Genome-wide association study to identify chromosomal regions associated with antibody response to *Mycobacterium avium* subspecies *paratuberculosis* in milk of Dutch Holstein-Friesians

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ABSTRACT

Heritability of susceptibility to Johne's disease in cattle has been shown to vary from 0.041 to 0.159. Although the presence of genetic variation involved in susceptibility to Johne's disease has been demonstrated, the understanding of genes contributing to the genetic variance is far from complete. The objective of this study was to contribute to further understanding of genetic variation involved in susceptibility to Johne's disease by identifying associated chromosomal regions using a genome-wide association approach. Log-transformed ELISA test results of 265,290 individual Holstein-Friesian cows from 3,927 herds from the Netherlands were analyzed to obtain sire estimated breeding values for *Mycobacterium avium* subspecies *paratuberculosis* (MAP)-specific antibody response in milk using a sire-maternal grandsire model with fixed effects for parity, year of birth, lactation stage, and herd; a covariate for milk yield on test day; and random effects for sire, maternal grandsire, and error. For 192 sires with estimated breeding values with a minimum reliability of 70%, single nucleotide polymorphism (SNP) typing was conducted by a multiple SNP analysis with a random polygenic effect fitting 37,869 SNP simultaneously. Five SNP associated with MAP-specific antibody response in milk were identified distributed over 4 chromosomal regions (chromosome 4, 15, 18, and 28). Thirteen putative SNP associated with MAP-specific antibody response in milk were identified distributed over 10 chromosomes (chromosome 4, 14, 16, 18, 19, 20, 21, 26, 27, and 29). This knowledge contributes to the current understanding of genetic variation involved in Johne's disease susceptibility and facilitates control of Johne's disease and improvement of health status by breeding.

Key words: genome-wide association approach, Johne's disease susceptibility, enzyme-linked immunosorbent assay, dairy cow

INTRODUCTION

Johne's disease, also known as paratuberculosis, occurs mainly in ruminants and is caused by the oral uptake of the causative agent *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from the environment. Johne's disease is characterized by granulomatous lesions in the distal part of the ileum. Ileal lesions elicit the deterioration of nutrient uptake and animals suffering from clinical Johne's disease show weight loss, diarrhea, and decreased milk production. Most animals infected with Johne's disease are not able to clear the infection. As a result, Johne's disease has a substantial economic impact on farms where the disease is endemic (Weber, 2006). Classical control strategies on the farm are based on management measures to improve hygiene and culling of infected animals but eradication of MAP from an infected herd has been shown to be difficult (Beyerbach et al., 2001). Additional approaches to address Johne's disease are needed. One approach is genetic selection for animals that are less susceptible to Johne's disease.

Earlier research showed a heritability of susceptibility to Johne's disease in cattle ranging from 0.041 to 0.159 (Koets et al., 2000; Mortensen et al., 2004; Gonda et al., 2006; Hinger et al., 2007; Attalla et al., 2010; van Hulzen et al., 2011). Nonzero heritability indicates that part of the phenotypic variation in the population is due to genetics. It also implies that susceptibility to Johne's disease can be successfully changed by means of genetic selection. Although the presence of genetic variation involved in susceptibility to Johne's disease has been demonstrated, the understanding of genes contributing to the genetic variance is far from complete. Research revealed contributions to Johne's disease susceptibility from polymorphisms in candidate

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genes *SLC11A1* (Pinedo et al., 2009b; Ruiz-Larrañaga et al., 2010), *TLR1* (Mucha et al., 2009), *TLR2* (Mucha et al., 2009; Koets et al., 2010), *TLR4* (Mucha et al., 2009), *CARD15/NOD2* (Pinedo et al., 2009a), and *PGLYRP1* (Pant et al., 2011); a QTL on chromosome 20 (Gonda et al., 2007); regions on chromosomes 3, 9 (Settles et al., 2009), and 12 (Minozzi et al., 2010); a set of 51 SNP covering several chromosomes that could be used as predictor of a MAP susceptibility breeding value in Holstein cattle (Kirkpatrick et al., 2011); and genomic regions on chromosome 1, 5, 6, 7, 10, 11, and 14 (Pant et al., 2010). Zanella et al. (2011a,b) revealed contributions to Johne's disease tolerance from regions on chromosomes 1, 2, 6, and 15.

The objective of this study was to contribute to further understanding of genetic variation in susceptibility to Johne's disease. A genome-wide association (GWA) approach was selected to perform a multiple SNP analysis fitting 37,869 SNP simultaneously to identify chromosomal regions associated with MAP specific antibody response in milk of Dutch Holstein-Friesians. Genes located in the associated chromosomal regions are discussed with respect to their potential role in Johne's disease susceptibility.

MATERIALS AND METHODS

Animals

Milk samples were collected from lactating cows during the routine milk production scheme. Collection, transportation, and storage of milk samples were described by van Hulzen et al. (2009). From January until December 2008, milk samples were sent to the Dutch Animal Health service (Deventer, the Netherlands) based on the decision of the farmer to test for antibodies specific for Johne's disease. Antibody testing was financially supported to stimulate Dutch dairy farmers to participate in the program. The raw data set contained over a million records. Pedigrees and milk production records of the animals were provided by the Dutch Cattle Improvement Organization (CRV, Arnhem, the Netherlands). The data set was edited to meet the minimum of 20 animals per herd and the minimum of 5 daughters plus granddaughters per sire. Only cows with at least 75% Holstein-Friesian genes were retained. Data from 265,290 cows from 3,927 herds with least 2 test positive animals for Johne's disease were used for breeding value estimation (van Hulzen et al., 2011).

Phenotype

All samples were tested using a commercially available ELISA kit (ELISA paratuberculosis antibody

screening; Institut Pourquier, Montpellier, France) according to the instructions of the manufacturer. Results were expressed as percentage of the sample-to-positive ratio, calculated as $100 \times [\text{the optical density (OD) value of the sample} - \text{the OD value of the negative control}] / [\text{the OD value of the positive control} - \text{the OD value of the negative control}]$.

Log-transformed sample-to-positive ratios of the individual cows were analyzed to obtain sire EBV for MAP-specific antibody response in milk using a sire-maternal grandsire model in ASReml (Gilmour et al., 2006), with fixed effects for parity, year of birth, lactation stage, and herd; a covariate for milk yield on test day; and random effects for sire, maternal grandsire, and error. van Hulzen et al. (2011) provided a detailed description of the model and presented results of quantitative genetic analysis of the data used in this study. The quantitative genetic analysis resulted in EBV for 2,702 sires. Reliabilities of EBV were calculated as $\frac{n}{n+k}$, where n is the number of daughters and $k = \frac{1}{\frac{1}{4}h^2} - 1$, with $h^2 = 0.041$ (van Hulzen et al., 2011).

Of the available 2,702 EBV, 192 EBV had a reliability of at least 70% and were used for further GWA analysis. Figure 1 shows the frequency distribution of 192 sire EBV used for GWA analysis.

Sire EBV were deregressed to account for 1) shrinkage of EBV using BLUP estimates and 2) differences in shrinkage of EBV of sires due to a differences in number of daughters used to calculate BLUP EBV (Thomsen et al., 2001; Garrick et al., 2009; Guo et al., 2010). Deregressed proofs (DRP) based on estimated daughter contributions (EDC) were calculated using the following formula (VanRaden et al., 2009):

$$DRP = PA + (EBV - PA) \times \left(\frac{EDC_{\text{parents+progeny}}}{EDC_{\text{progeny}}} \right),$$

where PA is the parent average. Estimated daughter contributions were derived from the reliabilities of the EBV using the following formulas (Mäntysaari et al., 2010):

$$EDC_{\text{parents+progeny}} = \frac{k \times REL_{EBV}}{1 - REL_{EBV}},$$

$$EDC_{\text{parents}} = \frac{k \times REL_{PA}}{1 - REL_{PA}},$$

$$EDC_{\text{progeny}} = EDC_{\text{parents+progeny}} - EDC_{\text{parents}},$$

Figure 1. Histogram of breeding values of 192 sires for *Mycobacterium avium* subspecies *paratuberculosis* (MAP)-specific antibody response in milk estimated on ELISA test results of 265,290 cows from 3,927 herds.

where REL_{EBV} is the reliability of the EBV and REL_{PA} is the reliability of the expected value of the animal (parent average).

DNA Extraction and Genotyping

Genomic DNA was extracted from sperm with a Qiagen M48 robotic station using the MagAttract DNA Mini M48 kit (Qiagen, Venlo, the Netherlands) according to the instructions of the manufacturer. The DNA concentration was determined using PicoGreen (Quand-iT PicoGreen; Life Technologies Corp., Grand Island, NY) according to the instructions of the manufacturer.

Single nucleotide polymorphism genotypes used were provided by the Dutch Cattle Improvement Cooperative (CRV, Arnhem, the Netherlands). Genotypes for 37,869 SNP were available, of which 464 SNP were not mapped to any of the 29 chromosomes. The SNP were mapped using the bovine genome assembly Btau_4.0. Following quality control checks, the SNP retained had a high call rate (>95%), minor allele frequency minimum of 0.025%, and differed 0.15 units from their expectation not to be in Hardy-Weinberg disequilibrium.

Multiple SNP Analysis

The multiple SNP analysis was performed using the following model (Meuwissen and Goddard, 2004):

$$Y_i = \mu + \sum_{j=1}^{37869} (q_{ij1} + q_{ij2})v_j + sire_i + e_i,$$

where Y_i is the DRP of the individual sire i ; μ is the general mean; v_j is the scale parameter of the QTL effect of the SNP at putative QTL position j ; $q_{ij1} + q_{ij2}$ is the size of the QTL effect for the paternal (maternal) allele of sire i at SNP j , drawn from a standard normal distribution $N(0,1)$; $sire_i$ is the random polygenic effect of sire i ; and e_i is the random residual component. The following assumptions were made for the random effects:

$$sire \sim N(0, \mathbf{A}\sigma_a^2),$$

$$\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2),$$

where **sire** is the vector included in the polygenic effects, **e** is the vector of the residual effects, **A** is the relationship matrix, σ_a^2 is the polygenic variance, **I** is the identity matrix, and σ_e^2 represents the residual variance. The prior mixture distribution was

$$v_j \sim \begin{cases} N(0, \sigma_v^2); & \text{with probability } \pi_0 \\ N\left(0, \frac{\sigma_v^2}{100}\right); & \text{with probability } \pi_1 = 1 - \pi_0 \end{cases},$$

where the null distribution modeled the majority of SNP with (virtually) no effect using the prior setting $\pi_0 = \frac{29}{37,869} = 0.001$, assuming 29 additive and independent QTL affecting the trait across the 29 chromosomes. The second distribution modeled SNP with large effects using the prior setting $\pi_1 = 1 - 0.001 = 0.999$. The variance of v_j (σ_v^2) was sampled from a scaled inverse chi-squared distribution with a prior variance. This prior variance was calculated as the additive genetic variance, divided by 29 (Calus et al., 2009). Unequal variances were assumed across SNP.

The presence of a QTL at SNP j was sampled from a Bernoulli distribution with probability equal to

$$\frac{P(v_j | \sigma_v^2) \times \Pr_j}{P(v_j | \sigma_v^2) \times \Pr_j + P\left(v_j \left| \frac{\sigma_v^2}{100} \right.\right) \times (1 - \Pr_j)},$$

where $P(v_j | \sigma_v^2)$ is the probability of v_j from $N(0, \sigma_v^2)$ (i.e., $\frac{1}{\sqrt{2\pi\sigma_v^2}} e^{-\frac{v_j^2}{2\sigma_v^2}}$), and $\Pr_j$ is the prior probability of the

presence of a QTL at SNP j . More details on the prior distributions and the full conditional distributions can be found in (Meuwissen and Goddard, 2004) and (Calus et al., 2008). The multiple SNP analysis was performed using a Markov chain Monte Carlo method using Gibbs sampling to obtain posterior estimates for all the effects in the model. The Gibbs sampler ran for 100,000 iterations and 10,000 iterations were removed as burn-in. In total, 4 chains were performed.

Significance Threshold

Bayes factors (BF) were calculated to determine the threshold on the posterior probabilities for the selection of significant associations by the following formula:

$$BF = \frac{\hat{p}_i / (1 - \hat{p}_i)}{(\pi_1 / \pi_0)},$$

where the estimated posterior probability was denoted by $\hat{p}_i$ and prior probabilities by π_0 and π_1 . Guidelines of Kass and Raftery (1995) were used to judge BF: a value above 3.2 is substantial, a value above 10 is strong, and a value above 100 is decisive.

Linkage Disequilibrium Analysis

Linkage disequilibrium (LD) was calculated according to Hill and Robertson (1968) for the SNP found to be strongly associated to MAP-specific antibody response in milk (BF >10) in this study with 200 surrounding SNP. The SNP found to be associated was located in the middle. The SNP were considered to be linked at a degree of LD (r^2) of at least 0.7.

Variance Explained by SNP

The proportion of genetic variance explained by a SNP was calculated using the following formula assuming no dominance:

$$\sigma_{SNP}^2 = 2 \times p \times q \times a^2,$$

where p was the allele frequency of the first allele of the SNP, q was the allele frequency of the second allele of the SNP, and a was the allele substitution effect. The allele substitution effect was calculated by the difference in estimated effects of both alleles ($v_j \times q_{i|1} - v_j \times q_{i|2}$). The proportion of genetic variance was calculated for the SNP found to be strongly associated to MAP-specific antibody response in milk (BF >10) in this study itself and for the SNP with 50 surrounding SNP.

The SNP found to be associated was located in the middle.

RESULTS

Genome-wide association analysis identified 4 chromosomal regions strongly associated with MAP-specific antibody response in milk on 4 chromosomes (Table 1). Genome-wide plots displaying the BF of 4 separate chains and average BF over 4 chains for different positions on the genome are shown in Figure 2.

Two SNP showed association with MAP-specific antibody response in milk in 4 chains. On chromosome 4, *ss66537488* at 51,425,144 bp showed association with an average BF of 81.5 (minimum = 32.9, maximum = 110.0). On chromosome 18, *ss61532938* at 22,546,064 bp showed association with an average BF of 21.8 (minimum = 16.9, maximum = 27.0).

Sixteen SNP showed association with MAP-specific antibody response in milk in 2 out of 4 chains. Although not significantly associated in all 4 chains of the analysis, the chromosomal region around the SNP may be in association with the trait of interest. Multiple linked SNP in one association area are drawn randomly from the null distribution of small effects or from the second distribution with SNP of large effects randomly, which can cause differences in estimates between chains. Single SNP may not show association in all of the chains and may not seem significant, but when a group of linked SNP is considered, the association area may often be found to have a high probability. Linkage disequilibrium analysis showed that of 16 putative associations, 3 sets of SNP strongly associated with MAP-specific antibody response in milk could be identified located in 2 chromosomal regions. First, on chromosome 15, *ss61536244* at 60,257,450 bp showed association with an average BF of 8.0 (minimum = 1.7, maximum = 15.3) and *ss61536232* at 60,260,849 bp showed association with an average BF of 14.7 (minimum = 0.8, maximum = 42.8). The degree of LD (r^2) between *ss61536244* and *ss61536232* was 0.7. Significant LD was also identified for *ss61536244* and *ss61536232* with *ss61536234* at 60,257,030 bp, *ss61536246* at 60,257,679 bp, and *ss61531353* at 60,733,532; $r^2 = 0.7$). Apparently, genetic variation of the chromosomal region associated with MAP-specific antibody response in milk was not attributed to *ss61536244* or *ss61536232* in all 4 chains, but genetic variation was attributed to a set of 5 SNP that were in high LD, indicating a chromosomal region associated with MAP-specific antibody response in milk on chromosome 15. Second, on chromosome 28, *rs41648898* at 11,839,054 bp showed association with an average BF of 7.2 (minimum = 1.0, maximum = 15.6). Significant LD was identified for *rs41648898*

Table 1. Single nucleotide polymorphisms found to be associated with *Mycobacterium avium* subspecies *paratuberculosis* (MAP)-specific antibody response in milk, with position (bp) on the *Bos taurus* chromosome (BTA), SNP identification (ID), Bayes factor, proportion of variance explained by the SNP (*Var_{SNP}*), and genes found in the 1 Mbp region

BTA	Position (bp)	SNP ID ¹	<i>Var_{SNP}</i> ³			Length of region (bp)	Length of chromosome (bp)	Genes found in 1 Mbp region
			Bayes factor ²	1 ⁴	50 ^{4,5}			
4	51,425,144	ss66537488	81.5	1.78E-02	1.98E-02	2,513,939	124,012,344	<i>DLD</i> , <i>Q28899</i> , <i>LAMB4</i> , <i>Q6Q146</i> , <i>PNPLA8</i> , <i>LOC784535</i> , <i>Q2YDK7</i> , <i>Q17QP5</i> , <i>EPDR1</i>
15	60,257,030–60,733,532	ss61536243 ss61536244 ss61536246 ss61536232 ss61531353	—	—	8.71E-04	3,999,824	84,598,801	<i>KCNA4</i> , <i>FSHB</i> , <i>LOC787432</i> , <i>A5P77</i> , <i>CK046</i>
18	22,546,064	ss61532938	21.8	6.83E-04	1.11E-03	23,010,241	66,118,950	<i>A2VDX5</i> , <i>IRX5</i>
28	11,823,511–11,839,054	rs41648897 rs41648896 rs41648895 rs41648899	—	—	4.81E-04	3,658,610	45,855,377	<i>ACM3</i> , <i>Q3SX15</i> , <i>ENSBTAG00000018960</i> , <i>ZNF25</i> , <i>ZNF334</i> , <i>LOC534200</i> , <i>ENSBTAG00000013592</i> , <i>BMS1</i> , <i>RET</i> , <i>C5GALNACT</i> , <i>Q5E957</i>

¹Single nucleotide polymorphism ID as displayed in the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP>).

²Average Bayes factor over 4 chains.

³Proportion of variance explained by the SNP and 50 adjacent SNPs, together with the length of the chromosome segment covered and the length of the chromosome.

⁴The *Var_{SNP}* is expressed as percentage of additive genetic variance and averaged over 4 chains.

⁵The SNP found to be associated with MAP-specific antibody response in milk was located in the middle.

with *rs41648897* at 11,823,511 bp and *rs41648896* at 11,823,594 bp ($r^2 = 0.8$), *rs41648895* at 11,823,663 bp, and *rs41648899* at 11,838,800 bp ($r^2 = 0.9$). Equal to chromosome 15, genetic variation of the chromosomal region associated with MAP-specific antibody response in milk was not attributed to *rs41648898* in all 4 chains but genetic variation was attributed to a set of 3 SNP that were linked, indicating a chromosomal region associated with MAP-specific antibody response in milk on chromosome 28. The remaining 13 SNPs, being putative associations, are shown in Table 2. In total, SNPs found to be strongly associated with MAP-specific antibody response in milk explained 0.021% of the genetic variation averaged over 4 chains.

DISCUSSION

Numerous studies have been carried out to increase the understanding of genetic variation involved in susceptibility and resistance to Johne's disease. Although the presence of genetic variation in the trait of interest has been demonstrated, the identification of genes contributing to the genetic variance is far from complete. Different approaches have been applied to identify genes and chromosomal regions, including testing of candidate genes and linkage analysis using low-density markers. Following the publication of the bovine genome sequence and the development of the high-density SNP chip, GWA analysis revealed information on several genomic regions involved in susceptibility to Johne's disease (Gonda et al., 2007; Settles et al., 2009; Minozzi et al., 2010; Pant et al., 2010; Kirkpatrick et al., 2011). Our study showed 4 chromosomal regions associated with susceptibility to Johne's disease on chromosome 4, 15, 18, and 28 and 13 chromosomal regions putatively associated with Johne's disease susceptibility on chromosome 4, 14, 16, 18, 19, 20, 21, 26, 27, and 29. Putative associations usually occur in situations where 1) QTL effects are small and, therefore, difficult to identify; 2) multiple linked SNPs in one association area are drawn randomly from the null distribution of small effects or from the second distribution with SNPs of large effects randomly, which can cause differences in estimates between chains; and 3) a trait displays low heritability. In this study, we applied multiple chains of GWA analysis and linkage analysis in an attempt to create a clear picture. This helped in some instances but the multiple chains approach is not able to eliminate putative associations completely. Further research involving additional data are needed to achieve that goal.

Comparison of results with GWA studies previously conducted showed one region in common: *ss61487847* and *ss86337081* on chromosome 27 (46,630,699 bp and

Figure 2. Genome-wide plot of Bayes factors for an association of loci with *Mycobacterium avium* subspecies *paratuberculosis*-specific antibody response in milk. Deregressed proofs of sires with a minimum reliability of 70% were used as response variable and 29 additive and independent QTL were assumed to affect the trait across the chromosomes. The Gibbs sampler ran for 100,000 iterations and 10,000 iterations were removed as burn-in. In total, 4 chains were performed represented by subgraphs a, b, c, and d. The average Bayes factor over 4 chains is represented by subgraph e.

46,767,632 bp) were found only 1.3 Mbp from a SNP found by Minozzi et al. (2010). Little congruence with other studies may be due to the low heritability of the trait, the analysis of different populations, and the use of different markers. Furthermore, Johne's disease progression within an animal contains multiple stages: 1) infection of cells by MAP, 2) a cell-mediated response restricts the expansion of MAP, and 3) for those animals that progress to Johne's disease, the cell-mediated response is replaced by the humoral response. The different stages involved in the development of Johne's disease and the way the phenotype is defined may also

reflect the different chromosomal regions found to be associated with Johne's disease susceptibility. In our study, Johne's disease susceptibility is defined as having MAP-specific antibodies in milk, whereas in the other studies, susceptibility was defined as having MAP-specific antibodies in serum or fecal culture of MAP or tissue culture of MAP, or both, which may all reflect another stage of disease. Zanella et al. (2011a,b) identified loci associated with tolerance to Johne's disease. Tolerance was defined as a cow's fitness at a given level of MAP infection intensity. Moreover, in contrast to other GWA studies on Johne's disease susceptibility

Table 2. Single nucleotide polymorphisms found to be putatively associated with *Mycobacterium avium* subspecies *paratuberculosis* (MAP)-specific antibody response in milk, with position (bp) on the *Bos taurus* chromosome (BTA), SNP identification (ID), and Bayes factor (minimum and maximum value in parentheses)

BTA	Position (bp)	SNP ID ¹	Bayes factor ²
4	50,973,953	<i>ss61554144</i>	17.9 (0.3; 46.5)
14	7,018,689	<i>rs109291899</i>	7.3 (0.3; 12.2)
16	55,936,466	<i>ss61508455</i>	7.2 (0.3; 15.3)
18	30,565,399	<i>ss61529077</i>	6.4 (0.5; 12.4)
19	23,716,787	<i>rs29012539</i>	6.9 (0.4; 14.2)
20	3,680,593	<i>rs41580314</i>	5.7 (0.4; 10.9)
21	38,012,768	<i>ss61485088</i>	8.3 (0.8; 18.1)
21	45,214,439	<i>rs29010390</i>	8.0 (1.7; 15.7)
26	36,889,435	<i>ss61549364</i>	6.8 (0.5; 15.4)
27	40,240,120	<i>rs29015783</i>	15.4 (0.8; 34.2)
27	46,630,699	<i>ss61487847</i>	10.8 (0.9; 18.1)
27	46,767,632	<i>ss86337081</i>	27.6 (1.2; 89.5)
29	332,626	<i>rs29018684</i>	7.0 (1.2; 13.0)

¹Single nucleotide polymorphism ID as displayed by NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP>).

²Average Bayes factor over 4 chains.

(Gonda et al., 2007; Settles et al., 2009; Minozzi et al., 2010; Pant et al., 2010; Kirkpatrick et al., 2011), a multiple SNP GWA approach was used. With multiple SNP analysis, simultaneous adjustment is made for all of the genetic variation that is captured by all SNP. This decreases the residual genetic variance, which is expected to result in higher power to detect other SNP associated with the trait of interest. This increase in power is similar to the principles of multiple QTL mapping (Jansen, 1993) and can lead to the detection of new chromosomal regions associated with the trait of interest, which might not be detected in a single-SNP analysis and may explain differences found between studies. Additionally, 2 simulation studies (Sillanpää and Arjas, 1998; Uleberg and Meuwissen, 2007) showed that the confidence interval became smaller when information from all QTL positions was used in multiple-QTL analysis compared with single-QTL analysis.

The sensitivity of the ELISA used in this study is low, especially for cows in early stages of disease. Low test sensitivity influences the number of infected cows detected by the test. Improvement of diagnosis by ELISA can be achieved by multiple testing. In this study, DRP of sires with a minimum reliability of 70% were used as input for the GWA analysis. Breeding value estimation was performed for sires based on ELISA test results of large numbers of daughters and, therefore, despite the low sensitivity of the ELISA, breeding values of sires reflect differences between sires in susceptibility to paratuberculosis defined as MAP-specific antibody response in milk.

Nine genes are located within 1 Mbp of the significant SNP on chromosome 4. Genes possibly involved in Johne's disease susceptibility and resistance from a biological point of view are suggested to be laminin β 4

(*LAMB4*) and dihydrolipoamide dehydrogenase (*DLD*). The presence of laminin increased interaction of MAP with epithelial cells in vitro (Lefrançois et al., 2011). Therefore, differences in *LAMB4* genotype in cattle may result in differences in development of infection occurring in the early stages. The *DLD* gene codes for an enzyme called dihydrolipoamide dehydrogenase in humans. Dihydrolipoamide dehydrogenase forms a subunit called the E3 component that is shared by several enzyme complexes. Branched-chain α -keto acid dehydrogenase, or BCKD, is one of the enzyme complexes that include the E3 component. The BCKD enzyme complex is responsible for one step in the normal breakdown of 3 AA: leucine, isoleucine, and valine, which are obtained from the diet. The breakdown of these AA produces energy beneficial for metabolic processes. In cattle, differences in *DLD* genotype and, therefore, the presumable ability to utilize nutrients may explain why some animals develop to the clinical stage of Johne's disease and others, despite intestinal lesions, do not. The *WNT2* gene is located on 1.7 Mbp (53,163,592–53,207,516 bp) of *ss66537488* on chromosome 4. The *WNT* genes encode small secreted proteins and *WNT* signaling is involved in virtually every aspect of embryonic development and also controls homeostatic self-renewal in several adult tissues, including the gut (Clevers, 2006). In animals infected with Johne's disease, MAP has the ability to cause granulomatous lesions in the distal part of the ileum, which may suggest a role for the *WNT* gene. Multiple genes are located within 1 Mbp of the significant SNP on chromosomes 15, 18, and 28. However, none of these genes is likely to be a candidate gene for involvement in Johne's disease susceptibility based on their function as described in the literature.

Five SNP found to be associated with Johne's disease susceptibility explained only a small part of the genetic variation (0.021%) in the population. Results of our study suggest that many QTL have a small effect and, therefore, breeding values estimated based on all SNP simultaneously might be more appropriate for selection to decrease MAP infection in the Holstein cattle population.

CONCLUSIONS

This study provides evidence for chromosomal regions involved in MAP-specific antibody response in milk. A multiple SNP GWA approach identified 5 SNP associated with MAP-specific antibody response in milk distributed over 4 chromosomal regions (chromosome 4, 15, 18, and 28) and 13 putative chromosomal regions. This knowledge contributes to the current understanding of genetic variation involved in Johne's disease susceptibility and facilitates control of Johne's disease and improvement of health status by breeding.

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