Original article

Quantitative trait loci linked to *PRNP* gene controlling health and production traits in INRA 401 sheep

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Abstract – In this study, the potential association of PrP genotypes with health and productive traits was investigated. Data were recorded on animals of the INRA 401 breed from the Bourges-La Sapinière INRA experimental farm. The population consisted of 30 rams and 852 ewes, which produced 1310 lambs. The animals were categorized into three PrP genotype classes: ARR homozygous, ARR heterozygous, and animals without any ARR allele. Two analyses differing in the approach considered were carried out. Firstly, the potential association of the PrP genotype with disease (*Salmonella* resistance) and production (wool and carcass) traits was studied. The data used included 1042, 1043 and 1013 genotyped animals for the *Salmonella* resistance, wool and carcass traits, respectively. The different traits were analyzed using an animal model, where the PrP genotype effect was included as a fixed effect. Association analyses do not indicate any evidence of an effect of PrP genotypes on traits studied in this breed. Secondly, a quantitative trait loci (QTL) detection approach using the *PRNP* gene as a marker was applied on ovine chromosome 13. Interval mapping was used. Evidence for one QTL affecting mean fiber diameter was found at 25 cM from the *PRNP* gene. However, a linkage between *PRNP* and this QTL does not imply unfavorable linkage disequilibrium for PRNP selection purposes.

association analysis / production trait / health trait / gene *PRNP* / sheep

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1. INTRODUCTION

Transmissible spongiform encephalopathies (TSE) are a group of fatal prion diseases. Scrapie is one TSE affecting sheep and goats. Susceptibility to scrapie in sheep is mainly controlled by the *PRNP* gene. The main polymorphic *PRNP* gene segments associated with natural scrapie are codons 136 (T, A, V), 154 (R, H) and 171 (R, Q, H, K) (*e.g.* [9]). The ARR allele is associated with resistance and VRQ is associated with susceptibility (*e.g.* [5,6]).

Even though to date no scientific evidence indicates that scrapie poses a risk to human health, a national selection program for scrapie resistance has been implemented in France as a precautionary measure in agreement with the European commission decision 2003/100/EC. The inclusion of PrP genotypes in breeding objectives might cause undesirable changes in other economically important production traits if the *PRNP* gene is involved in any of these traits. Thus, potential associations between production traits and PrP genotypes have to be studied.

Most results do not indicate any evidence of an effect of the PrP genotype on production traits [1, 2, 4, 18, 20, 22, 23]. These population studies, based on direct comparison of PrP genotypes for different traits of interest, allow the evaluation of a putative pleiotropic effect of the *PRNP* gene on these traits and the existence of a hypothetical gene in linkage disequilibrium with the *PRNP* gene. However, even in the case of linkage equilibrium, if a ram carries a favorable PrP allele on the same chromosome as that of an unfavorable allele of a gene (a QTL) controlling a production trait, its progeny inheriting this PrP allele will also inherit the unfavorable allele for the production trait. A QTL detection approach can be used to ascertain if such a gene close to the *PRNP* gene exists and to find its localization. The aim of this work was to investigate the potential associations of PrP genotypes with production (wool and carcass) and disease (*Salmonella* resistance) traits. First, association analyses were performed. Second, a QTL detection approach using the *PRNP* gene as a marker was applied on ovine chromosome 13 where the *PRNP* gene is located.

2. MATERIALS AND METHODS

Data were obtained from the Bourges-La Sapinière INRA experimental farm and were first described by Moreno *et al.* [13, 14]. The QTL experiment included animals of the synthetic INRA 401 breed: 30 rams and 852 ewes, producing 1310 lambs. This population was created to allow QTL detection for susceptibility to *Salmonella* infection, wool and carcass traits. After weaning,

lambs were put in conditions of intensive fattening. The lambs were inoculated intravenously with 10^8 *Salmonella abortusovis* Rv6 (vaccinal strain) at an average age of 113 days and mid-side wool samples were taken. They were slaughtered 10 days after the inoculation. The lambs were weighed at birth, weaning, vaccination and 2 days before slaughtering.

Several *Salmonella* resistance traits were measured at inoculation and/or slaughtering: specific antibody titers ($IgG1_0$, IgM_0, Var-IgG1, Var-IgM), body weight loss (loss-Wt), relative spleen weight (WtrS), pre-scapular node weights (WtLN, WtRN), and counts of *Salmonella* persisting in these organs (BgLN, BgRN, BgS). The traits showing a strong deviation from normality were transformed using a logarithmic transformation. More details about the different measures have been presented in Moreno *et al.* [14]. Wool samples were analyzed by Optical Fiber Diameter Analysis in order to determine the mean (MFD) and the coefficient of variation (CVFD) of the fiber diameter. The staple length (SL) was also measured. These measurements were first described by Ponz *et al.* [17]. Carcass measurements were taken from cold carcasses: characteristics of muscle mass (length, width, surface area of the *longissimus dorsi* muscle (Mus-s)), skeleton development (bone), carcass fatness (backfat thickness (backfat), kidney fat (Int-fat)) and the carcass yield (Yield). At slaughtering, a subjective carcass conformation score (Conform) was used. More details about the definition of the traits can be found in Moreno *et al.* [13].

For the *PRNP* gene, the animals were genotyped using the Taqman method [11]. Only four alleles were described in the INRA 401 breed: ARR, AHQ, VRQ, and AR-. The AR- allele includes ARQ and ARH alleles, which were confounded by the genotyping technique used. In this breed, the *PRNP* allelic frequencies reported by Palhière *et al.* [15] were 0.36, 0.07, 0.46, and 0.11 for ARR, AHQ, AR-, and VRQ alleles, respectively.

2.1. Association studies

To estimate a putative effect of the ARR allele, the animals were categorized into three classes: ARR homozygous (ARR/ARR), ARR heterozygous (ARR/—) and animals without the ARR allele (—/—). The trait values of the progeny (1310 lambs) were recorded. The data set included 1042, 1043 and 1013 records for the *Salmonella* resistance, wool and carcass traits, respectively. All these animals were genotyped (Tab. I).

The linear model used to analyze the data was

$$y_{ijkl} = \mathbf{x}_i\boldsymbol{\beta} + PrP_j + a_k + e_{ijkl},$$

Table I. Number of animals for each genotyped class for *Salmonella* resistance, wool and carcass traits.

Traits	ARR/ARR	ARR/—	—/—
Salmonella resistance	196	507	339
Wool	198	505	340
Carcass	190	487	336

where y_{ijkl} is the observation of the trait, $\mathbf{x}_i$ is an incidence row vector relating fixed effects $\boldsymbol{\beta}$ to the ith record, PrP_j is the PrP effect ($j = 1, 3$), a_k is the random additive genetic effect of the animal, and e_{ijkl} is the random residual effect. The fixed effects included in $\boldsymbol{\beta}$ (sex, batch, birth-rearing type, age class at vaccination, weight class at vaccination, weight variation class between vaccination and slaughter) changed according to the trait. These effects were similar to those used in previous studies [13, 14, 17]. The analyses were performed using AsReml [8]. In the analyses, pedigrees included all available ancestors of the animals in the data set (five generations).

Multiple testings were taken into account for different PrP genotype effect and traits. For contrast between the different levels of the PrP genotype effect, significances were based on the *t*-test considering the Bonferroni correction. This approach is conservative if the hypothesis tests are not independent (as is the case, when making all possible pairwise comparisons among PrP-genotype levels). For traits, a correction factor f was calculated using a principal component analysis applied to the matrix of genetic correlations between traits. Fifty-five percent of the variance was explained by 5, 2 and 5 eigenvalues for the *Salmonella* resistance, wool and carcass traits, respectively. Thus, f was equal to 12 (5 + 2 + 5). A similar correction was applied in QTL detection. In association studies, a significant association was declared if $P < 0.05/(3^*12)$.

2.2. QTL studies

Since the *PRNP* gene is located on sheep chromosome 13, this chromosome was chosen for further study. Parents and offspring were genotyped for four microsatellites on chromosome 13 (IL2RA, ILSTS0059, BMS1669, OARAE0016) and for the *PRNP* gene. Genetic distances between those five genes on the chromosome were estimated with the CRIMAP (http://linkage.rockefeller.edu/soft/crimap) software. To detect QTL, interval mapping analyses were performed using QTLMAP [7] on phenotypic measurements pre-corrected for known fixed effects (see below). Confidence intervals for the location of the QTL were calculated by bootstrapping [21].

Table II. Estimated contrasts (and standard errors) between PrP genotype classes for *Salmonella* resistance, wool and carcass traits.

Traits	ARR/ARR – ARR/—	ARR/ARR – —/—	ARR/— – —/—
Salmonella resistance			
IgM$_0$ (%)	0.661 (0.586)	−0.450 (0.721)	−1.112 (0.528)
Var-IgM (%)	−2.426 (2.901)	−1.241 (3.493)	1.185 (2.589)
Log-IgG1$_0$ (%)	−0.008 (0.023)	−0.004 (0.028)	0.004 (0.021)
Log-Var-IgG1 (%)	0.032 (0.019)	0.019 (0.023)	−0.014 (0.017)
Log-BgLN (g^{-1})	−0.044 (0.101)	0.035 (0.118)	0.079 (0.088)
Log-BgRN (g^{-1})	−0.023 (0.102)	0.163 (0.121)	0.186 (0.090)
Log-BgS (g^{-1})	−0.027 (0.031)	−0.026 (0.035)	0.001 (0.027)
Log-WtLN (g)	−0.001 (0.011)	−0.009 (0.014)	−0.008 (0.010)
Log-WtRN (g)	−0.006 (0.010)	0.001 (0.012)	0.007 (0.009)
Log-WtrS (mg·kg^{-1})	0.003 (0.006)	0.003 (0.007)	0.000 (0.005)
Log-loss-Wt (kg)	0.006 (0.012)	−0.003 (0.015)	0.003 (0.011)
Wool			
SL (mm)	−1.077 (1.196)	−0.833 (1.439)	0.243 (1.066)
MFD (micron)	−0.069 (0.198)	−0.121 (0.241)	−0.051 (0.177)
CVFD (%)	−1.488 (0.908)	−1.772 (1.117)	−0.284 (0.818)
Carcass			
Conform (Score)	−0.065 (0.117)	0.046 (0.140)	0.111 (0.104)
Width (cm)	0.059 (0.056)	0.077 (0.067)	0.018 (0.049)
Length (cm)	−0.006 (0.169)	0.006 (0.204)	0.012 (0.150)
Bone (mm)	−0.036 (0.123)	0.147 (0.146)	0.183 (0.108)
Yield (%)	0.090 (0.181)	−0.195 (0.221)	−0.286 (0.161)
Backfat (mm)	−0.096 (0.139)	−0.029 (0.115)	0.066 (0.102)
Int-fat (Score)	0.061 (0.052)	0.125 (0.061)	0.065 (0.046)
Mus-s (cm^2)	0.238 (0.161)	0.243 (0.225)	0.005 (0.184)

Log: logarithmic transformation applied to non-normal data.

The chromosome-wise threshold was obtained by permutation of the phenotypes [3] within families. For traits, the correction for multiple tests was calculated as in the association studies. In QTL studies, a significant association was declared if $P < 0.05/12$.

3. RESULTS

3.1. Association analyses

The estimated differences in *Salmonella* resistance and production (wool and carcass) traits among ARR/ARR, ARR/—, and —/— genotypes are presented in Table II. None of the differences in these traits was statistically significant after Bonferroni correction.

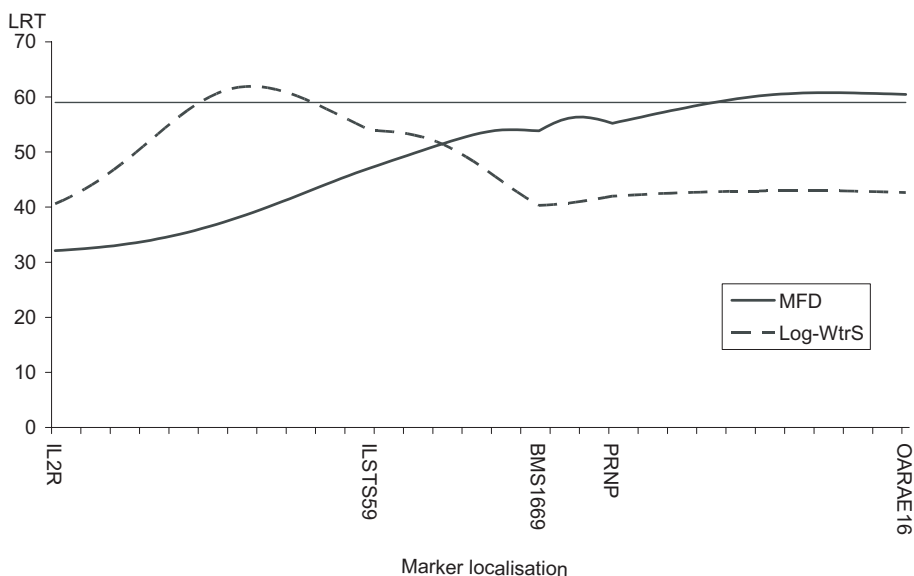**Figure 1.** Chromosomal location of markers on chromosome 13 (CRIMAP estimations).

Figure 2. Likelihood profile of QTL affecting MFD and WtrS traits on chromosome 13. LRT: likelihood ratio test; MFD: mean fiber diameter; WtrS: relative spleen weight. The thin horizontal line represents the 5% chromosomal threshold estimated by permutations [3].

3.2. QTL studies

The CRIMAP estimated marker locations (Fig. 1) are close to published maps (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9940).

For QTL analyses, Figure 2 shows the likelihood profile obtained on chromosome 13. Assuming a 5% chromosomal threshold, two significant results were found on chromosome 13 on an indirect trait of *Salmonella* resistance: the relative spleen weight (WtrS), and a wool trait, the mean of the fiber diameter (MFD).

The effects of the QTL were of 1.3 and 2.1 phenotypic standard deviation for WtrS and MFD respectively. The QTL for WtrS and MFD were located at positions 36 cM and 110 cM, respectively. The distance between the most

likely position of the QTL and *PRNP* gene was of 49 cM and 25 cM for WtrS and MFD respectively.

The power of the QTL detection design was calculated using the *powdaug.exe* program (Bovenhuis H., personal communication). For this design, the power was equal to 95% for a QTL effect of 47% of the phenotypic standard deviation for both traits: WtrS and MFD.

4. DISCUSSION

The results of association studies showed no evidence of a pleiotropic effect of the *PRNP* gene on *Salmonella* resistance, wool and carcass traits. At the population level, these analyses suggest that no gene affecting the traits is in linkage disequilibrium with the *PRNP* locus on chromosome 13.

Salmonella enterica serovar Abortusovis is pathogenic and can cause important health problems in sheep: abortion of ewes and death of lambs [16]. A QTL was found on relative spleen weight at the 5% chromosomal level. However, Moreno *et al.* [14] showed that the correlations of this indirect trait with antibody response and bacterial count traits were moderate (approximately 0.3). Since no QTL was found on antibody response and bacterial count traits, the small effect of the QTL for relative spleen weight is more probably due to differences in physiological and anatomical characteristics rather than in *Salmonella* resistance. Moreover, the IL-2Ra gene, where one of our markers is located, is clearly involved in host response to intracellular pathogens like *Salmonella* [12]. However, it is located at more than 50 cM from the *PRNP* gene and from this point of view does not represent a likely candidate for our QTL. Because no effect of the *PRNP* gene nor any QTL of *Salmonella* resistance close to *PRNP* was found, the selection for scrapie resistance does not represent a risk of introducing susceptibility to *Salmonella* disease.

QTL influencing wool production and quality have been identified in the INRA 401 breed [19]. No selection on wool characteristics has been made on the INRA 401 breed. The selection objectives of this breed include mainly maternal and meat traits. Whatever the breeding objectives, it is interesting to examine the possible relationship between *PRNP* locus and wool characteristics. This flock provides the opportunity to detect QTL affecting wool traits and to localize the QTL relative to the *PRNP* locus. For MFD, a QTL was found to be weakly linked to the *PRNP* gene (25 cM). This region had not been identified among the chromosomal regions associated with wool traits [19]. A linkage between *PRNP* and this QTL does not presuppose unfavorable linkage disequilibrium at the population level.

Carcass traits are economically important traits in livestock. Selection of animals with "good" carcass composition is of great significance to breeders and consumers. The carcass traits analyzed in this study were carcass weights, carcass conformation score, carcass dimension, fat and skeleton measurements, and surface area of musculus *longissimus dorsi*. A recent study [10] provides, as the authors commented, a limited evidence of associations between the *PRNP* gene and carcass and meat traits. In the present paper, these associations were not confirmed.

From the results of association and QTL studies, it can be concluded that selection on PrP genotypes should not have an effect on the health and production traits studied in this breed.

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