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Note

Identification of New Quantitative Trait Loci (Other Than the *PRNP* Gene) Modulating the Scrapie Incubation Period in Sheep

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ABSTRACT

Although susceptibility to scrapie is largely controlled by the *PRNP* gene, we have searched for additional genomic regions that affect scrapie incubation time in sheep, using two half-sib families with a susceptible *PRNP* genotype and naturally infected by scrapie. Quantitative trait loci were detected on OAR6 and OAR18.

TRANSMISSIBLE spongiform encephalopathies (TSE) are fatal neurodegenerative diseases that affect various mammalian species (humans, mice, cattle, sheep, goat., etc.). TSE are characterized by the accumulation of an abnormal form of a host-encoded protein in the central nervous system of affected individuals. Normal and abnormal forms are noted PrPC and PrPSc, respectively. In sheep, mice and humans, these diseases are genetically controlled and a large part of the natural susceptibility to TSE depends on inherited alleles of the *PRNP* gene encoding the PrP protein (Carlson *et al.* 1986; Goldmann *et al.* 1990; Palmer *et al.* 1991). However, not all individuals with similar *PRNP* alleles develop the disease, and if they do, they can present very different incubation periods. In fact, in addition to the *PRNP* locus, other environmental and genetic factors may act on the animal susceptibility.

Recently, some loci other than *PRNP* modulating susceptibility to prion diseases have been detected in mice and cattle. In mice, several studies using inbred crosses have detected loci affecting bovine spongiform encephalopathy (BSE) incubation time (Manolakou *et al.* 2001; Lloyd *et al.* 2002) and scrapie incubation time (Stephenson *et al.* 2000; Lloyd *et al.* 2001; Moreno *et al.* 2003). Some of these loci have been detected in more than one experiment on chromosomes 2, 4, 6, 7, 8, and 11 (Moreno *et al.* 2003). In cattle, data from BSE infected/healthy animals collected in half-sib families bred on farms have been analyzed with two approaches. First, the transmission-disequilibrium test (TDT) method permitted the detection of QTL on *Bos taurus* chromosomes (BTA) BTA5, -10, and -20 (Hernandez-Sanchez *et al.* 2002). Second, linkage and association methodologies led to the detection of QTL on BTA1, -6, -13, -17, -19, and -X (Zhang *et al.* 2004).

Díaz *et al.* (2005) have shown that, in a Romanov sheep flock naturally infected by scrapie, the *PRNP* gene explains only part of the total genetic variance of the survival time. This flock is maintained at the Langlade experimental farm and has been contaminated by scrapie with a very high incidence (near 30%) since 1993 (Elsen *et al.* 1999a). In this article, our aim was to identify loci that modulate scrapie incubation time in two sire families bred in the Langlade farm. ARQ/VRQ animals were produced by artificial insemination using two sires carrying the VRQ/VRQ susceptible genotype and dams carrying at least one ARQ allele.

The ARQ/VRQ genotype was determined by sequencing exon 3 of the *PRNP* gene, which revealed no other polymorphism, except silent mutations at codons 231 and 237. This genotype was chosen because it is known to be the most susceptible to scrapie after VRQ/

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TABLE 1

Scrapie status of animals in the sheep population

Scrapie status	No. of animals	
	Sire family 1	Sire family 2
Scrapie dead animals: positive histopathology, clinical signs, and positive immunohistochemistry	62	67
Infected scrapie animals (censored data): negative histopathology, no clinical sign, and positive immunohistochemistry	1	1
Uninfected scrapie animals: negative histopathology and immunohistochemistry	6	0
Total	69	68

A total of 137 ARQ/VRQ animals were bred: 69 animals were produced by sire 1 during spring 1998, and 68 animals by sire 2 during autumn 1999. The sex ratio was 1.4 in family 1 and 1.1 in family 2. The experimental population was observed for 8 years until the death of all animals. Animals were sacrificed when they were in the final phase of scrapie. The first symptoms of scrapie (mainly pruritus and lack of gait coordination) were recorded weekly by the technicians in charge of animal husbandry. Within 2 weeks after detection of clinical signs, animals were sent to the Veterinary School in Toulouse for euthanasia to establish a final diagnosis by brain histopathology, which reveals vacuolization of neurones specific to prion diseases in neuro-anatomical regions of the central nervous system. When the diagnosis was unclear, to check for scrapie infection in the brain, immunohistochemistry (IHC) of the obex was performed using two anti-PrP antibodies (polyclonal antiserum R521 and MAb 2G11) (Andreoletti *et al.* 2002a). "Scrapie dead animals" were animals culled for scrapie clinical signs and having a positive histopathology or IHC test and animals culled for reasons other than scrapie (other diseases, broken legs, etc.) but with a positive histopathology test. In fact, the histopathology test is positive at the final stage of the disease (Jeffrey and Gonzalez 2004). Animals without clinical signs and with a negative histopathology test but with a positive IHC test were considered as scrapie infected but their death was not due to scrapie (scrapie incubation time is censored). In fact, the IHC test in the brain is positive from the middle of the scrapie incubation period for VRQ/VRQ (Andreoletti *et al.* 2000) and ARQ/ARQ genotypes (van Keulen *et al.* 2007). Thus, the kinetics of infection is probably similar for the ARQ/VRQ genotype. Uninfected scrapie animals were animals with a negative or unclear histopathology test and a negative IHC test. The following analyses were performed only on 129 scrapie dead animals.

VRQ and because VRQ/VRQ animals are too difficult to produce in a scrapie-infected farm (VRQ/VRQ dams die of scrapie during their first lambing period at the age of 2 years). Observations were recorded during 8 years and although most animals were infected (95% of the tested animals showing a scrapie-infected status in Table 1), the data revealed differences in survival time after a natural scrapie infection for this genotype (death occurred between 17 and 75 months; see Figure 1 and Table 2).

Since infection occurs mainly during the lambing period via the placenta of infected ewes (Pattison *et al.* 1972, 1974; Hoinville 1996; Andreoletti *et al.* 2002b), the scrapie infection was assumed to occur at birth for all ARQ/VRQ animals. Accordingly, the age at which clinical scrapie appears was assumed to be a good approximation of the scrapie incubation time. In family 2, the mean incubation time is 30 months, while in family 1, it is, surprisingly, 1 year longer with a wider dispersion (Figure 1 and Table 2). Comparison with the whole Langlade population showed that family 1 has a significantly longer incubation time than all other ARQ/VRQ animals raised since 1991 (C. R. Moreno, personal communication). This atypical survival time could be due to a particularly low infection pressure in 1998 or to a specific resistance of sire 1 due to genes other than *PRNP*. Because of these differences between the two families, each one was considered independently inthe following analyses.

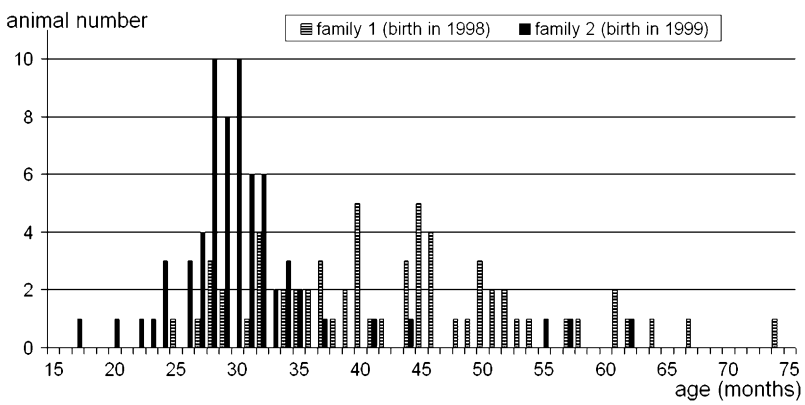

Figure 1.—Distribution of scrapie incubation time in families 1 and 2.

TABLE 2

Scrapie incubation time (months) in families 1 and 2

	No.	Mean ± SD	Minimum	Maximum
Family 1 (birth in 1998)	62	43 ± 11	25	75
Family 2 (birth in 1999)	67	30 ± 7	17	62

SD, standard deviation.

The six uninfected scrapie animals (Table 1) of family 1 were considered as missing values in the following analyses due to the uncertain scrapie infection during the birth period of these animals. The two censored animals were eliminated (Table 1) because Moreno *et al.* (2005) have shown that, when censoring is very weak, the use of QTL survival analyses is not really helpful. In this case, a classical Gaussian model was assumed to analyze the scrapie dead animals. A logarithmic transformation was used to normalize the incubation time distribution in both families for QTL analyses. To precorrect data for QTL analyses, several fixed effects were tested using the SAS GLM procedure (SAS Institute 1990) for variance analysis: sex, rearing type (natural or artificially suckled), *PRNP* maternal genotype, and scrapie status of dam. Only the rearing type was significant (>5% level) in each family. This effect of 3.6 months could be due to a lower infection pressure in artificially suckled lambs *vs.* naturally suckled lambs due to the shorter contact period with the scrapie-infected placenta during the lambing period.

No QTL was identified in family 1 but interval mapping approaches made it possible to identify two QTL regions in family 2, on OAR6 and -18. The chromosomal-wise significance levels were 1.8% for the QTL located on OAR6 and 0.7% for the QTL located on OAR18 (Figure 2). Following the definition of Lander and Kruglyak (1995), both QTL are genomewide suggestive QTL. The additive QTL effects are equal to 79 and 67% of the phenotypic standard deviation, which are equivalent to 5.7 and 4.9 months, respectively. The lack of QTL in family 1 is probably due to the high homozygosity of sire 1 in QTL regions on

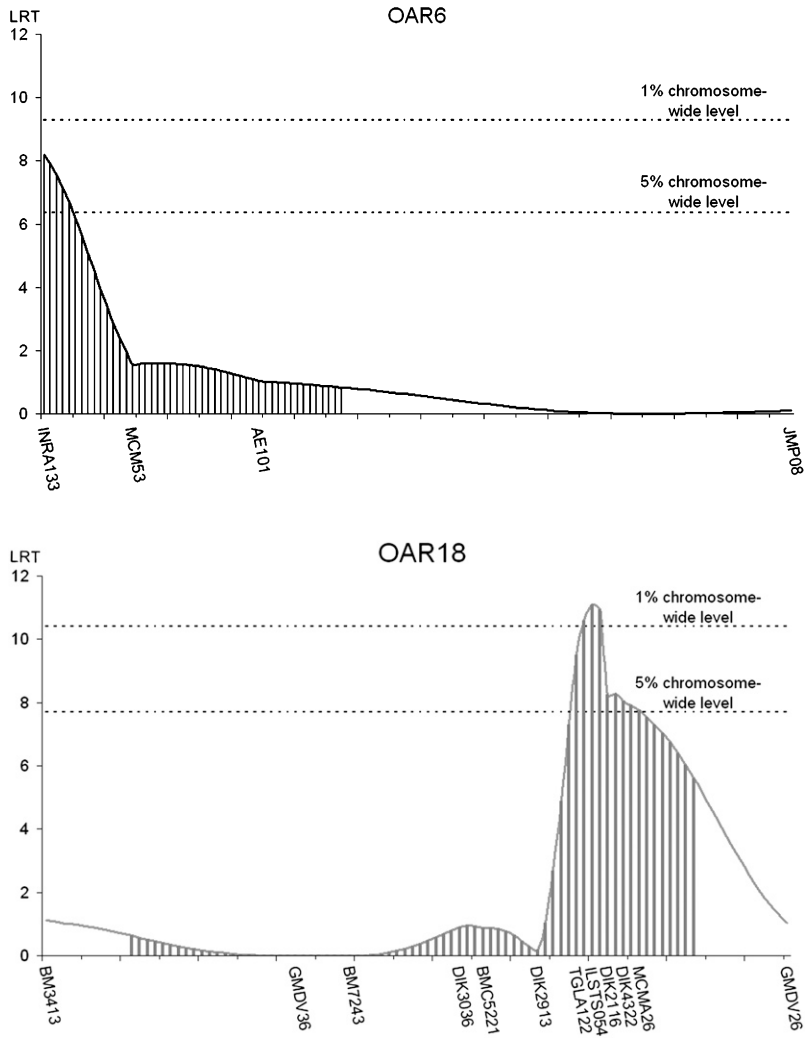

Figure 2.—Likelihood profiles of QTL-controlled scrapie incubation time of family 2 on OAR6 and -18. A total of 123 informative microsatellite markers were typed to cover 2270 cM of the 26 ovine autosomes. Subsequently, 8 additional markers were genotyped on OAR18 in the interval. Marker positions used were those estimated for the sex average map (from http://rubens.its.unimelb.edu.au/~jillm/jill.htm) except in cases when a marker was not present on the map. In that case, its position was then determined from physical map information and the relative position was calculated using the current population and the CRIMAP software (Green *et al.* 1990) and then placed on the map relative to markers of known position. QTL analyses were performed using an interval mapping method by maximum likelihood. The interval mapping analyses were performed using QTLMAP (Elsen *et al.* 1999b) on phenotypic measurements precorrected for significant fixed effects. The chromosome-wide significance level of the QTL analyses (in dotted line) were calculated using 10,000 permutations (Churchill and Doerge 1994). The confidential intervals (in hatched area) of QTL locations were constructed by bootstrapping the samples 1000 times (Visscher *et al.* 1996).

OAR6 and -18. In addition, because of a long survival time of some infected animals and the presence of some uninfected animal in family 1, the probability of a horizontal contamination after the birth for some infected animals of this family is not null.

The large confidential interval of the QTL regions makes difficult to propose candidate genes (Figure 2), but it was possible to compare them to putative orthologous regions related to TSE resistance in mice (Stephenson *et al.* 2000; Lloyd *et al.* 2001, 2002; Manolakou *et al.* 2001; Moreno *et al.* 2003) and cattle (Hernandez-Sanchez *et al.* 2002; Zhang *et al.* 2004). OAR6 and OAR18 are very conserved with BTA6 and BTA21, respectively (Cribiu *et al.* 2001). No QTL has been detected on BTA21 and the QTL region identified on OAR6 does not overlap with the QTL region on BTA6 (Zhang *et al.* 2004). Using the Ensembl program (http://www.ensembl.org/index), we determined that the OAR6 QTL region corresponds to the end of the MMU5 QTL region (Moreno *et al.* 2003) and that the OAR18 QTL region is partly common to the MMU12 QTL region (Lloyd *et al.* 2001, 2002).

In conclusion, QTL were found on OAR6 and -18 at a significant chromosome-wide level, indicating that genes outside *PRNP* affect scrapie resistance in sheep.

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