Association of the *PRNP* regulatory region polymorphisms with the occurrence of sporadic Creutzfeldt-Jakob disease

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Abstract

The prion protein (PrP) plays a central role in the pathogenesis of Creutzfeldt-Jakob disease and other transmissible spongiform encephalopathies (TSEs). Mutations in the coding region of the prion protein (PRNP) gene are linked to inherited forms of TSEs whereas aetiology of sporadic CJD (sCJD) remains obscure. It remains unclear whether the primary DNA sequence at non-coding region of PRNP gene influences development of the sCJD. Several recent reports showed non-coding region polymorphisms associated with sCJD but other could not support those findings. To test the hypothesis that there is a relationship between SNPs polymorphisms of PRNP non-coding regions and susceptibility to sCJD, we compared the primary structure of the regulatory region of the PRNP in 45 Dutch sCJD patients and in 135 healthy controls. We found a significant linkage of +310 C allele (OR 0.27, 95% CI 0.09-0.77; P = 0.009) and +310G/C genotype (OR 0.33, 95% CI 0.11-0.98; P = 0.048) with sCJD. No differences in frequencies of genotypes and allele of –101C/G and +258 G/A polymorphisms were found between sCJD patients and controls. We found two haplotypes protecting from sCJD (C-V in block 1 and G-C in block 2) and one susceptible haplotype for sCJD (G-G in block 2). Our findings support the hypothesis that polymorphism in the regulatory region of the PRNP gene may play an important role in the pathogenesis of sCJD.

Key words: Creutzfeldt-Jakob disease, prion disease, prion protein gene, prion protein promoter.

Introduction

Creutzfeldt-Jakob disease (CJD) is a rare fatal neurodegenerative disease associated with accumulation of the host-encoded abnormal isoform of the prion protein (PrP^{Sc}) in the brain and other tissues of affected individuals. The disease may be acquired (kuru and iatrogenic Creutzfeldt-Jakob disease), inherited, or of idiopathic (sporadic) origin [2,8]. The aetiology of the idiopathic form, sporadic Creutzfeldt-Jakob disease (sCJD),

remains obscure. However, the prion protein (*PRNP*) gene polymorphism, encoding methionine or valine at codon 129 is a strong susceptibility factor for sCJD. Patients with sporadic CJD are predominantly methionine or valine homozygous at codon 129 [11] whereas valine homozygosity acts as a susceptibility factor for iatrogenic CJD [7]. However, epidemiological data on sCJD incidence and codon 129 allelic frequencies [1] strongly indicate that this polymorphism cannot be the only factor influencing aetiology of sporadic CJD; therefore, factor or factors other than the coding region of *PRNP* polymorphisms must be involved in this process. Furthermore, it is believed that, in addition to such factors as the environmental exposition to prions, stochastic conversion of normal prion protein or a somatic *PRNP* mutation (albeit never found), which change *PRNP* expression leading to increase in PrP concentration is influencing susceptibility to sCJD. This suggestion comes from the studies of the transgenic mouse model which demonstrated that the level of *PrP* gene expression had a significant impact on the initiation and progression of the prion diseases. Transgenic mice containing multiple copies of hamster *PrP* gene had significantly reduced incubation time after hamster scrapie challenge, otherwise not pathogenic to mice, compared with a analogous challenge to wild-type mice [14], while hemizygous mice with only one copy of the *PrP* gene and with 50% normal *PrP* expression level had extended incubation period when compared with wild-type mice and *Prnp*$^{0/0}$ mice that did not express prion protein were completely resistant to infection with prions [6]. Different *PrP* expression levels could be due to the polymorphisms of the regulatory region of the *PRNP* gene. To date, several single nucleotide polymorphisms of the promoter region of the *PRNP* gene have been reported, a C to G transversion at position −101, a G to C transversion at position +310 and T to C transition at position +385. However, the links of these polymorphisms with the CJD development are inconsistent [10,12,15]. We have previously shown that the presence of −101G allele of *PRNP* may be considered as the risk factor for sCJD among codon 129 heterozygotes [4]. The purpose of this study was to reveal the possible links between SNPs located outside of the coding region of the *PRNP* gene and the overall susceptibility to sCJD in the Dutch population. To achieve this goal we investigated the genotype, allele and haplotype frequencies of the following *PRNP* polymorphisms: +258G/A, +310 G/C, and +385T/C in a sample of 45 patients with sporadic CJD and in 135 control individuals.

Material and methods

Subjects

The study included 45 neuropathologically confirmed sCJD cases of the Dutch origin. The control comprised 135 unrelated individuals with no history of neurodegenerative disease. All patients and controls were Caucasians. Informed consent for research purposes was obtained from all control subjects involved in the study and the project was approved by a local research ethics board. The patients in this study were diagnosed with the sporadic form of CJD and were classified as definite or probable according to criteria established earlier [5]. We sequenced the entire open reading frame of *PRNP* of all CJD cases to exclude from study the patients with the mutations in the prion protein gene.

Genotyping

Genomic DNA from peripheral blood leukocytes of controls and from post mortem brain tissue samples of CJD cases was isolated by the routine proteinase K-phenol-chloroform extraction. Polymorphism of codon 129 of the *PRNP* gene and polymorphism of −101 position was examined according to procedures described earlier [4]. The region downstream of exon I spanning nucleotides from 12742 to 13318 (GeneBank U29185) was amplified using the forward primer 5'-TTCTCCTCTCCTCACGACCG and the reverse primer 5'-TCTCTCCATCCCCCCAAGC. The PCR products were purified and then directly sequenced on an ABI 377 automatic sequencer using ABI Prism BigDye Terminator v 3.1 cycle sequencing kit (Applied Biosystems, USA). The results were analyzed using DNA Sequencing Analysis Software version 3.4.1.

Statistical analysis

Hardy-Weinberg equilibrium of all SNPs was determined in controls using a chi-squared test. The association between the rare alleles of *PRNP* regulatory region in SNPs carriers and the risk of sCJD onset was further analysed. Differences in allele and genotype distribution between cases and controls were tested using Fisher's exact test within STATISTICA StatSoft 8.2. Genetic association was expressed as odds ratio (OR) for heterozygotes and homozygotes of the rare allele versus homozygotes of the common allele.

To assess the association between sCJD and SNPs haplotypes, we used the Haplo.Stat program, which implements the test proposed by Schaid *et al.* [13]. Hap-

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lotypes with an estimated frequency lower than 0.01 were excluded from further analysis. The standardized measure of linkage disequilibrium (LD) was computed at pairs of SNP loci. Pairwise LD estimations were performed using Haploview software [3].

Haplotypes in two blocks were analyzed separately when D' > 0.9. Since frequency of minor allele at marker +385 was very low, haplotype analysis within block 2 was limited to polymorphism +285 and +310.

Results

Genotype and allele distribution

To examine the correlation between *PRNP* non-coding region polymorphisms and susceptibility to sporadic CJD (spanning nucleotides from 12742 to 13318 [GeneBank U29185]), we analyzed the genotype and allele frequencies of these polymorphisms and codon 129 polymorphism in 45 sCJD cases and 135 healthy controls. All genotype frequencies were in Hardy-Weinberg equilibrium (HWE) with the exception of the *PRNP* codon 129 polymorphism in controls ($P = 0.001$). This variation from HWE confuses the haplotype analyses and interpretation of these SNP results.

Distribution of codon 129 polymorphism of the *PRNP* gene was as follows: 15 (33%) CJD cases and 86 (64%) control individuals were M/V heterozygous, 23 (51%) CJD cases and 30 (22%) control individuals were M/M homozygous, and 7 (16%) CJD cases and 19 (14%) control individuals were V/V homozygous. These results are consistent with the earlier reports that codon 129 M/M homozygosity acts as a susceptibility factor whereas heterozygosity at codon 129 partially protects against development of sCJD (OR 4.40; 95% CI 2.03-9.51; $P = 0.0002$).

We found four single nucleotide polymorphisms within regulatory region of the *PRNP* gene: C to G transversion at position −101 occurring at an allele frequency of 0.11 in control individuals and 0.16 in CJD cases, G to A substitution at position +258 at an allele frequency of 0.03 in control individuals and 0.01 in CJD cases, G to C transversion at position +310 at an allele frequency of 0.15 in control individuals and 0.04 in CJD cases, and T to C substitution at position +385 at an allele frequency of 0.01 in control individuals (+385 C allele was not found in CJD cases).

Association of SNPs with sCJD

As shown in Table I, there were no statistical association between −101C/G (OR = 1.54, 95% CI: 0.79-3.01;

$P = 0.21$ for G vs. C allele and OR = 1.96, 95% CI: 0.93-4.16; $P = 0.1$ for C/G vs. C/C genotype) as well as +258 G/A (OR = 0.37, 95% CI: 0.45-2.98; $P = 0.46$ for A vs. G allele and OR = 0.36, 95% CI: 0.44-2.97; $P = 0.45$ for G/A vs. G/G genotype) polymorphisms and CJD susceptibility. Significantly decreased ORs were found in +310 C allele (OR = 0.27, 95% CI: 0.09-0.77; $P = 0.009$) and +310 GC genotype (OR = 0.33, 95% CI: 0.11-0.98; $P = 0.048$) carriers.

Haplotype frequency and linkage disequilibrium

Two haplotype blocks were constructed using Haploview, an LD-based partitioning algorithm, as shown in Fig. 1. Haplotype in two blocks was analyzed separately when D' value was over 0.9. Since frequency of minor allele of +385 position was very low, haplotype analysis within block 2 was limited to polymorphisms +258 and +310. Haplotypes with an estimated frequency lower than 0.01 were excluded. As shown in Table II, there was a significant negative association between haplotype C-V in block 1 and sCJD (Score = −2.47, $P = 0.014$). In block 2, haplotype G-C was associated with a reduced risk of sCJD (Score = −2.46, $P = 0.014$) whereas haplotype G-G was associated with an increased risk of sCJD (Score = 2.77, $P = 0.006$).

Discussion

Consistently with the earlier published data we found 129Met/Met homozygosity associated with an increased risk of sCJD (OR = 4.40, 95% CI: 2.03-9.51; $P = 0.0002$). However, the interpretation of these results is hindered by deviation of controls from HWE. Genotype frequencies may deviate from the expected values for several reasons such as genotyping error, small population size due to genetic drift and founder effect, and nonrandom mating. 129Met/Val *PRNP* polymorphism is a widely documented susceptibility sCJD factor but it does not explain all sCJD cases. Recently a lot of effort was put into identification of other genetic risk factors, especially located within the *PRNP* regulatory regions. Previous studies of non-coding regions of the *PRNP* gene showed presence of the regulatory sequence upstream of the transcription start site between −148 and −114 [9]. In addition, the intronic region of *PRNP* was proved to contain regulatory elements between +292 and +625 positions [10]. An association of sCJD with the polymorphisms in the regulatory regions of *PRNP* was investigated by several authors.
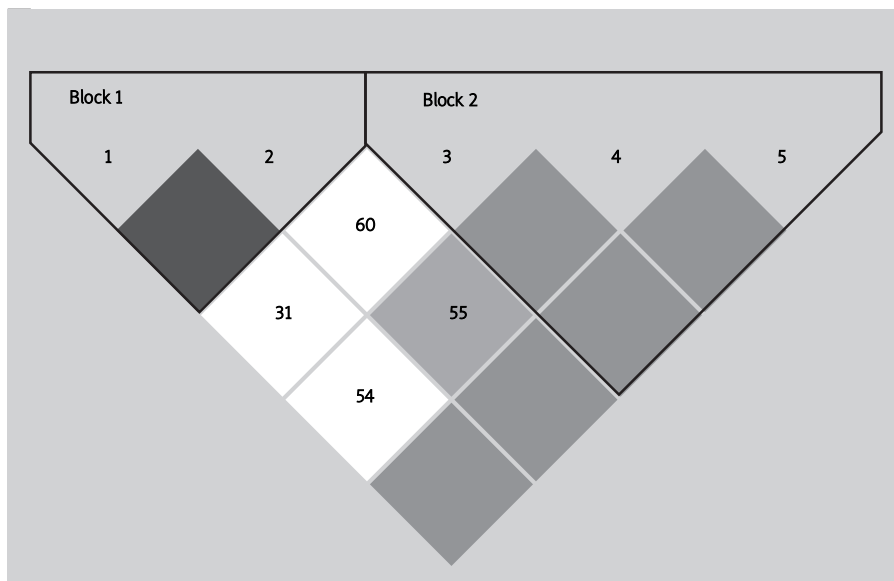
Table I. Distributions of SNP alleles and genotypes in sCJD patients and healthy controls

Polymorphism			CJD N	Control N	HWE	OR (95% CI)	*P*-value*
SNP –101					0.846		
	alleles	C	75	239		reference	
		G	15	31		1.54 (0.79-3.01)	0.21
	genotypes	C/C	30	106		reference	
		C/G	15	27		1.96 (0.93-4.16)	0.10
		G/G	0	2			
SNP 129					0.071		
	alleles	A	61	146		reference	
		G	29	124		0.55 (0.34-0.93)	0.03
						4.40 (2.03-9.51)	0.0002
	genotypes	A/A	23	30		reference	
		A/G	15	86		2.11 (0.77-5.89)	0.16
		G/G	7	19			
SNP 258					1.000		
	alleles	G	89	262		reference	
		A	1	8		0.37 (0.45-2.98)	0.46
	genotypes	G/G	44	127		reference	
		G/A	1	8		0.36 (0.44-2.97)	0.45
		A/A	0	0			
SNP 310					0.201		
	alleles	G	86	230		reference	
		C	4	40		0.27 (0.09-0.77)	0.009
	genotypes	G/G	41	100		reference	
		G/C	4	30		0.33 (0.11-0.98)	0.048
		C/C	0	5			0.32
SNP 385					1.000		
	alleles	T	90	268		reference	
		C	0	2			1.00
	genotypes	T/T	45	133		reference	1.00
		T/C	0	2			
		C/C	0	0			

*Estimated with two-sided Fisher's exact test
OR – odds ratio, CI – confidence interval

Analysis of studies of the association of –101 C/G polymorphism with sCJD lead to ambiguous conclusions. Previously we have found that -101G allele acts as a risk factor in codon 129 heterozygous subgroup (*P* = 0.047) [4]. In the present study we demonstrated a slight over-representation of –101G allele and –101G/C heterozygosity in the sCJD group, however these differences were not statistically significant. The influence of polymorphisms located within the *PRNP* non-coding regions was studied by McCormack *et al.* [10], who observed a non-significant overrepresentation of –101G allele in 129Met homozygous sCJD patients compared with controls. In this study, –101G allele seemed to be a susceptibility factor in 129Met homozygotes when the number of its carriers was compared with the number of individuals homozygous for the common allele at polymorphisms tested. On the contrary, no association between –101 *PRNP* polymorphism and sCJD was shown by

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Fig 1. Pairwise Linkage Disequilibrium between the *PRNP* SNPs. D' values and haplotype blocks. The plot shows the pairwise relationships for the studied SNPs: 1 (–101C/G), 2 (129A/G),3 (+258G/A), 4 (+310G/C), 5 (+385T/C). The associations estimated and graph constructed with the use of Haploview software.

Table II. Associations of PRNP haplotypes with sCJD

	SNP combination*	Haplotype-frequency	Haplotype score	*p*-value	simulated *p*-value
Block 1	CG	0.425	−2.467	0.014	0.016
	GA	0.128	1.307	0.191	0.139
	CA	0.447	1.377	0.169	0.131
Block 2	GC	0.122	−2.458	0.014	0.009
	GG	0.853	2.768	0.006	0.004

Haplotypes indicated by SNP alleles with markers placed in the order: block 1 ~101C/G, 129A/G (Met to Val substitution); block 2 +258G/A, +310G/C

Vollmert *et al.* in the study involving 534 sCJD cases and 740 controls [15].

The presented work showed deficiency of +258A allele among the sCJD group, however this difference was not statistically significant. We also found no association of +385 polymorphism with sCJD, though the rare allele occurs with very low frequency (+385C allele 0.007), which is in accordance with McCormack's report showing non-significant excess of +385C allele in sCJD129Met/Met homozygotes compared to an analogous control subgroup.

While evaluating the association of +310G/C SNP with sCJD in the Dutch population, we have found a significant relation of +310 G/C SNP with the sporadic form of CJD. The presence of +310 G/C genotype results in an almost three-fold decreased risk of sCJD (OR = 0.33,

95% CI: 0.11-0.98; *P* = 0.048). This effect is even more noticeable if +310C allele frequency was compared (OR = 0.27, 95% CI: 0.09-0.77; *P* = 0.009).

McCormack *et al.* found a non-significant overre-presentation of +310C allele carriers among 129Met/Met homozygous sCJD patients compared to 129Met/Met control subgroup. However, their study comprised limited numbers of subjects (25 sCJD cases and 100 controls). Interestingly, Sanchez-Juan *et al.* recently found that carrying +310 C allele is associated with 2.4 increase of developing sCJD when adjusted with *PRNP* 129Met/Val genotype [12].

Here, we demonstrated that five SNPs are formed into two haploblocks. We found two haplotypes protecting from sCJD (C-V in block 1 and G-C in block 2) and one susceptible haplotype for sCJD (G-G in block 2).

Because the genetic variations of the *PRNP* gene leading to alterations of the protein structure are involved in minority of CJD cases, polymorphisms in the regulatory region of the prion protein gene are considered as the CJD susceptibility factors. The study of the regulatory region of the *PRNP* gene may provide a key to solving the problem of individual susceptibility to sporadic and acquired forms of prion diseases. Studies of a correlation between *PRNP* polymorphisms and CJD in different populations revealed contradictory results. There may be various explanations for these differences across studies. One possibility may be a relatively small sample size of CJD patients and control subjects. The other explanation is that the distribution of the *PRNP* genotype between studied ethnic groups is different. Further meta-analysis is needed to solve this enigma. Moreover, the research on the physiological role of *PRNP* promoter polymorphisms including their influence on PrP expression levels should be performed in the future.

Acknowledgements

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