TRANSPOSITION TO SHEEP OF MOUSE QUANTITATIVE TRAIT LOCI (QTL) INFLUENCING SUSCEPTIBILITY TO PRION DISEASES

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INTRODUCTION

Transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative diseases of a number of mammalian species. TSE are characterised by the accumulation of an abnormal form of a host-encoded protein, PrP, in the central nervous system of affected individuals. In sheep, mouse and human, these diseases are genetically controlled and a large part of the natural susceptibility to TSE depends on inherited alleles of the *Prnp* gene encoding PrP protein. However, all individuals with similar PrP alleles may not contract the disease and if they do, they may have very different incubation periods, suggesting that other environmental and genetic factors influence susceptibility to TSE. This paper presents and compares several studies searching QTL, other than the PrP gene, influencing susceptibility to TSE in mice and sheep. Firstly, mouse inbred lines with defined *Prnp* alleles and different incubation periods offer the opportunity to look for genes influencing the outcome of the disease using the QTL methodology. In that way, several research groups identified genetic loci involved in mouse susceptibility to TSE (Stephenson *et al.,* 2000 ; Manolakou *et al*., 2001 ; Lloyd *et al.,* 2001 ; Moreno *et al*., in preparation). Secondly, locations of a few QTLs found in the mouse were used to determine homologous regions on the sheep genome. In these regions, microsatellite markers were typed on two half-sib families of ARQ-VRQ sheep (susceptible PrP genotype) to search QTL. The first results are presented.

MATERIALS AND METHODS

Most of information gathered from mouse studies is summarized in table 1. Details are only given for our own experiments. In the following, Stephenson *et al.* (2000), Lloyd *et al.* (2001) and Manolakou *et al*. (2001) papers are abbreviated St, Ll and Ma, respectively.

Populations. *Mice.* see table 1.
Sheep. Two half-sib families were created from 2 sires carrying the VRQ/VRQ susceptible genotype and ARQ/ARQ dams. A total of 79 animals were born in 1998, and a set of 73 animals in spring 2000. The animals were bred in a farm (Langlade, France) known to be infected at a large scale by scrapie (Elsen *et al.,* 1999a). Two binary traits and one continuous trait were recorded : presence or not of abnormal PrP (PrPsc) in tonsils, death or not of scrapie and age at death of scrapie. At present, available information is the presence or not of PrPsc in tonsils in the 2 families and the death or not of scrapie in the first family.

DNA Isolation and Genotyping. *Mice*. In our study, a panel of 472 markers was tested on DNA from the parental strains. Final genotypes were obtained for 72 markers spread throughout the genome.
Sheep. 100 microsatellites spread throughout the genome are to be tested. To date 39 markers have been typed. These markers are located across 20 chromosomes and correspond to homologous mice regions where the largest mouse QTLs have been found.

Table 1. Comparison of the designs of 4 QTL studies in mice

	Study St : Stephenson *et al.* 2000	**Study Ll : Loyd *et al.* 2001**	**Study Ma : Manolakou *et al.* 2001**	**Our study : Moreno *et al.* 2002**
Resistant parental line	**CAST/Ei** $\mu t = 172$ j	**CAST/Ei** $\mu t = 188$ j	**C57BL** $\mu t = 540$ j	**C57BL** $\mu t = 167$ j
Susceptible parental line	**SJL/J** $\mu t = 105$ j	**NZW/OlaHsd** $\mu t = 108$ j	**RIII** $\mu t = 442$ j	**RIII** $\mu t = 161$ j
Cross	**F2 :** n=163 $\mu t_F = 126$ j $\mu t_M = 129$ j	**F2 :** n=1009 $\mu t_F = 157$ j $\mu t_M = 158$ j	**BC :** n=1027 $\mu t_F = 493$ j $\mu t_M = 485$ j	**F2 :** n=282 $\mu t_F = 164$ j $\mu t_M = 171$ j
Sex effect	No	No	Yes	Yes
Infectious agent	Chandler murine **scrapie**	Chandler murine **scrapie**	**BSE** resulting from 7 cows	**scrapie** C506M3
Way of inoculation	Intracerebrally	Intracerebrally	intracerebrally	intracerebrally
Age at inoculation	6-7 weeks	-	3-8 weeks	18 weeks
End of the inoculation period	appearance of clinical signs	appearance of clinical signs	appearance of clinical signs	Death of scrapie
Number of markers	153	157	90	72

μt is the mean of incubation period, μt_F for females, μt_M for males ; n is the number of mice.

Data analysis. *Mice*. In our study, QTL were searched using the MAP MANAGER QT software (Manly, 1998) which performs both unimarker analysis and interval mapping analysis (Lander and Bostein, 1989). Genomewise significance thresholds were calculated using permutations (Churchill and Doerge, 1994). Two significance levels were considered : significant and suggestive linkage (Lander and Kruglyak, 1995).
Sheep. QTLs for binary traits were tested with threshold models. The GENMOD procedure of SAS Institute (1990) was used to realise unimarker analysis. The QTLMAP software (Elsen *et al.,* 1999b) was extended to realise an interval mapping analysis with threshold model. Significant thresholds were calculated using permutations.

RESULTS AND DISCUSSION

Mice. ***Results from our experiment.*** As a large sex effect was found on survival times, three sets of data have been analysed to search QTL : females (137 mice), males (145 mice) and all animals after correction for the sex effect (282 mice). It enables us to control for the presence or not of interaction between sex effect and a putative QTL. Four QTL out of 6 (table2) were detected at suggestive genome threshold (chromosome 4, 6, 8, 17) and 2 other QTL at significant genome threshold (chromosome 5, 7). Those QTL were not simultaneously detected for the three sets of data, excepted for the one on chromosome 7.

Table 2. Results of the 4 QTL studies in mice (lod scores of detected QTs)

	Study St : Stephenson *et al.* 2000	**Study Ll : Loyd *et al*. 2001**	**Study Ma : Manolakou *et al*. 2001**	**Our study : Moreno *et al.* 2002**
Chromosome 2	-	8.2	5.7	-
Chromosome 4	-	-	4.7	2.1^{f}
Chromosome 5	-	-	-	4.7^{f}
Chromosome 6	-	3.9	-	2^{c}, 3.2^{f}
Chromosome 7	2.2	3.6	-	3.5^{c}, 2.1^{m},2^{f}
Chromosome 8	-	-	5	1.9^{c}, 2.8^{m}
Chromosome 9	5.7	-	-	-
Chromosome 10	2.1	-	-	-
Chromosome 11	5.6	57.6	-	-
Chromosome 12	-	6.8	-	-
Chromosome 15	-	-	3.7	-
Chromosome 17	-	-	-	2.4^{m}, 2.5^{f}
Chromosome 18	2.7	-	-	-
Chromosome 19	2.5	-	-	-

$^{f, m, c}$ indicate that the analysis is realised on females, males or corrected data for sex effect.

Comparison between the 4 experimental designs. Three major differences exist between designs : the type of parental mouse strains, the inoculated infectious isolate and the measured trait (table 1). The conditions of St and Ll studies were very similar (parental strains, isolate, measured trait were identical), but were quite different from ours. The parental strains were the same for Ma and our studies but Ma used a BSE isolate whereas we used a scrapie isolate. Moreover Ma measured the length of life until the appearance of the clinical signs whereas we measured the length of life until the death of scrapie. In spite of these experimental differences, QTLs were found on identical chromosomes in several studies. As evidenced by simulation studies (not detailed here), the rather different values of lod scores for QTL located in the same areas could be explained by differences in power due to population sizes.

Identical QTLs for scrapie susceptibility were detected in several studies. A QTL on chromosome 11 was detected at the same position in St and Ll studies but was not present in our study. This QTL seems specific to the Cast/Ei line. A QTL on chromosome 7 was detected in the three scrapie studies although the genetic resources were different. However this QTL was located at very different locations (beginning of the chromosome for our and Ll studies, end of the chromosome for St study). This inconsistency could either correspond to the existence of 2 different QTLs or to large imprecision of the location. At last, on chromosome 6, a QTL with a weak effect was detected by Ll and our studies.

Some of the QTLs controlling susceptibility to scrapie and BSE are identical, other are different. The genetic determinism of BSE and scrapie resistance may be compared from Ma and our studies which used the same parental strains, but different isolates. Three common QTLs, located in overlapping intervals on chromosomes 4, 8 and 15 (this latter QTL being detected at a threshold close to suggestive threshold in our study) were found, suggesting that both diseases result from common mechanisms in infected individuals. On the opposite, QTLs specific of our scrapie study were found on chromosomes 7, 6, 5, 17 and a specific QTL of the Ma BSE study on chromosome 2. The effects of these QTLs could be due to scrapie or BSE isolate, but other experimental differences between studies could affect the QTL detection : the

used genetic crosses (F2 *vs.* BC), the measured traits (age at clinical sign or at death) or the markers density.

Sex is a factor influencing resistance to TSE diseases. The sex effect is weak or null in Ma, Ll or St studies, which measure the length of life until the appearance of clinical signs, but is highly significant in our study, which measures total length of life : females died more rapidly than males. Sex depending factors (hormones, growth factors, fat mass, stoutness, size, appetite,…) are possibly implicated in mechanisms affecting late steps of disease or life. Elsen *et al.,* (1999a) also found a sex effect on the length of life of sheep bred in scrapie contaminated environment.

Sheep. Preliminary results show a QTL on chromosome 18 at 5 % significant chromosome level. This QTL is between TGLA122 and MCMA26, TGLA122 being cytogenetically located at in the cytogenetic 18q24 band (Schibler *et al.*, 1998). Mice and sheep map comparisons indicate that sheep 18q24 corresponds to mouse chromosomes 2 and 12 where QTLs were found. Uncertainties of comparative mapping between rodents and ruminants does not allow yet to discriminate between these two possibilities.

CONCLUSION

The advantages of the mouse species (availability of inbred lines, low cost of breeding, high control of recording processes, very informative genetic map) allow the power of QTL experiments to be very high. Transposition to other mammals, as the sheep species, is possible using a comparative map approach. We show in this paper that such an approach might be successfully applied to the study of genetic susceptibility to TSEs.

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