Detection of New Quantitative Trait Loci for Susceptibility to Transmissible Spongiform Encephalopathies in Mice

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ABSTRACT

Susceptibility to scrapie is largely controlled by the *PRNP* gene in mice and in several other species. However, individuals with identical scrapie susceptibility *Prnp* alleles may have very different incubation periods, suggesting the influence of other environmental and genetic factors. To detect loci influencing susceptibility to TSE, two mouse lines carrying the same *PRNP* genotype (C57BL and RIII) were crossed to produce an F_2 population inoculated intracerebrally with a mouse-adapted scrapie strain. Linkage was studied between 72 markers and the age of death of F_2 animals. Six QTL were detected, two at a genome-wide significant level (chromosomes 5 and 7) and four at a genome-wide suggestive level (chromosomes 4, 6, 8, and 17). Our results confirmed the existence of some QTL that were detected previously (chromosomes 4, 6, 7, and 8) while others were found only in the present study (chromosomes 5 and 17). Furthermore, it seems that some QTL (chromosomes 4 and 8) are involved in resistance to scrapie as well as to BSE.

TRANSMISSIBLE spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases in a number of mammalian species, including ruminants, felines, and primates (Dickinson 1976; Prusiner 1982). A long incubation period is typical of TSE: from several months in experimental mouse models to >10 years in humans (Gajdusek 1967). Other characteristics common to these diseases are the accumulation of a conformationally abnormal and proteinase-K-resistant isoform of the prion protein (PrPsc) in the brain, preceding neuronal death, gliosis, vacuolation, and neurological disorders (Dormont 1998). Although the pathogenesis of these diseases is well documented (Andréoletti *et al.* 2000; Aguzzi *et al.* 2001; Huang *et al.* 2002), the mechanisms of initial infection and dissemination of the agent from the intestine or any other peripheral site of entry to the brain are still poorly understood. At least in sheep, mice, and humans, the probability of infection and the outcome of the disease are genetically controlled (Dickinson 1976; Hunter *et al.* 1989; Prusiner and Scott 1997).

In these species, a large part of the natural susceptibility to TSE depends on inherited alleles of the *PRNP* gene coding for both the normal and abnormal forms of the prion protein (PrP). However, all individuals with identical scrapie susceptibility *Prnp* alleles do not contract the disease and, if they do, they can have very different incubation periods (Elsen *et al.* 1999; Lloyd *et al.* 2001), suggesting the influence of other environmental and genetic factors influencing susceptibility to TSE. In addition, the low number of bovine spongiform encephalopathy (BSE) cases observed in each affected herd suggests a genetic influence on the probability for an individual to develop the mad cow disease. No linkage with *Prnp* alleles could be found, which suggests that genes other than *PRNP* may be involved in the bovine disease.

Mouse inbred lines with defined *Prnp* alleles and different incubation periods offer the opportunity to identify genes influencing the outcome of the disease using the quantitative trait loci (QTL) methodology. The main advantages of this approach are its capacity to scan the whole genome without any *a priori* assumption about the mechanisms and genes involved and to screen only the genes influencing the observed phenotype. Several research groups have recently applied this approach to the identification of additional genetic loci involved in mouse susceptibility to TSE. Stephenson *et al.* (2000) and Lloyd *et al.* (2001) both used the Chandler scrapie agent strain to challenge a mouse F_2 population issued from a cross between two subspecies of *Mus musculus*, *M. musculus musculus* and *M. musculus domesticus*, chosen to maximize between-line polymorphism. Manolakou *et al.* (2001) used the BSE strain and the C57BL and RIII mouse inbred lines (from the *M. musculus domesticus* subspecies) to produce backcross mice.

Here we report the results of a QTL detection study using a cross between C57BL and RIII mouse inbred strains to produce an F_2 population that was inoculated with the mouse-adapted scrapie strain C506-M3. This

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offered the opportunity to compare the locations of the QTL identified in two experiments (Manolakou *et al.* 2001; this study), which essentially differ by the TSE challenge strain.

MATERIALS AND METHODS

Mouse infections: The two parental mouse lines used were C57BL/Fa/Dk and RIII/Fa/Dk, originating from the Neuropathogenesis Unit, Edinburgh, United Kingdom (a gift from M. Bruce). In a first experiment, reciprocal crosses were performed to generate a first F_1 population (female C57BL × male RIII and female RIII × male C57BL) and 282 F_2 mice were generated by crosses between the F_1 progeny (female C57BL × male RIII). New F_1 animals were generated for a second experiment using the same reciprocal crosses as in the first experiment (see Table 1 for the total number of inoculated animals of each generation).

Mice were challenged with the C506-M3 mouse-adapted scrapie strain passaged once in the C57BL mice in our facilities. The C506-M3 strain has been claimed to originate from the ME7 scrapie strain (D. Dormont, personal communication). The length of incubation periods in C57BL and RIII mice was in agreement with this statement. The inoculum was prepared by pooling the brains of 12 C57BL mice at the terminal stage of the disease. Mice were inoculated intracerebrally with 20 μl of a 1% suspension of the brain pool in a 5% glucose solution at the age of 18 and 13 weeks in the first and second experiments, respectively. A previous experiment had shown that 100% of the C57BL female mice inoculated with this strain under these conditions had survival times between 151 and 173 days and showed symptoms characteristic of the disease, *i.e.*, gait disturbances, ataxia, and rigidity of the tail or prostration.

Mice were observed weekly up to 120 days postinoculation and then every day for scrapie symptoms. The animals were sacrificed at the terminal stage of the disease. Survival time was calculated for each mouse as the interval between the day of injection and the day of sacrifice. Mice dying accidentally or of intercurrent diseases with no scrapie symptoms were removed from the experimental population.

DNA isolation and genotyping: Genomic DNA was isolated from tail snips. Approximately 1 cm of the tail was removed just after the death of the animals. Tail tips were incubated overnight at 50° in 0.5 ml of extraction buffer (0.01 m Tris·HCl, pH 8, 0.025 m EDTA/0.075 m NaCl/1% SDS) containing 500 μg/ml proteinase K. The samples were then extracted twice with phenol-chloroform and a third time with chloroform. High-molecular-weight DNA was obtained after isopropanol precipitation and redissolved in 100 μl TE (10 mm Tris/1 mm EDTA, pH 7.5). DNA for genotyping was resuspended in distilled water at 50 ng/μl. This stock DNA (1.5 μl) was used as the template in a 10-μl PCR. All PCRs were carried out in 96-well plates by using a T1 mouse MapPairs set (Research Genetics, Huntsville, AL), and additional unlabeled or labeled primers were obtained from Isoprim (Toulouse, France). A panel of 472 markers was tested on DNA from the parental strains. Final genotypes were obtained for 72 markers spread throughout the genome. PCR reactions were carried out in 1.5 mm $MgCl_2$ with Taq DNA polymerase (Promega, Madison, WI) in the buffer provided. Cycling conditions were as follows: 94° for 2 min, 55° for 45 sec, 72° for 45 sec, 94° for 45 sec for 35 cycles; 55° for 45 sec, and 72° for 7 min; and then 4° before storage at −20°. The alleles were detected by electrophoresis on either a 4% agarose gel for the 53 unlabeled primers or an ABI 310 capillary system (Applied Biosystems, Foster City, CA) for the 20 labeled primers.

TABLE 1

Incubation time by sex in the experimental population

Genetic type	Sex	No. of animals	Means[a]
C57BL	M	40	173.4 ± 5.2
	F	41	162.8 ± 4.8
RIII	M	40	164.9 ± 4.7
	F	38	160.3 ± 3.7
F_1 (C57BL × RIII)	M	40	177.0 ± 3.6
	F	40	168.0 ± 4.3
F_1 (RIII × C57BL)	M	40	175.7 ± 4.5
	F	42	170.4 ± 4.5
F_2 (C57BL × RIII) × (C57BL × RIII)	M	145	173.0 ± 6.5
	F	137	165.8 ± 5.7

Probability value for all genetic types is $P < 0.0001$.
[a] Means of survival times corrected for the batch effect.

Data analysis: The distance between the markers on the chromosomes was estimated using Map Manager QTX software and the Mouse Genome Database (http://www.informatics.jax.org).

Deviation from normality of the trait (duration of life) was assessed from the asymmetry coefficient g1 and kurtosis coefficient g2 (SAS UNIVARIATE procedure; SAS Institute 1990b). The general linear model (GLM) procedure of the SAS package (SAS Institute 1990a) was used to estimate fixed effects: batch, sex, and genetic type (pure lines, F_1 and F_2 crosses). To search QTL, several methods were used. Single-marker analyses were performed using the GLM procedure (SAS Institute 1990a) and MAP MANAGER QT, version b29 (Manly 1998; http://mapmgr.roswellpark.org/mmQT.html). The latter software was also used to perform interval mapping analyses (Lander and Botstein 1989) and composite interval mapping (CIM) analyses (Jansen 1993; Zeng 1993), which combine interval mapping with multiple regressions. Genome-wide significance thresholds were calculated using the permutation method (Churchill and Doerge 1994; Doerge and Churchill 1996). These thresholds were used to calculate two genome-wide thresholds for suggestive and significant linkages (Lander and Kruglyak 1995). Lander and Kruglyak (1995) defined significant linkage as a 5% genome-wide significance threshold and suggestive linkage as equivalent to an expectation of one false-positive result on a whole-genome scan. The 1-LOD score support confidence interval (C.I.) was chosen to calculate the confidence interval for the position and effects of the QTL (Lander and Botstein 1989).

We also used a multiple-interval mapping strategy based on a genetic algorithm strategy (Nakamichi *et al.* 2001; http://wheat.ab.a.u-tokyo.ac.jp/naka/soft1.html). This analysis allows us to search simultaneously for linked and unlinked QTL in the whole genome but it does not allow us to test the different models, which are directly compared using the Akaike information criterion (Akaike 1973).

RESULTS

Survival time of our mouse population: Within genetic type × sex, the means of survival time were corrected for the batch effect (Table 1). The differences in survival time between parental strains, 8.5 days for

TABLE 2

Interval mapping results in the F_2 population

Chromosome	Data	LOD score	P^a	C.I. of localization (cM)	Variance (%) explained by the QTL	Standardized additive effect[b]	Standardized dominant effect[b]
4	Females	2.1	*	34–64	5	0.37	0.26
5	Females	4.7	**	51–72	13	−0.44	0.35
6	Corrected	2.0	*	25–67	2	−0.28	0.00
	Females	3.2	*	25–59	9	−0.53	0.11
7	Corrected	3.5	**	2–18	5	0.33	0.16
	Males	2.1	*	37–70	5	0.20	0.42
	Females	2.0	*	2–19	5	0.35	0.21
8	Corrected	1.9	*	22–64	2	0.26	0.00
	Males	2.8	*	24–64	7	0.45	0.38
17	Males	2.0	*	7–21	5	0.35	0.00
	Females	2.5	*	36–46	7	0.30	0.42

[a] Single (*) and double (**) asterisks represent the suggestive and significant statistics, respectively, calculated using the permutation method (Churchill and Doerge 1994) for each set of data.

[b] Estimations of additive and dominant effects are divided by the standard deviation of the data set.

males and 2.5 days for females, were significant ($P = 0.0001$ and $P = 0.01$, respectively). As previously reported by others, RIII mice appeared to be more susceptible to the scrapie C506-M3 strain than C57BL mice, whatever the sex. Otherwise, there was a highly significant sex effect both in parental lines (C57BL and RIII) and in crosses (F_1 and F_2): males died from scrapie later than females (Table 1).

For F_2 survival times, F_2 survival times of males, and F_2 survival times of females, the skewness values were equal to −0.39, −0.61, and −0.03, respectively, and the kurtosis values were equal to 0.30, 0.42, and 0, respectively. Therefore, these three sets of data were assumed to be normally distributed.

QTL mapping: To identify possible interactions between sex and QTL location, the genome scan was performed on three data sets: males (145 mice), females (137 mice), and both sexes after precorrection for the sex effect (282 mice). The results are presented in Table 2 and Figure 1.

QTL detection on X would show effects for getting different copies of X. These analyses are often done within sexes separately because in males it will be X1Y *vs.* X2Y, while in females (for the present cross) it will be X1X2 *vs.* X1X1 (only one type of F_1 was used). None of the markers from the X chromosome showed a QTL effect. However, these QTL analyses for X are not expected to necessarily reveal any QTL that explain differences between the sexes.

From the six QTL observed, four were detected at a suggestive level on chromosomes 4, 6, 8, and 17 and two at a significant level on chromosomes 5 and 7. The estimates of the additive effects showed that the alleles increasing resistance came from the C57BL line (the "resistant" line) for chromosomes 4, 7, 8, and 17 and from the RIII line (the "susceptible" line) for chromosomes 5 and 6. In the latter two chromosomes, the resistant allele had a recessive effect, while it had a weak-to-moderate dominant effect in all other chromosomes.

The results differed among the data files analyzed. Among the QTL detected in the females (chromosomes 4, 5, and 6), only the QTL located on chromosome 6 was still detected at a suggestive level, when considering all data corrected for the sex effect, while the other two QTL showed a LOD score just under the suggestive threshold. The QTL found on chromosome 8 was evidenced in both the male data set and the sex-corrected data set. Finally, the QTL located on chromosome 7 was detected in the three data files, but not located in the same confidence interval in the male data set as compared to the female and sex-corrected data set (Figure 1).

Data were also analyzed using the CIM method (Zeng 1993) and the multiple-interval mapping method (Nakamichi *et al.* 2001) to discriminate between one or two distinct QTL segregating on chromosome 7 in the corrected data set (Table 3). Both techniques showed the existence of two QTL located 20 cM apart, which had additive effects of opposite sign and a weak-to-moderate dominant effect. The CIM analysis showed a significant effect for the QTL located at 6 cM from the end of the linkage group and a suggestive effect for the QTL

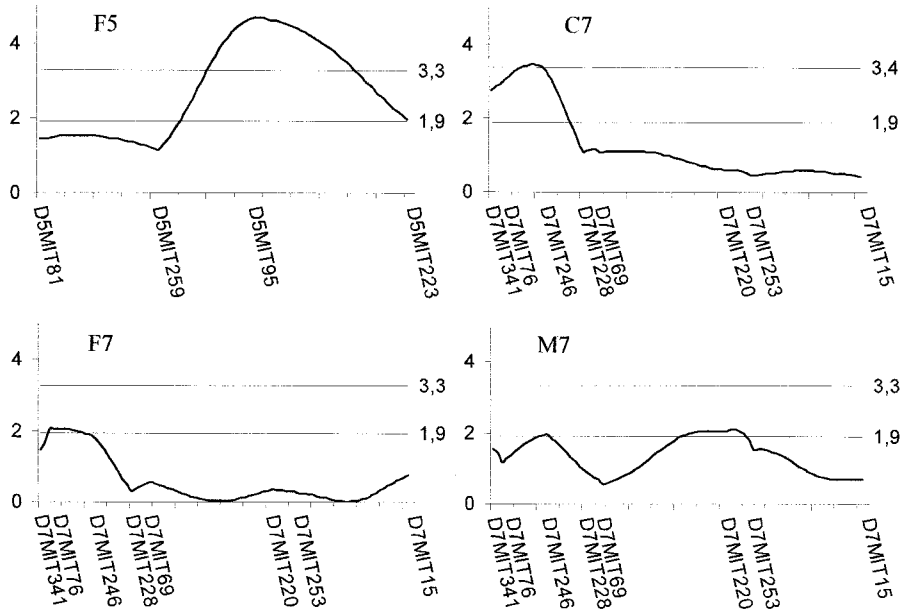

FIGURE 1.—LOD score plot for chromosomes 5 and 7 for the three data sets. LOD score plots are presented for chromosomes 5 (F5) and 7 (C7, F7, and M7). The *x*-axes indicate the chromosomal linkage maps in centimorgans; the *y*-axes indicate the value of LOD scores obtained using Map Manager QTX with an interval mapping model that allowed us to estimate the additive and dominant effects of the QTL. The thin horizontal lines represent the suggestive and significant thresholds calculated for each data set: male (M), female (F), and corrected (C).

located at 26 cM. Since the two QTL were close together and had an opposite additive effect, the hypothesis that the second QTL is a statistical artifact cannot be ruled out. The presence of two QTL, however, was supported by the similarity of characteristics (localization and effects) of both QTL detected with the two different methods used.

DISCUSSION

The results of the four genome scans for QTL controlling susceptibility to TSE in mice are now available. The following discussion focuses on a comparison of these studies (Table 4).

TABLE 3

Results of different interval mapping analyses on chromosome 7 for the sex-corrected data set

Methods	No. of QTL	QTL position (cM)	Standardized additive effect[a]	Standardized dominance effect[a]
SIM	1	9	0.33	0.16
CIM	2	6[b]	0.52	0.25
		26[c]	−0.31	0.20
MIM-GA	2	9	0.54	0.02
		25	−0.30	0.21

SIM, simple interval mapping (LANDER and BOTSTEIN 1989); CIM (JANSEN 1993; ZENG 1993); MIM-GA, multiple-interval mapping using the genetic algorithm strategy (NAKAMICHI *et al.* 2001).

[a] Estimations of additive and dominant effects are divided by standard deviation of the sex-corrected data set.

[b] QTL found considering D7MIT246 (26 cM) as the cofactor in the model.

[c] QTL found considering D7MIT69 (13 cM) as the cofactor in the model.

Susceptibility to TSE is influenced by the sex: In the studies of STEPHENSON *et al.* (2000) and LLOYD *et al.* (2001), no sex effect was observed. MANOLAKOU *et al.* (2001) found a sex effect in their F_1 population and in one of their backcross populations (with an inversion of this effect) and attributed this observation to an interaction between a maternal effect and a chromosome X effect. In our study (Tables 1 and 2), the sex effect was highly significant with a shorter survival time in females. A possible interpretation of the importance of the sex effect in our study may come from a late difference between males and females: the trait we measured was the age at death and we considered the whole life span of the animals. Such an effect was also found by ELSEN *et al.* (1999) in a natural scrapie epidemic in sheep.

This observation may explain the differences between the results obtained, depending on the data considered in our study. The mechanisms playing a role at the end of the survival time (hormonal factor, body size, fat composition, appetite, etc.) could be the source of this sex effect. Indeed, in the RIII and C57BL lines, as in the lines used by STEPHENSON *et al.* (2000) and LLOYD *et al.* (2001), noninoculated males also had a longer life than noninoculated females. Thus, some QTL whose survival time was detected in our study may partially reflect the polymorphism for survival in general. Even if the age at death is probably partly determined by nonspecific mechanisms of TSE, this trait is objective and easy to measure, whereas the age at the first clinical signs used in the other studies is a subjective trait, which does not have a fully clear biological significance and reflects only part of the phenomenon analyzed.

The choice of the QTL detection model: In our study,

TABLE 4

Comparison of four studies dealing with QTL for TSE in mice

	Stephenson *et al.* (2000)	Lloyd *et al.* (2001)	Manolakou *et al.* (2001)	Our study
		Populations		
Resistant line	CAST/Ei	CAST/Ei	C57BL	C57BL
Incubation time (days)	172	188	540	167
Susceptible line	SJL/J	NZW/OlaHsd	RIII	RIII
Incubation time (days)	105	108	442	161
Segregating cross	F_2: $n = 163$	F_2: $n = 1009$	BC: $n = 1027$	F_2: $n = 282$
Incubation time (female, male)	126, 129	157, 158	493, 485	166, 173
		Design		
Infectious agent	Chandler	Chandler	BSE Isolate	Scrapie ME7
Inoculation route	Intracerebrally	Intracerebrally	Intracerebrally	Intracerebrally
Age at inoculation	6–7 wk	—	3–8 wk	18 wk
Trait recorded	Clinical signs	Clinical signs	Clinical signs	Death
		Results		
No. of markers	153	157	90	72
QTL chromosomes	*7*, 9, 10, 11, 18, 19	2, *6*, *7*, 11, 12	2, *4*, *8*, 15	*4*, 5, *6*, *7*, *8*, 17
	LOD scores for QTL found in at least two studies			
Chromosome 2	—	8.2	5.7[a]	—
Chromosome 4	—	—	4.7[a]	2.1[b]
Chromosome 6	—	3.9	—	2[c], 3.2[b]
Chromosome 7	2.2	3.6	—	3.5[c], 2.1[d], 2[b]
Chromosome 8	—	—	5[a]	1.9[c], 2.8[d]
Chromosome 11	5.6	57.6	—	—

Italic numerals indicate those chromosomes in which a QTL was detected in at least two of four studies. *n*, number of mice.

[a] From the LOD score plot of Manolakou *et al.* (2001).

[b] Female data.

[c] Sex-corrected data.

[d] Male data.

we chose to consider three data sets (male only, female only, or sex-corrected data) to perform the QTL analysis, taking into account sex and QTL interactions. This solution is not the most powerful but the software used was unable to perform interval mapping analysis with a model estimating QTL and sex interactions.

Experimental designs and their power to detect QTL: The designs differed mostly in the parental lines, the inoculated TSE strain, and the recorded trait (Table 4). Studies by Stephenson *et al.* (2000) and Lloyd *et al.* (2001) were performed under very similar conditions (same parental lines, same inoculated TSE strain, and same recorded trait). These conditions are different from ours, except for the use of the scrapie strain. Manolakou *et al.* (2001) used the same mouse lines (RIII and C57BL) as ours but, in contrast to our study, the mice were inoculated with BSE and the trait recorded was the age at first clinical sign. Despite these differences, QTL were found on six chromosomes in at least two studies out of four, but with different significance levels and locations (Table 4).

To gain insight into the origin of the differences among the four studies, the power of the four designs was calculated. This depends on the QTL effect, marker density, and population size. The theoretical and empirical studies of Lynch and Walsh (1998) have shown that the power of a QTL detection design estimated considering a single marker analysis is quite similar to the power from interval mapping techniques, in particular when marker distances are <20 cM. Considering a dominant QTL in an F_2 or a backcross population, Darvasi (1998) showed that a global risk at the $\alpha = 0.05$ level is obtained with a single test level at $\alpha = 10^{-4}$. Thus a QTL explaining 5% of the phenotypic variance should be detected at the 10^{-4} level with a power of 72% with our design, close to 100% in Lloyd *et al.* (2001) and Manolakou *et al.* (2001), but with a power of only 30% with the Stephenson *et al.* (2000) design.

These differences indicate that the same QTL could be observed with a lower LOD score in Stephenson *et al.* (2000) as compared to Lloyd *et al.* (2001; chromosomes 7 and 11), and in our study as compared to Manolakou *et al.* (2001; chromosomes 4 and 8).

QTL controlling resistance to scrapie were evidenced in different studies: A QTL with a major effect was found in the same region of chromosome 11 in the studies by Stephenson *et al.* (2000) and Lloyd *et al.* (2001), but not in our study. This suggests that this QTL could be specific to the Cast/Ei mouse line. Otherwise, despite the differences in mouse lines, a QTL was found on chromosome 7 with the three designs using a scrapie isolate. The location of this QTL varies considerably from one study to another: in the proximal part of the chromosome in Lloyd *et al.* (2001) and in the distal part in Stephenson *et al.* (2000). In our study, one QTL and probably two QTL with opposite effects were detected in the proximal part of the chromosome. It can be hypothesized that the QTL found by Lloyd *et al.* (2001) is similar to one or both of the QTL that we found. Considering the power of the Stephenson *et al.* (2000) design (see above) and the limited number of markers that they used, we cannot exclude that their QTL on chromosome 7 is the same. Finally, the small QTL detected on chromosome 6 in Lloyd *et al.* (2001) and in our study should be shared by the resistant lines NZW/OlaHsd and the susceptible line RIII.

Agreement and divergence between QTL controlling scrapie and BSE inoculation responses: The pure lines used in Manolakou *et al.* (2001) and in our study were the same. The genetic determinism of the resistance to both diseases was only partially the same. Shared QTL were found on chromosomes 4 and 8 and perhaps on chromosome 15 (which has a significance level just below the suggestive linkage in our study). These three QTL showing similar effects are located in overlapping confidence intervals, but were detected with much lower LOD scores in our study as compared to Manolakou *et al.* (2001), due to the size of our respective population.

Finding shared QTL is not very surprising considering the similarity in the pathogenesis of these diseases. By contrast, finding QTL expressed in only one of the challenges is more surprising (on chromosomes 7, 6, 5, and 17 for the scrapie challenge and on chromosome 2 for the BSE). These discrepancies, however, cannot be explained by a few experimental differences between the studies: different segregating crosses (F_2 and backcross), marker density, phenotypic measurement [we measured the age at death while Manolakou *et al.* (2001) considered the age at first signs of scrapie].

Candidate genes in the confidence interval of the QTL found: As in any QTL study, chromosomal regions significantly influencing the incubation period are rather large and contain a number of potential "candidate" genes. However, although this might be pure coincidence, the products of several genes that directly interact with the PrP protein, or are known to be involved in scrapie pathogenesis, have been located on each of the chromosomal segments defined by the QTL confidence intervals. Among the phenomena related to scrapie pathogenesis, we should mention inflammation, apoptosis, signaling pathways, and, of course, PrP expression.

We found two genes located in the QTL region of mouse chromosome 4, coding for potential PrP ligand proteins: complement component factor C1q (Klein *et al.* 2001) and PrP ligand 8 (Yehiely *et al.* 1997). Another candidate gene is cathepsin D, which has an increased expression during scrapie and Alzheimer's diseases (Diedrich *et al.* 1991). A significant QTL found in female mice on chromosome 5 corresponds to a region containing the ubiquitin C and the neuronal NO synthase (NOS1) genes. The increasing interest in the products of these two genes results from their involvement in the normal, although unknown, function of the PrPc protein as well as neuropathology (Alves-Rodriguez *et al.* 1998; Wong *et al.* 2001a,b; Yedidia *et al.* 2001). On mouse chromosome 6, the QTL region includes the laminin receptor-1-like gene, which is also a PrP receptor (Hundt *et al.* 2001). The gene coding for APOE, whose expression increases during scrapie and Alzheimer's diseases, is located in the QTL region described on chromosome 7 (Diedrich *et al.* 1991). The same region of mouse chromosome 7 also carries genes coding for TGFb and NGF factors, which are involved in the scrapie disease (Tashiro *et al.* 1998; Kuwahara *et al.* 2000). The QTL region defined on mouse chromosome 8 contains the Scrg1 gene (scrapie-responsive gene 1), the expression of which is increased during scrapie (Dandoy-Dron *et al.* 1998). The QTL found on chromosome 17 in our study are in surprisingly different locations in males and females. The gene coding for plasminogen, a plasma protein identified as a carrier of PrP (Maissen *et al.* 2001), is located in the male region. The mouse major histocompatibility complex H-2, which is probably involved in susceptibility to prion disease, is located in the female region.

The most interesting candidate genes are cited here, but other genes were considered. In most cases, these genes were involved in phenomena related to scrapie pathogenesis: inflammation, apoptosis, signaling pathways, and, of course, PrP expression.

Conclusion: In conclusion, the present study confirms that genes other than the prion protein gene (*PRNP*) affect susceptibility to TSE diseases in mice. Some QTL were detected previously (chromosomes 4, 6, 7, and 8) while others were found only in the present study (chromosomes 5 and 17). Furthermore, it seems that some QTL (chromosomes 4 and 8) are involved in resistance to scrapie as well as to BSE.

The knowledge of these chromosomal regions could be used directly to identify homologous regions in farm animals and humans and to detect the QTL affecting susceptibility to TSE diseases in these species. Moreover, additional studies could allow us to identify the genes

and their causal mutation responsible for the susceptible and resistant effects of the QTL. These results could provide potential candidate genes for other animal species, but could also allow us to better understand the partially unknown mechanisms behind TSE diseases.

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LITERATURE CITED

Aguzzi, A., S. Brandner, M. B. Fischer, H. Furukawa, M. Glatzel *et al.*, 2001 Spongiform encephalopathies: insights from transgenic models. Adv. Virus Res. **56:** 313–352.

Akaike, H., 1973 Information theory and an extension of the maximum likelihood principle, pp. 267–281 in *2nd International Symposium on Information Theory*. Akademisi Kiado, Budapest.

Alves-Rodrigues, A., L. Gregori and M. E. Figueiredo-Pereira, 1998 Ubiquitin, cellular inclusions and their role in neurodegeneration. Trends Neurosci. **21** (12): 516–520.

Andréoletti, O., P. Berthon, D. Marc, P. Sarradin, J. Grosclaude *et al.*, 2000 Early accumulation of PrPsc in gut associated lymphoid and nervous tissues of susceptible lambs from a Romanov flock with natural scrapie. J. Gen. Virol. **81:** 3115–3126.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

Dandoy-Dron, F., F. Guillo, L. Benboudjema, J. P. Deslys, C. Lasmezas *et al.*, 1998 Gene expression in scrapie. Cloning of a new scrapie-responsive gene and the identification of increased levels of seven other mRNA transcripts. J. Biol. Chem. **273** (13): 7691–7697.

Darvasi, A., 1998 Experimental strategies for the genetic dissection of complex traits in animal models. Nat. Genet. **18:** 19–24.

Dickinson, A. G., 1976 Scrapie in sheep and goats, pp. 209–241 in *Slow Virus Diseases of Animals and Man*, edited by H. R. Kimberlin. North-Holland, Amsterdam.

Diedrich, J. F., H. Minnigan, R. I. Carp, J. N. Whitaker, R. Race *et al.*, 1991 Neuropathological changes in scrapie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin D in astrocytes. Virology **65** (9): 4759–4768.

Doerge, R. W., and G. A. Churchill, 1996 Permutation tests for multiple loci affecting a quantitative character. Genetics **142:** 285–294.

Dormont, D., 1998 Prions, new molecular forms of biological information. Res. Immunol. **149** (7–8): 711–717.

Elsen, J. M., Y. Amigues, F. Schelcher, V. Ducrocq, O. Andréoletti *et al.*, 1999 Genetic susceptibility and transmission factors in scrapie: detailed analysis of an epidemic in a closed flock of Romanov. Arch. Virol. **144:** 431–445.

Gajdusek, C., 1967 Discussion on kuru, scrapie and the experimental kuru-like syndrome in chimpanzees. Curr. Top. Microbiol. Immunol. **40:** 59–63.

Huang, F. P., C. F. Farquhar, N. A. Mabbott, M. E. Bruce and G. G. MacPherson, 2002 Migrating intestinal dendritic cells transport PrP (Sc) from the gut. J. Gen. Virol. **83** (Pt 1): 267–271.

Hundt, C., J. M. Peyrin, S. Haik, S. Gauczynski, C. Leucht *et al.*, 2001 Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor. EMBO J. **20** (21): 5876–5886.

Hunter, N., J. D. Foster, A. J. Dickinson and J. Hope, 1989 Linkage of the gene for the scrapie-associated fibril protein (PrP) to the Sip gene in Cheviot sheep. Vet. Rec. **124:** 364–366.

Jansen, R. C., 1993 Interval mapping of multiple quantitative trait loci. Genetics **135:** 205–211.

Klein, M. A., P. S. Kaeser, P. Schwarz, H. Weyd, I. Xenarios *et al.*, 2001 Complement facilitates early prion pathogenesis. Nat. Med. **7** (4): 488–492.

Kuwahara, C., A. Kubosaki, T. Nishimura, Y. Nasu, Y. Nakamura *et al.*, 2000 Enhanced expression of cellular prion protein gene by insulin or nerve growth factor in immortalized mouse neuronal precursor cell lines. Biochem. Biophys. Res. Commun. **268** (3): 763–766.

Lander, E. S., and D. Botstein, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

Lander, E., and L. Kruglyak, 1995 Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. **11:** 241–247.

Lloyd, S. E., O. N. Onwuazor, J. A. Beck, G. Mallinson, M. Farrall *et al.*, 2001 Identification of multiple quantitative trait loci linked to prion disease incubation period in mice. Proc. Natl. Acad. Sci. USA **98:** 6279–6283.

Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.

Maissen, M., C. Roeckl, M. Glatzel, W. Goldmann and A. Aguzzi, 2001 Plasminogen binds to disease-associated prion protein of multiple species. Lancet **357** (9273): 2026–2028.

Manly, K. F., 1998 *User's Manual for Map Manager Classic and Map Manager QT* (http://mapmgr.roswellpark.org/MMM/MMMBookTOC.html).

Manolakou, K., J. Beaton, I. McConnell, C. Farquar, J. Manson *et al.*, 2001 Genetic and environmental factors modify bovine spongiform encephalopathy incubation period in mice. Proc. Natl. Acad. Sci. USA **98:** 7402–7407.

Nakamichi, R., Y. Ukai and H. Kishino, 2001 Detection of closely linked multiple quantitative trait loci using a genetic algorithm. Genetics **158:** 463–475.

Prusiner, S. B., 1982 Novel proteinaceous infectious particles cause scrapie. Science **216** (4542): 136–144.

Prusiner, S. B., and M. R. Scott, 1997 Genetics of prions. Annu. Rev. Genet. **31:** 139–175.

SAS Institute, 1990a *SAS/STAT User's Guide*, Version 6, Vol. 2, Ed. 4. SAS Institute, Cary, NC.

SAS Institute, 1990b *SAS Procedures Guide*, Version 6, Ed. 3. SAS Institute, Cary, NC.

Stephenson, D. A., K. Chiotti, C. Ebeling, D. Groth, S. J. DeArmond *et al.*, 2000 Quantitative trait loci affecting prion incubation time in mice. Genomics **69:** 47–53.

Tashiro, H., K. Dohura and T. Iwaki, 1998 Differential expression of transforming growth factor-beta isoforms in human prion diseases. Neuropathol. Appl. Neurobiol. **24** (4): 284–292.

Wong, B. S., T. Liu, R. Li, T. Pan, R. B. Petersen *et al.*, 2001a Increased levels of oxidative stress markers detected in the brains of mice devoid of prion protein. J. Neurochem. **76** (2): 565–557.

Wong, B. S., T. Liu, D. Paisley, R. Li, T. Pan *et al.*, 2001b Induction of HO-1 and NOS in doppel-expressing mice devoid of PrP: implications for doppel function. Mol. Cell. Neurosci. **17** (4): 768–775.

Yedidia, Y., L. Horonchik, S. Tzaban, A. Yanai and A. Taraboulos, 2001 Proteasomes and ubiquitin are involved in the turnover of the wild-type prion protein. EMBO J. **20** (19): 5383–5391.

Yehiely, F., P. Bamborough, M. Da Costa, B. J. Perry, G. Thinakaran *et al.*, 1997 Identification of candidate proteins binding to prion protein. Neurobiol. Dis. **3** (4): 339–355.

Zeng, Z. H., 1993 Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA **90:** 10972–10976.

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