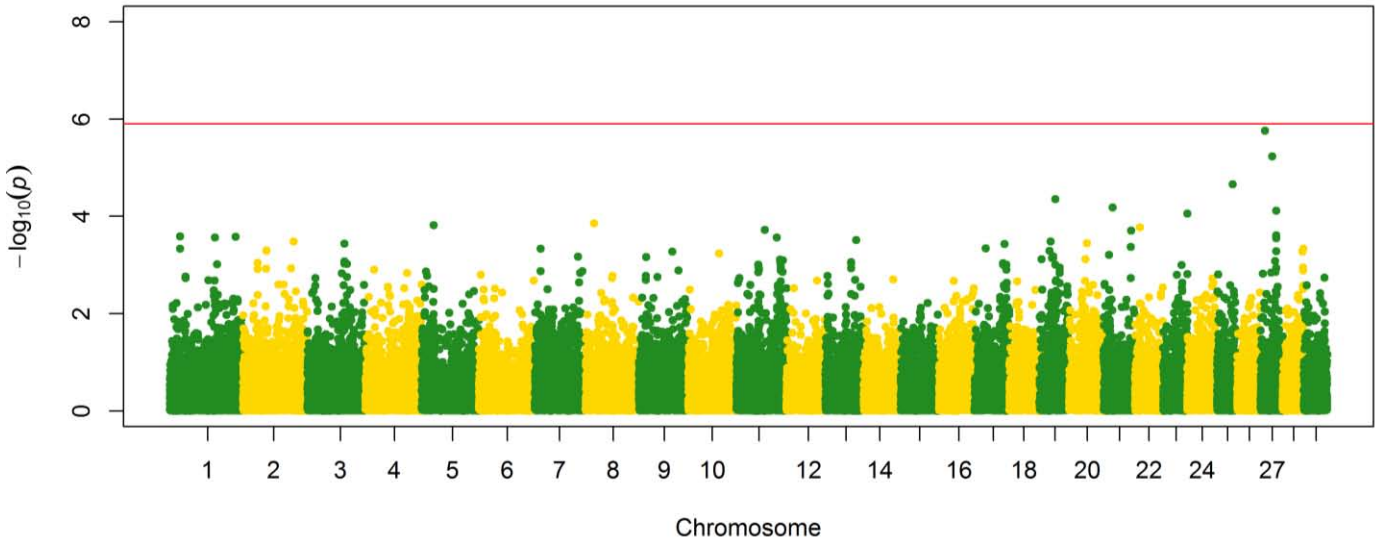

Figure 34: Significance levels (minus-log-P-values) for maternal stillbirth after correction

Even with the FDR approach (q- value threshold) no significant SNPs were detected. However one marker on chromosome 27 was near the Bonferroni line and there seemed to be a peak.

To detect something of potential importance the 20 most significant SNPs were used for the gene search, see table 5.

CHR	SNP	BP	A1	BETA	P-Value	MAF	Variance
27	ARS-BFGL-NGS-103904	9845495	A	1,029	1,72E-06	0,373	43,76
27	Hapmap52620-rs29010181	25421630	C	1,312	5,86E-06	0,1523	30,80
25	ARS-BFGL-NGS-73511	34258184	G	1,074	2,20E-05	0,2195	33,45
19	Hapmap32042-BTA-133010	34622653	G	0,858	4,46E-05	0,3796	36,74
21	ARS-BFGL-NGS-13218	19648985	C	-1,425	6,54E-05	0,09207	-21,66
27	ARS-BFGL-NGS-14674	34762381	G	-0,832	7,67E-05	0,382	-35,71
23	Hapmap47467-BTA-100234	53329481	A	-1,174	8,88E-05	0,1339	-24,75
8	Hapmap25285-BTA-145628	18048903	A	-1,453	1,41E-04	0,07955	-19,34
5	BTA-72957-no-rs	26044533	A	-1,038	1,53E-04	0,1752	-27,27
22	BTA-55233-no-rs	10388190	C	-0,790	1,68E-04	0,4	-34,49
11	BTB-00475179	62286212	G	1,554	1,90E-04	0,06627	17,48
21	ARS-BFGL-NGS-104428	60665642	A	-1,187	1,98E-04	0,1229	-23,26
27	ARS-BFGL-NGS-16551	34570064	G	0,808	2,45E-04	0,3289	32,43
1	Hapmap26157-BTA-148092	22253338	G	-1,388	2,59E-04	0,0796	-18,49
1	ARS-BFGL-NGS-31728	144559050	A	0,769	2,65E-04	0,3875	33,20
11	ARS-BFGL-NGS-115717	88794226	A	-0,830	2,72E-04	0,2927	-31,25
1	Hapmap49174-BTA-43310	98944629	G	-0,835	2,75E-04	0,2871	-31,07
27	ARS-BFGL-NGS-71338	34654594	G	-0,746	2,82E-04	0,4773	-33,82
13	ARS-BFGL-NGS-81601	68269103	G	0,755	3,08E-04	0,4212	33,48
19	ARS-BFGL-NGS-14187	24654047	A	1,019	3,28E-04	0,1626	25,23

Table 5: 20 most significant SNPs for maternal stillbirth

As indicated in the table the most significant marker lies on chromosome 27 but it is not close to any significant gene for the trait. Only 3 of those markers brought interesting results.
The second marker on chromosome 27 indeed plays an important role. This SNP is explaining around 30.80% of the additive genetic variance and contains at least one stillbirth-connected gene, namely DCTN6 or Dynactin 6 which is up-regulated during preeclampsia (Nishizawa et al., 2007). This disorder can lead to perinatal mortality and is affecting approximately 2 % of pregnant women worldwide. Like in fetal growth restriction there are a lot of factors leading to the disorder including genetic predisposition (Pennings et al., 2011).

Additionally GTSF1 on chromosome 5 near BTA-72957-no-rs is down regulated during preeclampsia, which is normally expressed in embryonic male and female gonads. However for its function in ovarian failure, more investigation would need to be done. ERICH 1 was also found in this study to be up regulated during preeclampsia (Yan et al., 2013). This gene can be found on chromosome 21 near the ARS-BFGL-NGS-104428 marker on a base position of 60,665,642 base pairs.

SERPINA3 is another gene that is connected with preeclampsia and intrauterine growth restriction in human. If one of those problems occur the SERPINA3 mRNA levels are 2- and 7 folds higher than in normal placentas (Chelbi et al., 2012). Also this gene lies in a one mega-base area of ARS-BFGL-NGS-104428.

Like in the current study, Olsen et al (2010) did not find a lot of signals for maternal stillbirth. However, they were using Norwegian Red cattle and speculate that the frequency of stillbirth is much lower than in other breeds, and therefore the QTLs have not been detected (Olsen et al., 2010).

3.5 Stillbirth with high gestation length

3.5.1 Direct stillbirth with high gestation length

Seperation of stillbirth data into sets with high and low gestation lengths was performed to investigate whether stillbirth is caused by different genes in early calving and late calving .However the number of animals was low and only few significant markers were detected.
In the first case the deviation between observed and estimated -log p-value was only mild before the correction procedure.

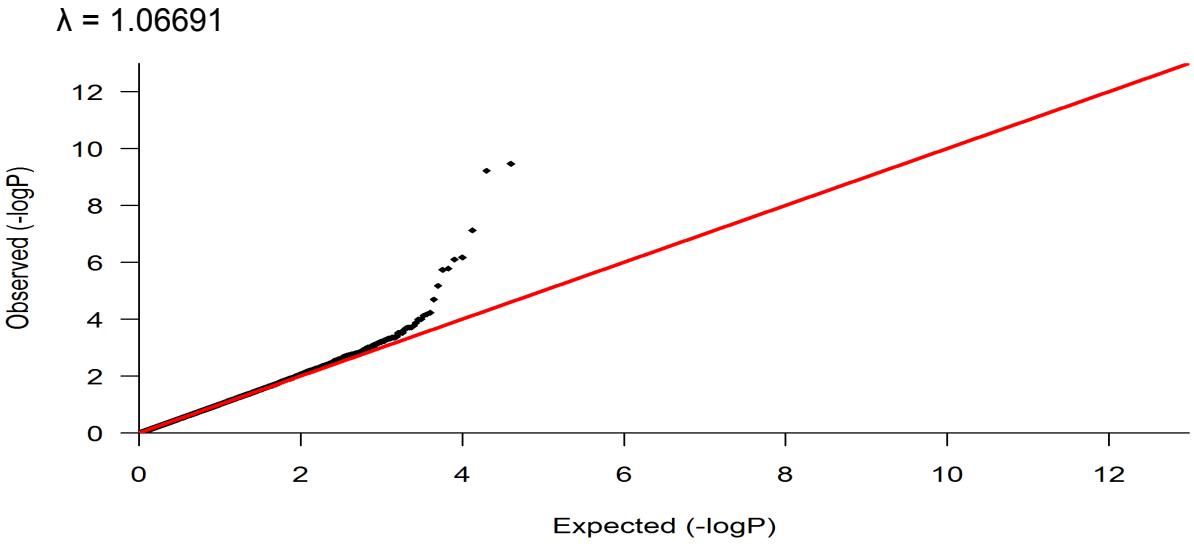

Figure 35: QQ- Plot for direct stillbirth with high gestation length before correction

The peak on chromosome 14 was there too, but the other one on chromosome 21 was not visible.

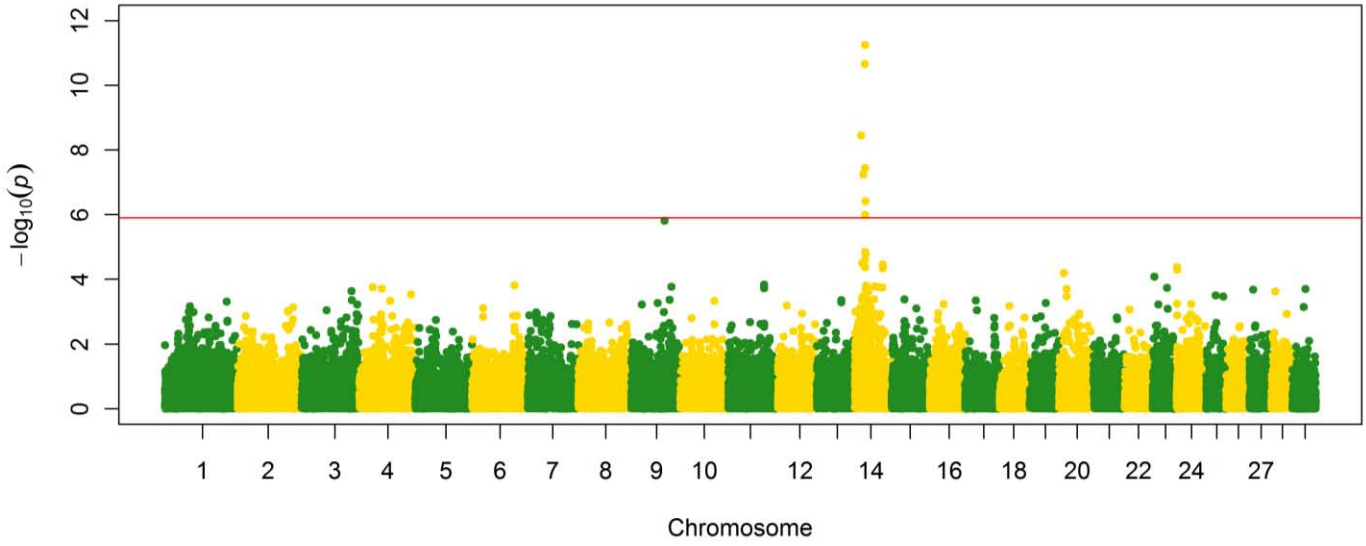

Figure 36: Significance levels (minus-log-P-values) for direct stillbirth with high GL without correction

However, a correction procedure was performed like in all other traits, but brought no big differences to light. So the deviation was reduced, since it was not big before not much change was visible. Interestingly there were more changes on the Manhattan plot after the correction, because two markers from other chromosomes were now significant.
Applying the q-value threshold gave similar results, only three other markers from chromosome 14 appeared.

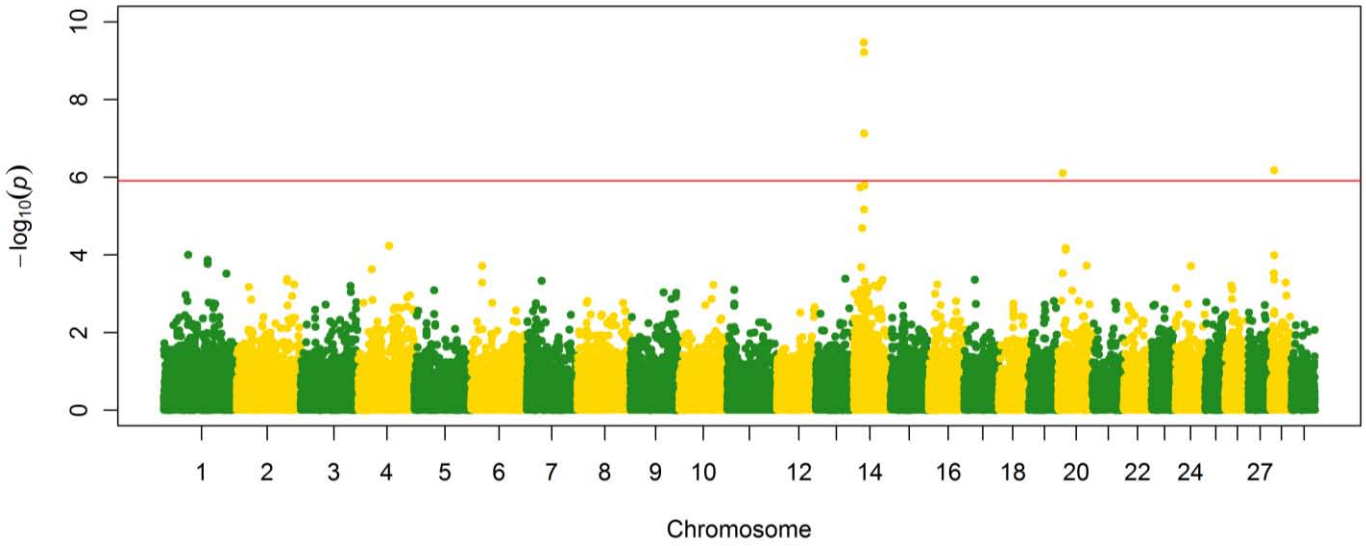

Figure 37: Significance levels (minus-log-P-values) for direct stillbirth with high GL after correction

The most significant SNP in this trait was ARS-BFGL-NGS-104268 which not even appears in the conventional analysis of stillbirth. The next SNPs were then the same like in the other trait. All three of them are near each other and cover an area from 22,260,373 to 22,382,727 base pairs. Within the surrounding area the SNTG1 gene is situated which has already been described to be connected with longevity in cattle

(Mészáros et al., 2014), even if there is no direct link to stillbirth but a indirect one via calving ease through their connection. The DERL1 gene which is on chromosome 14 too, near ARS-BFGL-NGS-28867 was discovered in both searches as well.

On chromosome 28 where one marker was found to be above the Bonferroni threshold and another one within the twenty most significant, but no gene relevant could be detected.
For chromosome 20 the situation was different, the marker above the Bonferroni threshold, BTB-00772171 on base position 10,06 mega bases, delivered some relevant genes. Around one mega base of this position lies the CCDC125 gene which is one of a few genes down-regulated in fetal growth restriction. This can enlarge the risk for perinatal mortality, and itself can be the result of heterogeneous causes like maternal, fetal or placental factors (Nishizawa et al., 2011).

Another gene within this area is CCNB1 on base position from 10.44 to 10.45 mega bases. This gene plays as well a role in fetal growth restriction together with the HLX gene, as both of them show reduced levels (Murthi et al., 2011).
The third gene in the surrounding of BTB-00772171 is SMN1. A mutation in this gene causes spinal muscular atrophies which is the second most common autosomal recessive inherited disorder in humans. If such a mutation comes together with a deletion of SMN2 too, an embryo is not viable (Monani et al., 2000).

Additionally to this marker above the line three other SNPs on this chromosome were situated inside the top twenty, but none of them delivered significant genes.
If there had been a greater number of animals available for the analysis, maybe more genes would appear. However it was interestingly that most of the detected genes were not the same like for the “normal” part of the trait. So here most of the genes are situated at chromosome 20, which doesn´t appear in the direct stillbirth search with all animals.

3.5.2 Maternal stillbirth with high gestation length

For this trait 370 animals have been available for the analysis. Maybe this is the reason that not much variation exists, so the deviation is not very big (λ =1.08273).

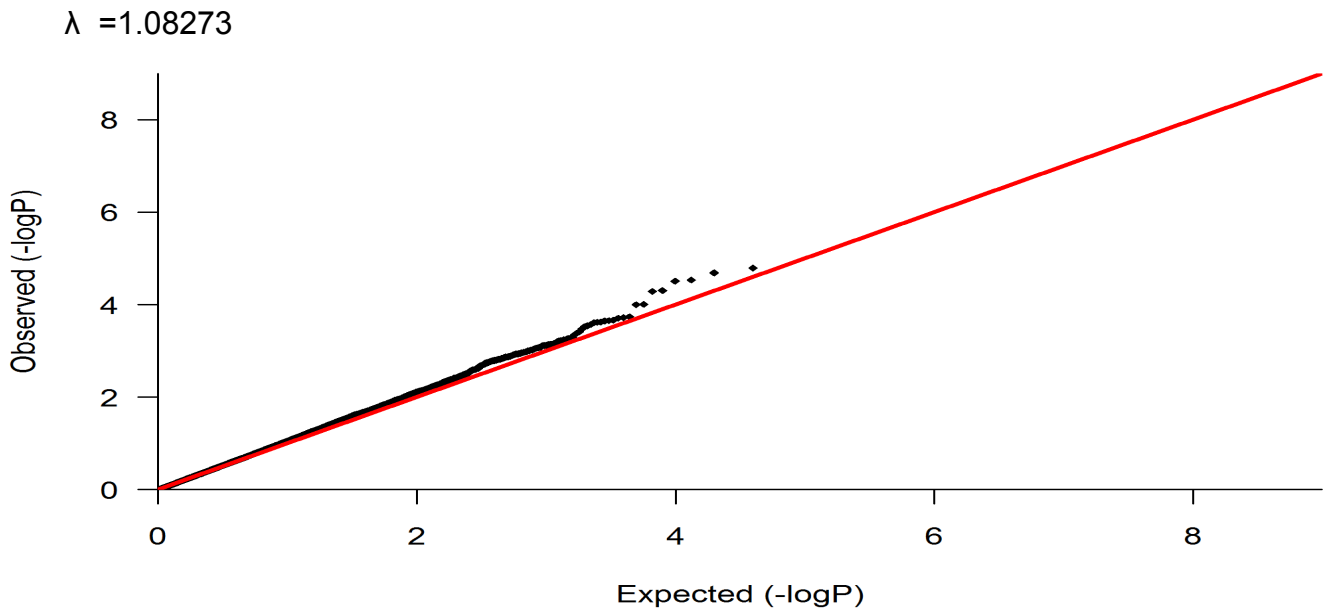

Figure 38: QQ-Plof for maternal stillbirth (high GL) without correction

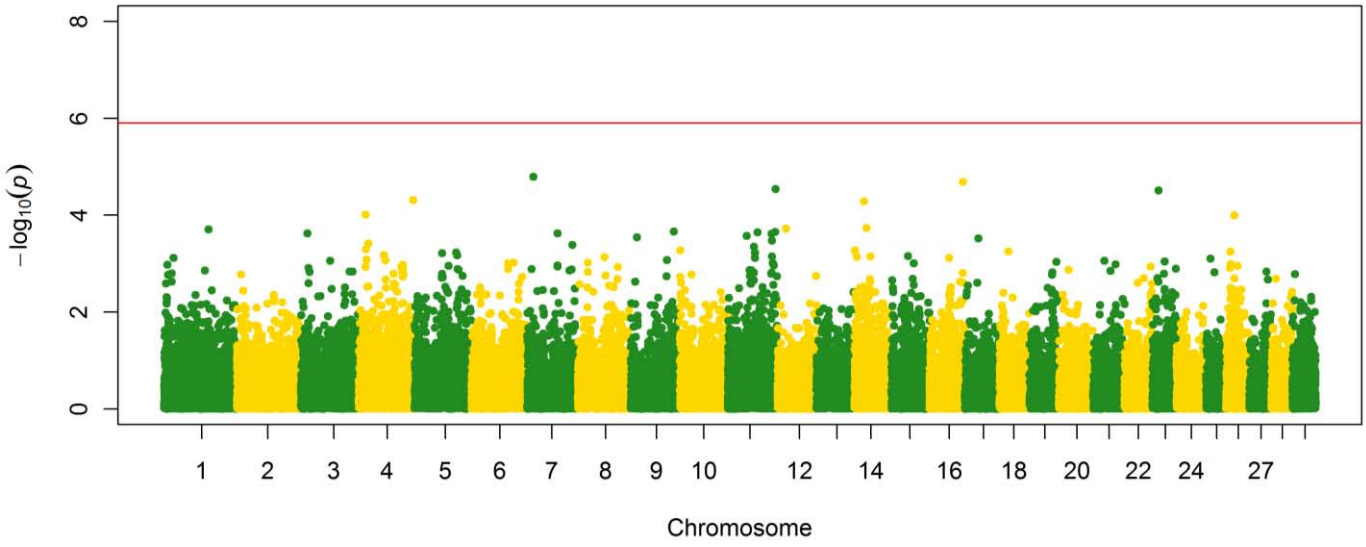

Figure 39: Significance levels (minus-log-P-values) for maternal stillbirth (high GL) without correction

Despite this outcome a correction procedure was performed, but didn´t brought very different results either, see figures 40 and 41.

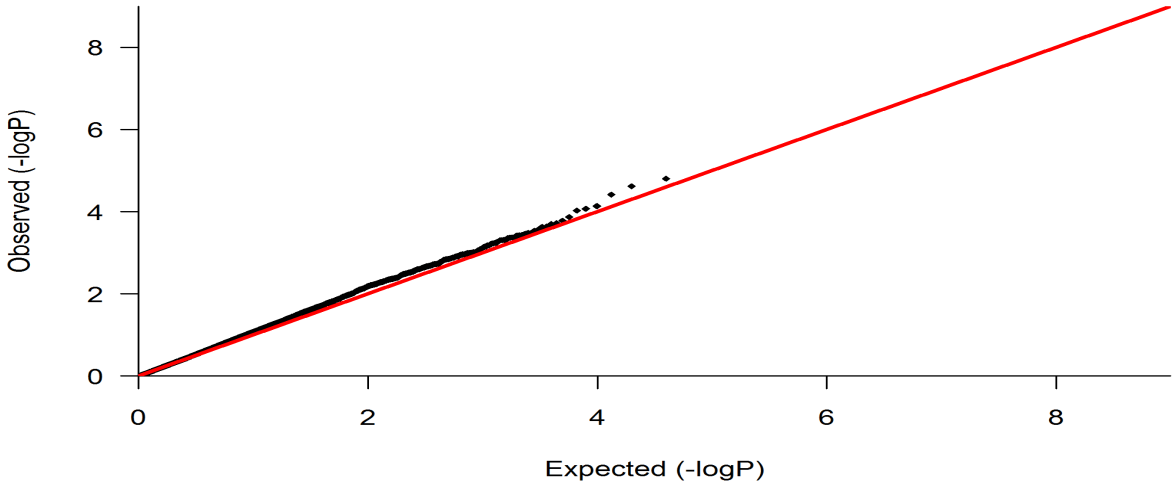

Figure 40: QQ-Plot for maternal stillbirth (high GL) after correction

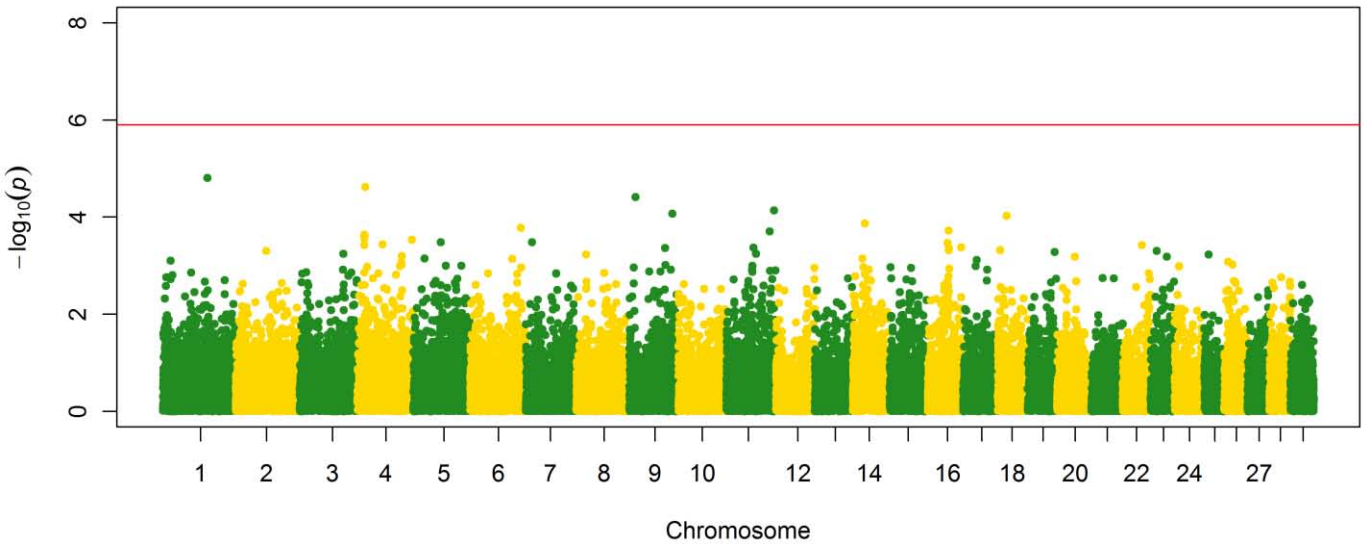

Figure 41: Significance levels (minus-log-P-values) for maternal stillbirth (high GL) after correction

The q-value threshold was calculated additionally, but did not include any significant markers. Since the analysis for maternal stillbirth with all animals did not show any better result, this outcome is not really surprising.
Still, a gene search with the twenty most significant markers was performed as well. Like in the direct part of this trait the genes found during the search were different to them of the search with the whole set of animals.

From those twenty markers only four are situated near stillbirth connected genes. So Chromosome 16 contains two important SNPs, namely ARS-BFGL-NGS-54860 on a base position of 45.94 MB and ARS-BFGL-NGS-28769 (43.33MB). A few base pairs away from the first one is the ERRFI1 gene situated. By comparing placental tissues from pregnant women with and without preeclampsia it is apparent that this gene is

overexpressed through the disorder (Trifonova et al., 2014), which has already been described earlier in this thesis.
Near the second SNP lies the UBIAD1 gene, which is a vitamin K2 biosynthetic enzyme. In a strict sense it has nothing to do with stillbirth, however mouse who are lacking this gene totally (which means they didn´t got it from their parents) die as embryos (Nakagawa et al., 2014).

C1GALT1 is another gene without a direct connection to stillbirth. Yet, it is decreased in placentas of women with recurrent miscarriage (Rull et al., 2013). Stillbirth and miscarriage are not the same problems, but maybe a gene causing miscarriage in human could cause stillbirth in cattle.

3.6 Stillbirth with low gestation length

3.6.1 Direct stillbirth with low gestation length

Even before the correction for population structure there were no markers above the Bonferroni line.

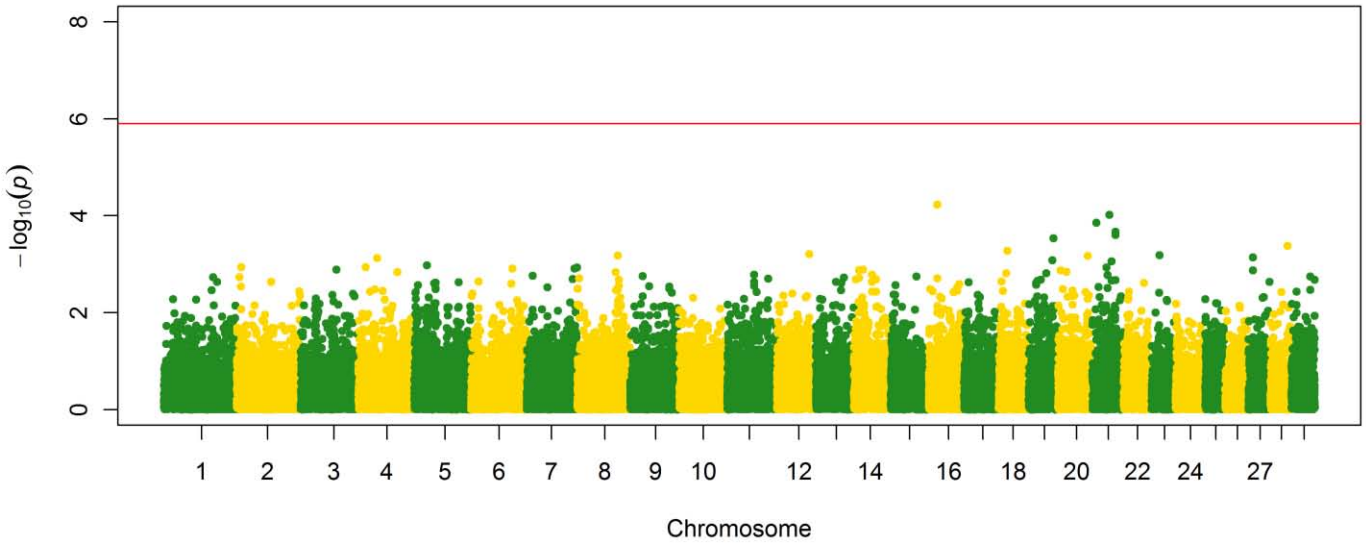

Figure 42: Significance levels (minus-log-P-values) for maternal stillbirth (low GL) without correction

Figure 42 provides a QQ-Plot for this trait without correction, where it can be seen that the inflation factor is 1 so there are effetely no differences between the animals.

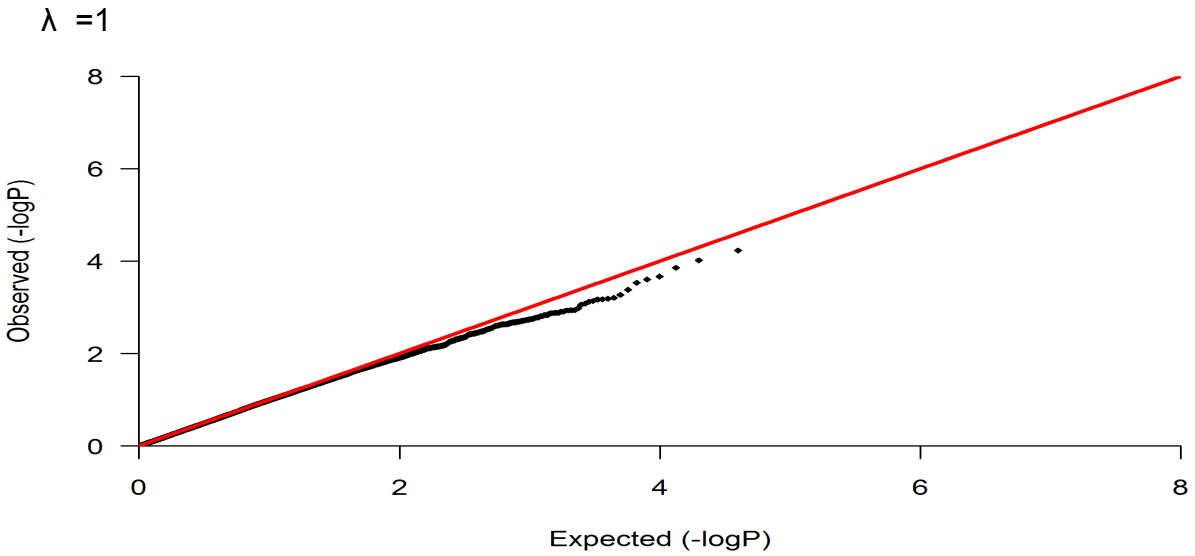

Figure 43: QQ-Plot for direct stillbirth (low GL) without correction

Like in every analysis before a correction procedure was carried out, with a gene search afterwards.
Not surprisingly no markers where showing up above the Bonferroni threshold and even with the q-value nothing significant appeared, so the search was performed with the top twenty markers like in a few traits before.
Only three SNPs were close to genes previously connected to stillbirth.

Of these three markers one is on chromosome 25 on base-pair position of 2,219,666 and is called ARS-BFGL-NGS-15062. Around 200 000 base pairs away is the claudin 6 (CLDN6) gene. An experiment with transgenic mice overexpressing this gene showed that too high levels affect the formation of the epidermal permeability barrier which can lead to neonatal lethality because of poor temperature stability, outflow of water and infections through the skin (Turksen and Troy, 2002).
Another gene of the claudin family, namely CLDN7 on chromosome 19 on base pair position 27,602,595 near Hapmap48138-BTA-96848 was also detected in this search. It was found by Turksen and Troy (2002) to be connected with the overexpression of claudin 6, even if its expression was decreased.
Additionally to these two genes another one has been found on chromosome 1 around 200,000 bases away from Hapmap59715-rs29022224. In a proper meaning of the sense this gene, namely IL12A has nothing to do with direct stillbirth, it is in fact influencing maternal stillbirth. Its levels are particularly increased during preterm delivery which is a significant cause of infant mortality (El-Shazly et al., 2004).

3.6.2 Maternal stillbirth with low gestation length

The first run of the analysis for this part of the trait revealed nothing very new. Like in the analysis for maternal stillbirth with high gestation length, there was nearly nothing to figure out of the Manhattan plot.

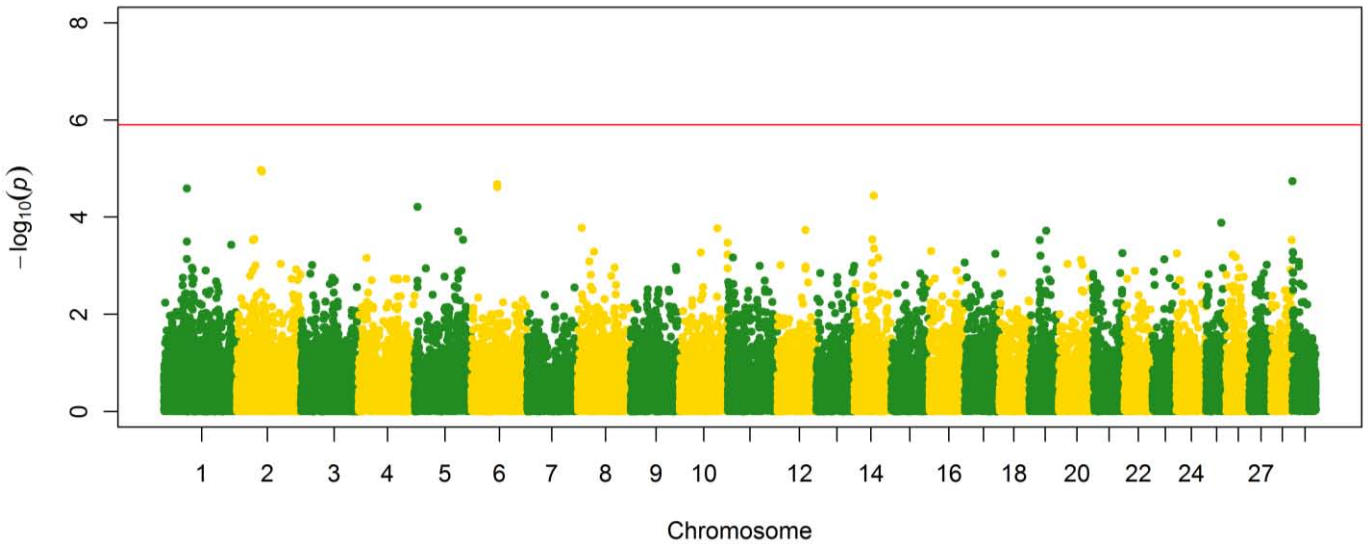

Figure 44: Significance levels (minus-log-P-values) for maternal stillbirth (low GL) without correction

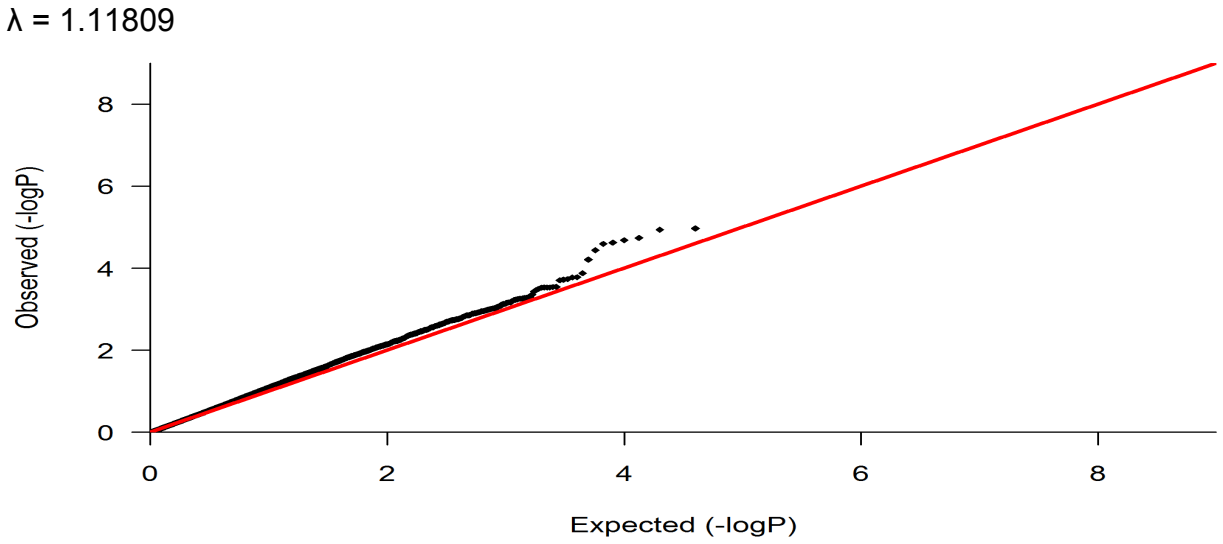

Figure 45: QQ- Plot for maternal stillbirth (low GL) without correction

Despite the mild deviation of 1.11809 a correction procedure was performed as in all other traits.

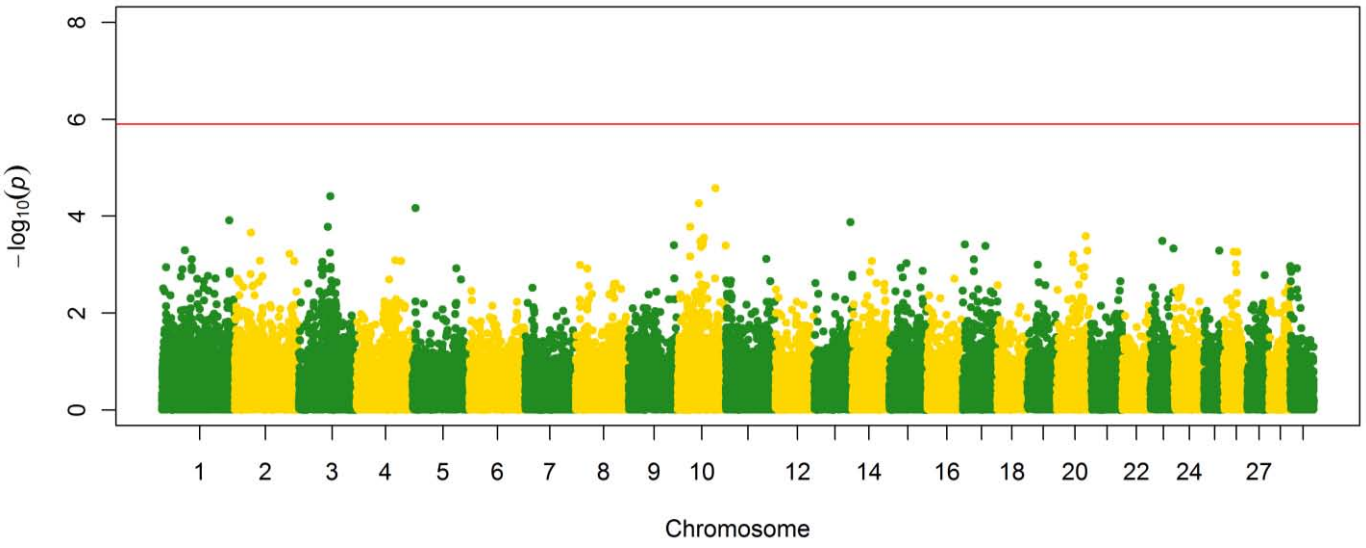

Figure 46: Significance levels (minus-log-P-values) for maternal stillbirth (low GL) without correction

After the correction process the inflation factor was a bit reduced (λ = 1.01319). As expected by the way, nothing was discovered to be associated, not even with the q-value approach, therefore the top twenty SNPs were used in this case as well.

CHR	SNP	BP	A1	BETA	P-Value	MAF
1	BTA-19847-no-rs	148224421	A	-3,645	1,23E-04	0,2003
2	ARS-BFGL-NGS-44341	35010647	A	-3,434	2,19E-04	0,2563
3	Hapmap50439-BTA-68100	64564074	G	3,768	1,66E-04	0,2279
3	Hapmap48578-BTA-105324	69718616	G	3,823	3,88E-05	0,241
5	BTA-73200-no-rs	5857304	A	3,564	6,80E-05	0,241
9	ARS-BFGL-NGS-100350	100169525	A	2,801	3,98E-04	0,3936
10	BTB-00414590	27946898	G	2,905	1,66E-04	0,4489
10	UA-IFASA-4326	46415087	C	-3,993	5,46E-05	0,1969
10	ARS-BFGL-NGS-70374	50297273	C	-2,860	3,30E-04	0,4763
10	ARS-BFGL-NGS-92694	51627569	A	-2,963	4,37E-04	0,3356
10	Hapmap28067-BTA-71220	55700822	A	-4,526	3,83E-04	0,1039
10	BTA-71218-no-rs	55743358	C	-4,524	3,56E-04	0,105
10	BTA-105432-no-rs	57744635	A	-3,519	2,77E-04	0,2285
10	BTB-00437757	83036529	A	-5,553	2,67E-05	0,08702
10	BTA-96648-no-rs	105950060	A	3,424	4,07E-04	0,1975
13	ARS-BFGL-NGS-16572	79538675	G	3,618	1,33E-04	0,2224
17	ARS-BFGL-NGS-11273	4893936	A	-3,176	3,87E-04	0,3149
17	ARS-BFGL-NGS-78287	50267264	A	-8,957	4,10E-04	0,02535
20	Hapmap24007-BTA-153345	63607529	A	-6,985	2,59E-04	0,04661
23	ARS-BFGL-NGS-54047	26298418	G	3,190	3,23E-04	0,2089

Table 6: SNPs used for the gene search for maternal stillbirth (low GL)

As can be seen from the Manhattan plot and in Table 6 most of the markers are located on chromosome 10, but only one of them is surrounded by stillbirth connected genes. This marker is ARS-BFGL-NGS-92694with two relevant genes in its surrounding namely FAM63B and ADAM10.
Both of them had been detected earlier in a study about preeclampsia and other pregnancy related hypertensive disorders in Taiwanese women. In comparison with placentas from women with normal pregnancies it was shown that the expression of ADAM10 is decreased in preeclampsia. In the course of this survey superimposed preeclampsia on hypertension has been analysed as well, which is defined by worsening hypertension and proteinuria occurring in women with documented hypertension. FAM63B was found to be increased in such kind of disorders in comparison with normal preeclampsia(S.-D. Chang et al., 2011).
A third gene of our analysis was also found to be decreased during preeclampsia in this study, namely ARFIP1 which is situated at chromosome 17 around 30 000 base pairs away from ARS-BFGL-NGS-11273.
TRIM2 is another gene around one mega-base of this marker, which has as well been found to be up-regulated during preeclampsia in another survey (Yan et al., 2013).

Additionally to these findings one other gene on chromosome 13 has been detected as well, even if this is not directly connected to stillbirth.
NFATC2 is documented to be down-regulated in maternal deciduas (tissue of endometrial origin) at the time when spontaneous abortions in human occur (Wang et al., 2014). Even if this is not the same as stillbirth, because it arises earlier in pregnancy, the gene could be involved in stillbirth influencing cattle too.

The results of the analysis for parts of with high and low duration of pregnancies indicate that larger samples would be useful.

Conclusions

The purpose of this study was to find candidate genes for gestation length (direct/maternal), calving ease (direct/maternal), stillbirth (direct/maternal), early fertility disorders (direct).

For gestation length, two peaks on chromosomes 21 and 4 were detected for the direct and maternal expression of the trait. However, in the surrounding area of the most significant markers no genes of relevance due to the results of other studies, including human, have been detected.
Interestingly, the peak on chromosome 21 was on the same position as for two other traits in this analysis, namely calving ease (direct and maternal) and stillbirth (only direct). This peak includes the region for Prader-Willi-Syndrome, a disorder increasing the risk of Caesarean section in humans, with three relevant genes (MAGEL2, NDN and SNRPB). It seems that this region is very important in cattle too.
Additionally to this region on chromosome 21, a high peak on chromosome 14 has been discovered. A few of the genes detected there are involved in growth traits, which is correlated with difficulties during birth. Those genes are CHCHD7, MOS, LYN, PLAG1, PENK, RPS20, SDR16C5 and XKR4.
The genes for calving ease maternal were nearly the same as for direct calving ease.

The gene search for stillbirth provided similar results. For the direct part of the trait, the peaks on chromosomes 14 and 21 were nearly identical to those for calving ease. Only a few genes were different, for example SNAI2 on chromosome 14, causing congenital heart defects which is an important cause of infant mortality in humans. The maternal part of this trait was different, no peaks came up and only a few genes could be discovered. Some of them like SERPINA3 play a role during preeclampsia, a disorder leading to perinatal mortality in human.

The analysis for early fertility disorders did mostly not deliver significant results. No peaks were visible and only one marker deemed significant, but around its surrounding area no genes were detected connected to retained placenta, inflammation of the uterus or puerperal disease. A separation of the composite trait provided to this study into the three component traits retained placenta, inflammation of the uterus and puerperal disease will potentially provide better results. .

Separate analysis for stillbirth with low and high gestation lengths were performed to look for genes linked to stillbirth at different gestation lengths. Only the analysis for direct stillbirth with high gestation length resulted in a peak on chromosome 14. This could be due to the low number of animals used for the search.

Overall, it seems that calving ease, stillbirth and gestation length are partly affected by the same genes.

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Appendix

Figures:

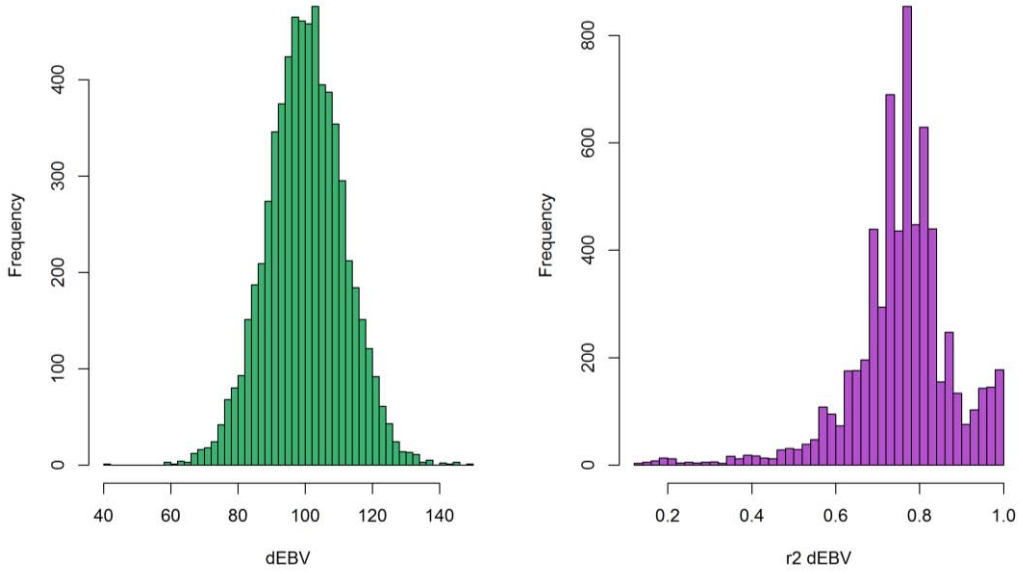

Figure 47: Distribution of deregressed breeding values and reliabilities (r2) for maternal calving ease

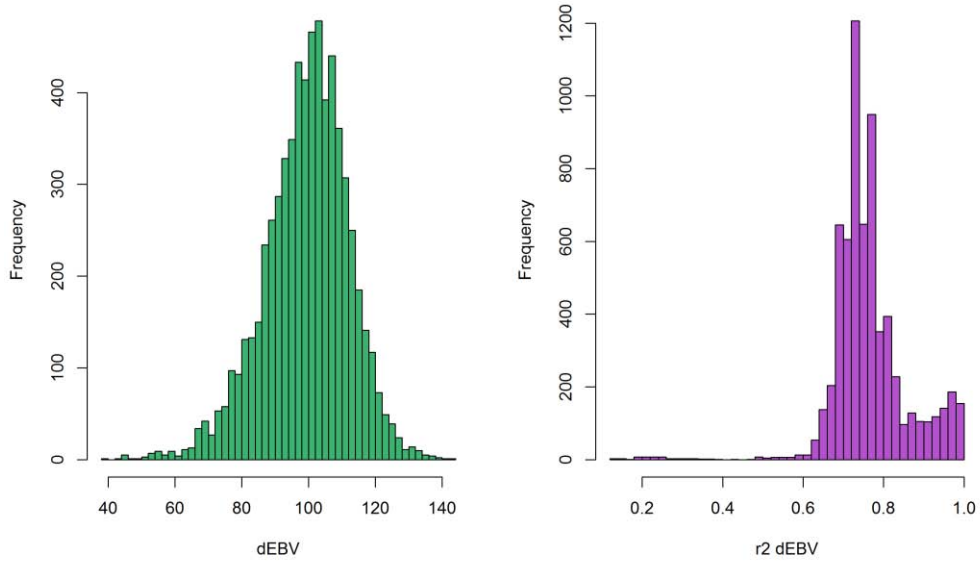

Figure 48: Distribution of deregressed breeding values and reliabilities (r2) for direct stillbirth

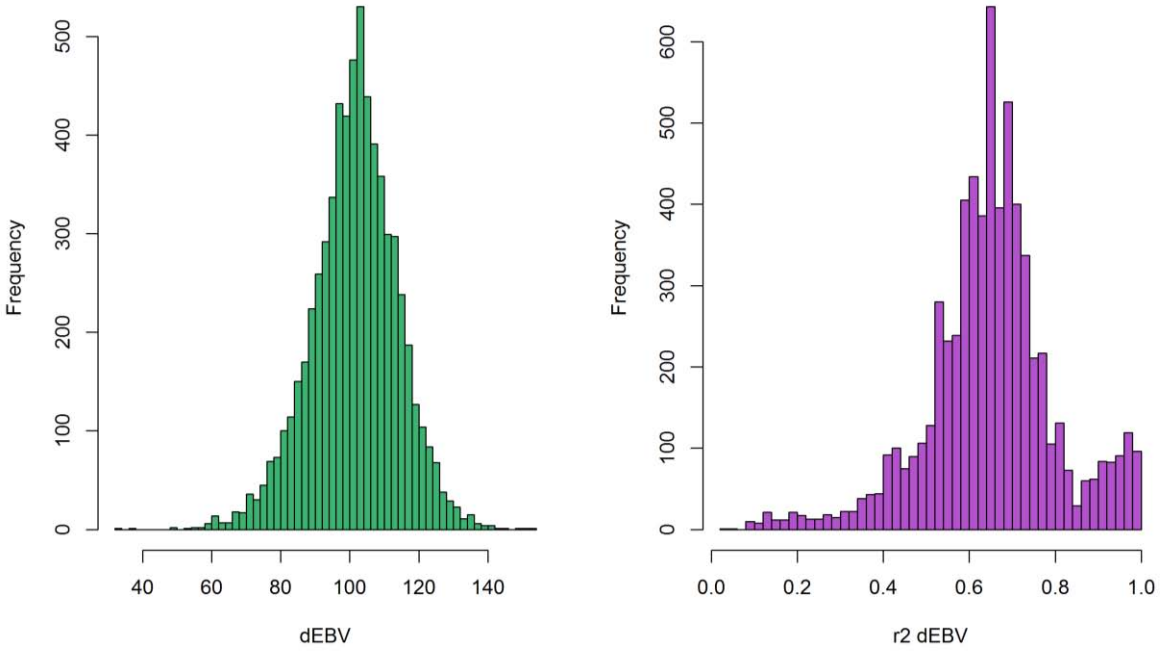

Figure 49: Distribution of deregressed breeding values and reliabilities (r2) for maternal stillbirth

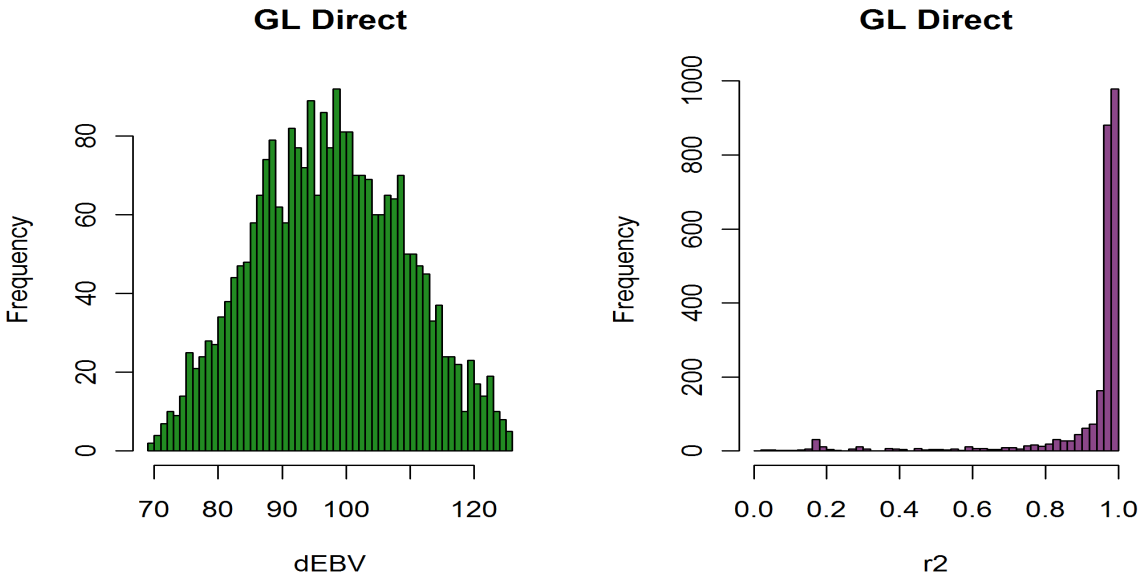

Figure 50: Distribution of deregressed breeding values and reliabilities (r2) for direct gestation length

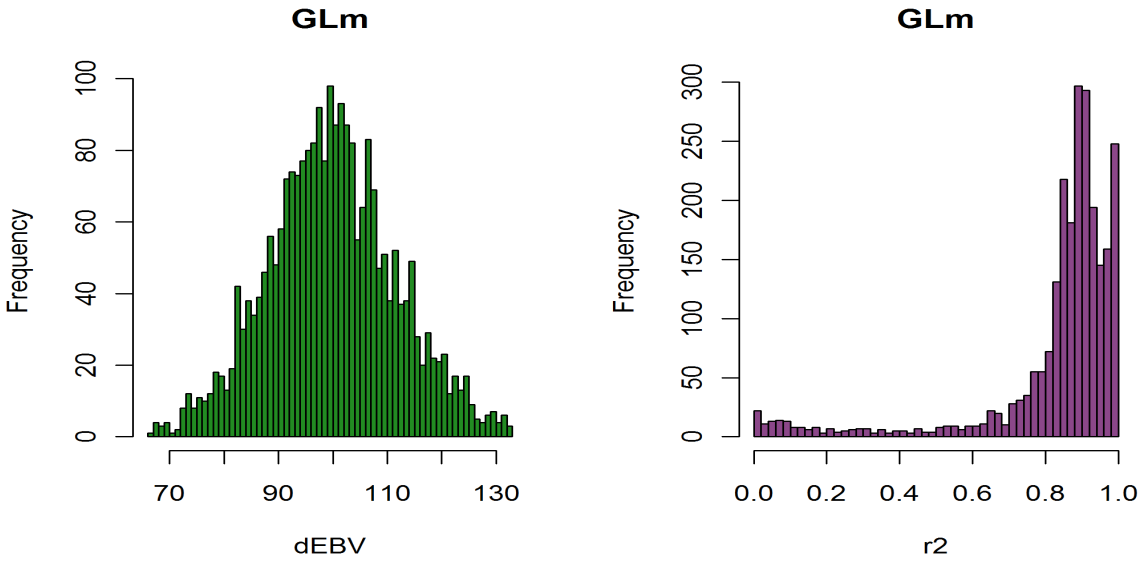

Figure 51: Distribution of deregressed breeding values and reliabilities (r2) for maternal gestation length

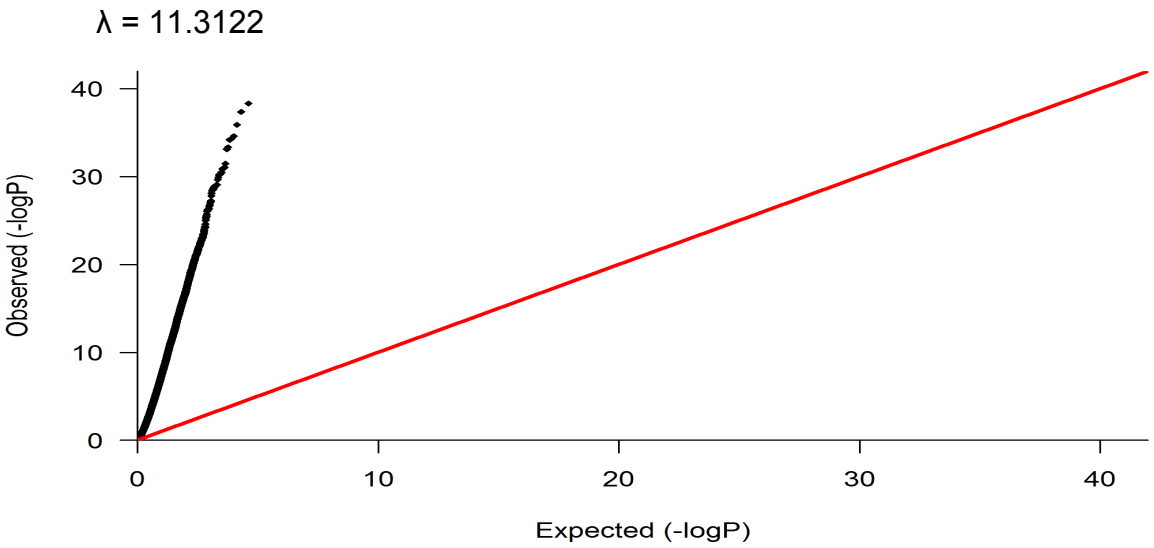

Figure 52: Q-Q plot for maternal calving ease without correction

List of Tables