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Multiplicative genetic effects in scrapie disease susceptibility

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Abstract – Despite experimental evidence that scrapie is an infectious disease of sheep, variations of the occurrence of the natural disease suggest an influence of host genetic factors. It has been established that the genetic polymorphism of the prion protein (PrP) gene is correlated to the incidence of scrapie and to the survival time: five polymorphisms have been described by variations at amino-acid codons 136, 154 and 171. In this paper we study the effect on scrapie susceptibility of the pairing of the five allelic variants known to exist: we show that scrapie susceptibility is given by the produce of the elementary allelic factors. This first well-documented evidence of a multiplicative property of genetic risk factors could give hints on the underlying mechanisms of prion-induced neurodegenerative diseases. ***To cite this article: M.-A. Dubois et al., C. R. Biologies 325 (2002) 565–570.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

multiplicative effects / risk factors / allelic variants / genetic susceptibility / prion disease

Résumé – Effets multiplicatifs des facteurs de risques génétiques de sensibilité à la tremblante du mouton. Malgré l'évidence expérimentale d'une origine infectieuse de la tremblante du mouton, l'existence d'une variabilité importante de la forme naturelle de la maladie au sein des élevages suggère une influence des facteurs génétiques. Il a été établi que le polymorphisme du gène de la protéine prion (PrP) pouvait être corrélé à l'incidence de la tremblante ainsi qu'à la durée de survie des individus infectés. Cinq formes alléliques déterminantes ont ainsi été décrites à partir des variations des séquences d'acides aminés aux codons 136, 154 et 171. Dans cet article, nous étudions l'effet de l'appariement des cinq variants connus sur la sensibilité à la tremblante. Nous montrons que la sensibilité d'un individu donné à la tremblante est le produit des effets élémentaires de chacun de ses deux allèles. Cette propriété multiplicative des facteurs de risques alléliques de la maladie est une piste pour comprendre les différentes cinétiques du processus dégénératif de cette maladie à prion. ***Pour citer cet article : M.-A. Dubois et al., C. R. Biologies 325 (2002) 565–570.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

effets multiplicatifs / facteurs de risques génétiques / variants alléliques / susceptibilité génétique / maladie à prion

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Version abrégée

La tremblante du mouton est une encéphalopathie spongiforme transmissible, dont l'agent infectieux n'a pas été totalement caractérisé. L'infection se traduit par une accumulation au niveau du cerveau d'un isomorphe de la protéine prion (PrP) codée par l'hôte. Malgré l'évidence expérimentale d'une origine infectieuse de la maladie, l'existence d'une variabilité importante de la forme naturelle de la maladie au sein des élevages suggère une influence des facteurs génétiques. Il a été établi que le polymorphisme du gène de la PrP pouvait être corrélé à l'incidence de la tremblante ainsi qu'à la durée de survie des individus infectés. Cinq formes alléliques déterminantes ont ainsi été décrites à partir des variations des séquences d'acides aminés aux codons 136, 154 et 171. Deux formes alléliques ont été associées à une « résistance à la maladie », un à une « hypersensibilité à la maladie » et deux à un impact intermédiaire. L'objectif de ce travail est de proposer une méthode de calcul explicite du risque de tremblante. Nous étudions l'effet de l'appariement des cinq variants alléliques sur la sensibilité génétique à la tremblante. Nous montrons que la sensibilité d'un individu donné à la tremblante est le produit des effets élémentaires de chacun de ses deux allèles. Les facteurs de risque associés aux six catégories génétiques rencontrées sont les produits des facteurs de risques associés aux catégories alléliques correspondantes. Cette propriété multiplicative des facteurs de risques alléliques de la maladie constitue une piste pour comprendre les différentes cinétiques du processus dégénératif des maladies à prion.

1. Introduction

Scrapie is a fatal neurodegenerative disease of sheep, reported more than 200 years ago [1], which belongs to the group of transmissible spongiform encephalopathies, also known as prion diseases. Other members of this group include bovine spongiform encephalopathy (BSE) and Creutzfeldt–Jakob disease (CJD) in man. Prion diseases are characterised by the accumulation of an anomalous protease-resistant isoform of a natural host-encoded protein (prion protein or PrP) in the brain of affected animals and humans [2]. These diseases are characterised by a long incubation period and a prolonged clinical phase. But there is still much to learn about the epidemiology and the pathogenesis of the natural prion diseases. The nature of the agent has not been completely resolved: it is thought to be composed largely, if not entirely, of these anomalous PrP molecules [3]. Several polymorphisms in the gene encoding this protein are known to be associated with the incidence, the susceptibility, and the pathology of these diseases in different species. Therefore, it can be said that prion diseases result of infectious and/or inherited disorders [4].

Scrapie is an infectious disease [5] of sheep, but variations of the occurrence of the natural disease suggest an influence of host genetic factors [6]. In this specific case, it was first proposed that this variation was mediated by a gene called SIP (Scrapie Incubation Period), presenting two allelic forms sA (for 'short') and pA (for 'prolonged') [7]. Later molecular genetic studies confirmed that SIP was the gene encoding PrP, and identified several variant alleles correlated to the incidence of scrapie in sheep and to the survival time [8]. Five polymorphisms are described by variations at codons 136, 154 and 171: ARQ (which is the probable candidate as the ancestral allele), ARR, AHQ, ARH (rarely observed), VRQ (the first letter corresponds to the 136th codon, the second to the 154th codon, the third to the 171th codon, and A = alanine, H = histidine, Q = glutamine, R = arginine, and V = valine) [9–15]. Genotypes result from the homozygous or heterozygous pairing of alleles inherited from ram and ewe (for example VRQ/VRQ). This pairing allows from three to 15 PrP genotype variants: from three genotypes for a two-alleles breed (Hampshire Down flock) to 15 genotypes for a five-alleles breed (Texel flock). It has been established that the PrP genotype contributes to the determination of the survival times of scrapie-affected sheep [16]. The goal of this study is to propose a way to compute explicitly the risk of scrapie as a function of the elementary risks associated with each allelic variant.

2. PrP allelic variants and their association with natural scrapie

Combined detection of the polymorphisms at codon 136, 154 and 171 suggests that VRQ and ARR are antagonist in determining disease susceptibility: VRQ is associated with a high incidence of natural scrapie and ARR is associated with a low incidence of natural scrapie. Data obtained by screening both scrapie-affected sheep and control sheep of Manech Tête Rousse breed in France (Table 1) show that the VRQ allelic variant is present in 32.29% of all scrapie cases and in only 3.50% of control sheep. This allele is associated with a high incidence of disease with a χ^2

Table 1. Occurrences of allelic variants in scrapie-affected sheep and in control sheep.

Allelic variant		Scrapie*	% Scrapie	Control*	% Control	χ^2	p
R	**ARR**	0	0.00	88	30.77	38.38	5.8×10^{-10}
	AHQ	0	0.00	4	1.40	—	—
S	**ARQ**	95	98.96	274	95.80	2.18	0.14
	ARH	—	—	—	—	—	—
H	**VRQ**	31	32.29	10	3.50	62.20	3.1×10^{-15}
Number of sheep		96	100.00	286	100.00		

* Sheep collected through the French department 'Pyrénées-Atlantiques' in 1995 among Manech Tête Rousse (MTR) flocks. Affected sheep (n = 96) are MTR histo + ewes, collected from different flocks, and aged between 2 and 5 yr. Age-matched control sheep (n = 286) are the MTR rams from the Artificial Insemination Centre flock (INRA, unpublished, 1998).

ratio of 62.20 ($p = 3.1\times10^{-15}$). The ARR allelic variant is present in 30.77% of control sheep, but in 0% of scrapie cases: it is associated with a low incidence of disease with a χ^2 ratio of 38.38 ($p = 5.8\times10^{-10}$). The AHQ variant, which generally has a very low occurrence in sheep breeds, can be tentatively associated with a low incidence of disease similar to that of the ARR variant (presently available data are insufficient to suggest a different incidence value). The ARQ variant does not show association with disease incidence ($\chi^2 = 2.18$, $p = 0.14$), neither does the ARH variant (which is rarely distinguished from the ARQ variant). Therefore, the five alleles can be classified into three allelic groups:

R = resistant = ARR or AHQ

S = susceptible = ARQ or ARH

H = hypersusceptible = VRQ

Following this classification, appurtenance to the ***S*** class (the ARQ and ARH alleles) has no effect on the disease incidence; appurtenance to the ***R*** class (the ARR and AHQ alleles) has a negative effect on the disease incidence; appurtenance to the ***H*** class (the VRQ allele) has a positive effect on the disease incidence.

3. Pairing allelic variants to obtain genotypes

A genotype is obtained by pairing two alleles chosen among five possible variants; a three-class allelic model allows us to define six genotypic groups:

– **G1** genotypes result from two alleles both belonging to the **R** class (corresponding to the ARR/ARR, ARR/AHQ and AHQ/AHQ genotypes);

– **G2** genotypes result from two alleles belonging to the **R** and **S** classes respectively (corresponding to the ARR/ARQ, ARR/ARH, AHQ/ARQ and AHQ/ARH genotypes);

– **G3** genotypes result from two alleles belonging to the **R** and **H** classes respectively (corresponding to the ARR/VRQ and AHQ/VRQ genotypes);

– **G4** genotypes result from two alleles both belonging to the **S** class (corresponding to the ARQ/ARQ, ARQ/ARH and ARH/ARH genotypes);

– **G5** genotypes result from two alleles belonging to the **S** and **H** classes respectively (corresponding to the ARQ/VRQ and ARH/VRQ genotypes);

– **G6** genotypes result from two alleles both belonging to the **H** class (corresponding to the VRQ/VRQ genotype).

Table 2 shows the frequencies of genotypes in scrapie-affected sheep and in control sheep.

For each one of the genotypic groups **G*i*** that are represented both in the scrapie-affected and in the control groups, we compute an observed genetic risk $R(\mathbf{Gi})_{\text{obs}}$ as the ratio of the proportion of this group in control sheep, by the proportion of this group in scrapie-affected sheep. It is remarkable, in Table 3, that genotypes of the **G4** group are found in the scrapie-affected group at the same frequency as in the control group (67.71%, 64.69%): the observed genetic risk, $R(\mathbf{G4})_{\text{obs}}$, is $1.05 \approx 1$. The same calculation for the **G5** group gives an observed genetic risk $R(\mathbf{G5})_{\text{obs}}$ of $9.93 \approx 10$. Because there is no scrapie-affected sheep belonging to the **G2** genotypic group, the observed genetic risk $R(\mathbf{G2})_{\text{obs}}$ is 0, which, given the size of the control group, can be estimated as $\leq 2\times10^{-2}$ (conversely, as there is no **G6** sheep present in the control group, the genetic risk $R(\mathbf{G6})$ cannot be estimated).

Table 2. Frequencies of genotypes in scrapie-affected sheep and in control sheep.

Genotype	Scrapie	Control
ARR/ARR	0	11
ARR/ARQ	0	76
ARQ/AHQ	0	4
ARR/VRQ	0	1
ARQ/ARQ	65	185
ARQ/VRQ	30	9
VRQ/VRQ	1	0
Total	96	286

Table 3. Frequencies of G2, **G4**, **G5** genotypic groups in scrapie-affected sheep and in control sheep. **G2** = {ARR or AHQ}/{ARQ or ARH}, **G4** = {ARQ or ARH}/{ARQ or ARH}, **G5** = VRQ/{ARQ or ARH}.

Genotypes	Scrapie*	% Scrapie	Control*	% Control	$R(\mathbf{Gi})_{obs}$ **	$R(\mathbf{Gi})$***
G2	0	0.00	80	27.97	0.00	$\leq 2\times10^{-2}$
G4	65	67.71	185	64.69	1.05	≈ 1
G5	30	31.25	9	3.15	9.93	≈ 10
G6	1	1.04	0	0		
Number of sheep	96	100.00	286	100.00		

* Sheep collected throughout the 'Pyrénées-Atlantiques' in 1995 in Manech Tête Rousse (MTR) breeds (INRA, unpublished, 1998).
** Ratio of the proportion of a given genotypic group in control sheep to the proportion of the same group in scrapie-affected sheep.
*** Approximate values.

4. Calculation of genetic scrapie susceptibility

From the data above, it is tempting to speculate about the quantitative effect of pairing PrP allelic variants on scrapie susceptibility. Let us suppose that the risk factor $R(\mathbf{Gi})$ associated with the genotype **G*i*** (**G1** to **G6**) is the produce of elementary factors r_j and r_k, with the indices j and k chosen among the three allelic susceptibility classes **R**, **S**, **H** defined above. Data from Table 3 allow us to calculate the values of the elementary risk factors $r_{\mathbf{S}}$, $r_{\mathbf{H}}$ and $r_{\mathbf{R}}$: the value $\boldsymbol{R}(\mathbf{G4})$ for the susceptible–susceptible genotypic group **G4** suggests that $r_{\mathbf{S}} \approx 1$. Hence, the relative risk factor associated to the hypersusceptible–susceptible genotypic group **G5** suggests that $r_{\mathbf{H}} \approx 10$. In the same way, the relative risk factor associated with the resistant–susceptible genotypic group **G2** suggests that $r_{\mathbf{R}} \approx 2\times10^{-2}$. For the other genotypic groups, the calculated relative genetic risk assuming a multiplicative effect is consistent with the published data [17, 18]. These studies compare the PrP genotypes at codon 136, 154, and 171 of natural scrapie-affected sheep with those of control sheep of the same age range from the same flock. Table 4 presents all the data collected from the different monitored flocks: Texel, Halfbred, Herdwick, Merino, Shetland, Suffolk, Poll Dorset, Soay, Manech Tête Rousse.

Table 4 shows, for each genotypic group, the observed relative genetic risk and the calculated relative genetic risk. Sheep distributions according to genotypes are

Table 4. Relative risks and frequencies of genotype in scrapie-affected and control sheep.

Genotypes			Scrapie*	% Scrapie	Control*	% Control	$R(\mathbf{Gi})_{obs}$***	$R(\mathbf{Gi})_{cal}$***	χ^2**	p**
$\mathbf{G_1}$	{ARR, AHQ}	{ARR, AHQ}	0	0.00	72	12.41	0.00	4×10^{-4}	25.8	< 0.000 01
$\mathbf{G_2}$	{ARR, AHQ}	{ARQ, ARH}	0	0.00	155	26.72	0.00	2×10^{-2}	62.9	< 0.000 01
$\mathbf{G_3}$	{ARR, AHQ}	{VRQ}	3	1.60	40	6.90	0.23	0.2	7.55	0.006
G4	{ARQ, ARH}	{ARQ, ARH}	76	40.43	282	48.62	0.83	1	3.83	0.05
$\mathbf{G_5}$	{ARQ, ARH}	{VRQ}	88	46.81	30	5.17	9.05	10	189.3	< 0.000 01
$\mathbf{G_6}$	{VRQ}	{VRQ}	21	11.17	1	0.17	64.79	10^2	61.7	< 0.000 01
Total			188	100.00	580	100.00			302.9	< 0.000 01

* Data grouped from control studies presented here and published. Sheep into different flocks: Texel (affected: 34, control: 91), Halfbred (affected: 17, control: 23), Herdwick (affected: 6, control: 35), Merino × Shetland (affected: 3, control: 45), Shetland (affected: 22, control: 38), Suffolk (affected: 5, control: 21), Poll Dorset (affected: 4, control: 16), Soay (affected: 1, control: 25), Manech Tête Rousse (affected: 96, control: 286).
** Calculated by comparing a given genotype with other possible genotypes in the scrapie-affected group and control group.
*** $R(\mathbf{Gi})_{obs}$ is the ratio between the frequencies of the **G*i*** genotype in scrapie-affected group and control group, and $R(\mathbf{Gi})_{cal}$ is calculated as the product of the genetic risk associated with each allelic variant class.

significantly different in the scrapie-affected population and in the control population ($\chi^2 = 302.8913$, $p = 2.3935\times10^{-63}$). Most of the scrapie cases are in the **G5** group, and the frequency of **G5** animals in affected population (46.81%) is about ten times higher in this population than in the control population (5.17%): $R(\mathbf{G5})_{obs} = 9.05$. The calculated relative risk has a good predictive value ($r_S\, r_H = 10$). Many scrapie cases are also in the **G4** group, and the frequency of **G4** animals in the affected population (40.43%) is approximately the same in the control population (48.62%). The genetic risk $R(\mathbf{G4})_{obs}$ equals 0.83. Again, the calculated relative risk has a good predictive value ($r_S\, r_S = 1$). The frequency of **G6** animals in the affected population (11.17%) is nearly a hundred times higher than in the control population (0.17%): $R(\mathbf{G6})_{obs} = 64.79$. This is in accordance with the calculated value of the relative risk ($r_H\, r_H = 10^2$). The relative frequency of **G2** and **G3** sheep in affected population (0 and 1.60%), compared with the same frequencies in the control population (26.72 and 6.90%), are in accordance with the calculated values ($r_R\, r_S = 2\times10^{-2}$ and $r_R\, r_H = 0.2$). Finally, in the most resistant genotypic group, **G1**, no case of scrapie have been found in sheep neither in Europe nor in the USA. Only one affected Suffolk sheep with the genotype ARR/ARR has been reported in Japan [19]. Our multiplicative calculation method takes into account a risk of four affected **G1** sheep for ten thousand healthy sheep ($rr = r_R\, r_R = 4\times10^{-4}$). It is to be noted that in a recent case-control study involving 18 British flocks of various breeds, Hoek and al. [20] obtained very similar results: the risks associated with different genotypes, compared to the risk associated with the ARQ/ARQ ancestral genotype gave odds-ratios similar to our $R(\mathbf{Gi})_{cal}$ values: 0.23 for the AHQ/VRQ genotype and 0.51 for the ARR/VRQ genotype (**G3** group), 15.9 for the ARH/VRQ genotype and 14.6 for the ARQ/VRQ genotype (**G5** group), 0.04 for the ARR/ARQ genotype (**G2** group), and finally 65.0 for the VRQ/VRQ genotype (**G6** group).

5. Biological discussion of the framework

In the outbreaks of natural scrapie described here, the primary risk factor associated with the occurrence of the disease is unambiguously the PrP genotype. Recent analysis of the human PrP gene in cases of sporadic and familial Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler syndrome have revealed a number of mutant alleles of this gene that appear to control the incidence of these diseases [21]. Similar association of a normal allele with disease onset has been proposed for Huntington disease [22]. But scrapie cannot be entirely genetic in origin, as proposed by Parry [23]: this disease is also transmissible. Experimental challenges of sheep of different genotypes, with different scrapie isolates, are consistent with these observations of natural outbreaks [12]. {ARR, AHQ}/VRQ are antagonist: the first is associated with low ($r_R \approx 2\times10^{-2}$) and the second with high ($r_H \approx 10$) disease susceptibility. This fact suggests that the relative dominance of these allelic variants account for all scrapie sources (‘strains’). Only one reversed association between the SSBP/1 isolates and the BSE isolates is described by Goldmann et al. [9] for sheep heterozygous at both codon 136 and 171 (VQ/AR), but this inversion has been obtained in a very specific intracerebral challenge.

Differentiating a genetic origin and a genetic susceptibility in a contaminated environment (infectious dose) is very difficult. It is not clearly decidable whether resistant sheep cannot become infected, or whether the incubation period in these sheep is so long in relation to sheep life expectancy that clinical signs are never observed. In the first case, a resistant sheep might be able to limit multiplication of the infectious agent, and to tolerate a low level of scrapie infection. In the second case, resistant genotype sheep might act as carriers: without the appearance of the disease, a resistant sheep could act as a source of infection of more susceptible sheep. Unable to limit multiplication and spread of scrapie infection, susceptible sheep become more quickly ill [24]. Hunter [25] says that it is possible that the rate-limiting step is neuro-invasion without which infection, from peripheral tissues, could not spread to the brain to cause illness and death. In this context, the multiplicative effect should be linked to a serial process determining firstly the multiplication and secondly the neuro-invasion of the infectious agent. Additional data will help refining the risk factors associated with each allele, and will probably allow a clearer understanding of the processes controlled by the genotypic factors. This evidence of a multiplicative effect of scrapie genetic susceptibility is very likely to exist also in other neurodegenerative diseases.

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