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ELECTRONIC LETTER

Significant association of a M129V independent polymorphism in the 5′ UTR of the *PRNP* gene with sporadic Creutzfeldt-Jakob disease in a large German case-control study

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Background: A single nucleotide polymorphism (SNP) in the coding region of the prion protein gene (*PRNP*) at codon 129 has been repeatedly shown to be an associated factor to sporadic Creutzfeldt-Jakob disease (sCJD), but additional major predisposing DNA variants for sCJD are still unknown. Several previous studies focused on the characterisation of polymorphisms in *PRNP* and the prion-like doppel gene (*PRND*), generating contradictory results on relatively small sample sets. Thus, extensive studies are required for validation of the polymorphisms in *PRNP* and *PRND*.

Methods: We evaluated a set of nine SNPs of *PRNP* and one SNP of *PRND* in 593 German sCJD patients and 748 German healthy controls. Genotyping was performed using MALDI-TOF mass spectrometry.

Results: In addition to *PRNP* 129, we detected a significant association between sCJD and allele frequencies of six further *PRNP* SNPs. No significant association of *PRND* T174M with sCJD was shown. We observed strong linkage disequilibrium within eight adjacent *PRNP* SNPs, including *PRNP* 129. However, the association of sCJD with *PRNP* 1368 and *PRNP* 34296 appeared to be independent on the genotype of *PRNP* 129. We additionally identified the most common haplotypes of *PRNP* to be over-represented or under-represented in our cohort of patients with sCJD.

Conclusion: Our study evaluated previous findings of the association of SNPs in the *PRNP* and *PRND* genes in the largest cohorts for association study in sCJD to date, and extends previous findings by defining for the first time the haplotypes associated with sCJD in a large population of the German CJD surveillance study.

Creutzfeldt-Jakob disease (CJD) is a fatal transmissible neurodegenerative disease in humans, which can present in acquired, familial, or sporadic forms. A minority of CJD cases is caused by transmission from material contaminated with infectious human CJD or bovine spongiform encephalopathy (BSE). Around 10% of cases are inherited and are associated with mutations in the prion protein gene (*PRNP*), located on chromosome 20p12.[1–3] The majority of CJD cases (more than 90% of the CJD cases) occur sporadically, representing the most common form of CJD worldwide. Prion diseases such as CJD in humans, BSE in cattle, and scrapie in goats and sheep are characterised by spongiform degeneration, neuronal cell loss, astrogliosis, and deposition of the scrapie isoform of the prion protein, PrP^{Sc}, in the brain. The latter is derived through a posttranslational conformational conversion from the cellular prion protein, PrP^{C}.

The aetiology of sporadic CJD (sCJD) is not known. A common polymorphism in the coding region of the *PRNP* gene at codon 129 (M129V) is a recognised genetic risk factor. Homozygotes for methionine and to a lesser extent for valine are over-represented in every group of sCJD examined to date.[1 4–6] In addition, this polymorphism has been shown to modify the clinical and neuropathological phenotype of the disease.[5] In white populations, according to studies in several countries, 37.5–45% of individuals are homozygous for methionine and 40–51% heterozygous, and 10–15% are homozygous for valine.[6] However, *PRNP* M129V may not be the sole genetic factor predisposing to the disease. In mouse models of prion disease, inbred mouse strains with the same genotype of the prion protein gene show notable differences in the incubation time following experimental infection. Detailed studies have detected several quantitative trait loci for susceptibility to prion disease in mice, suggesting the influence of genetic factors other than *PRNP*.[7–9]

There is growing interest in polymorphisms outside the coding region of the *PRNP* gene and in other candidate genes. Mead *et al* identified 56 polymorphic sites within 25 kb of the *PRNP* locus, including sites within the *PRNP* promoter and the *PRNP* 3′ untranslated region. In an association study comprising 93 sCJD patients from the UK and 652 healthy controls from families in the UK and those registered with CEPH (the Centre d'Etude du Polymorphisme Humain), a significant association between an SNP upstream of *PRNP* exon 1 (designated SNP 1368) and sCJD was demonstrated, in addition to the strong susceptibility conferred by codon 129.[10] However, this finding could not be confirmed by Croes *et al* in a study based on a Dutch cohort.[11] McCormack *et al* evaluated three SNPs at position 101 bp upstream of exon 1 and at 310 bp and 385 bp downstream of exon 1 of human *PRNP*, which are within or adjacent to the regulatory regions of *PRNP*.[12] This group suggested an association of CJD with the polymorphisms in the regulatory regions of *PRNP*. However, their data were based on a case-control study with limited sample size (sCJD, n = 25; controls, n = 100). Thus,

Abbreviations: BSE, bovine spongiform encephalopathy; KORA S4, Kooperative Gesundheitsforschung im Raum Augsburg, survey 4; LD, linkage disequilibrium; MALDI-TOF, matrix assisted laser desorption ionisation-time of flight; MS, mass spectrometry; *PRND*, prion-like doppel gene; *PRNP*, prion protein gene; SAP, shrimp alkaline phosphatase; sCJD, sporadic Creutzfeldt-Jakob disease; SNP, single nucleotide polymorphism; SSCP, single strand conformational polymorphism

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extensive studies are required for validation of the polymorphisms of *PRNP* in larger cohorts.

Another gene of interest is the prion-like doppel gene (*PRND*), located 16 kb downstream of *PRNP*.[13] *PRND* shares 24% coding sequence identity with *PRNP*, and its product, the doppel protein (Dpl), was suggested to have biological properties antagonistic to PrP.[14] Although there have been several published studies on the association of SNPs in *PRND* with CJD, particularly *PRND* T174M, other studies could not confirm this association.[10 11 15–17]

To evaluate the published data, which in most studies were derived from small cohorts and in part produced conflicting data, we investigated the association of *PRNP* and *PRND* polymorphisms with sporadic CJD. The association study was performed in the largest case and control cohorts to date. We analysed the genomic DNA of 593 sCJD patients and 748 healthy controls matched for age and sex from a population based German study (Kooperative Gesundheitsforschung im Raum Augsburg, survey 4, (KORA S4), 2000). Analysing nine polymorphic positions in the *PRNP* locus and one SNP of the *PRND*, we identified two SNPs that act as possible risk factors for sCJD in addition to the *PRNP* 129 polymorphism. Additionally, we found the most common haplotypes to be differentially distributed in healthy German controls and German patients with sCJD.

METHODS

Subjects

CJD suspects were referred to the German CJD surveillance unit (http://www.neuropathologie-lmu-muenchen.de/inp/ and http://www.cjd-goettingen.de/) and were clinically classified as ''probable'' or ''possible'' CJD or ''other''.[18 19] The diagnosis of ''definite'' CJD was made on post-mortem examination using neuropathological criteria.[20] DNA of patients with ''probable'' and ''definite'' CJD were collected and screened for pathogenic mutations in the *PRNP* gene. To screen for pathogenic mutations, the coding region of *PRNP* was amplified by PCR and subjected to either SSCP analysis or direct sequence analysis (n = 316 of all analysed cases).[1] Familial cases with pathogenic mutations in *PRNP*[1] were excluded. The remaining 593 cases, including 387 ''definite'' sCJD and 206 ''probable'' sCJD cases were enrolled in our association study.

In total, 748 age and sex matched healthy controls were taken from a population based German study performed in the city and region of Augsburg (KORA S4),[21 22] which is a representative sample of the adult general population of German nationality.

All study participants gave informed written consent according to the Bavarian ethics committee and the ethics committee of the Ludwig Maximilians University of Munich, and every attempt was made to ensure anonymity of the participants.

DNA preparation and analysis

Genomic DNA of sCJD patients was extracted in most cases from blood or in some cases from frozen postmortem brain tissue using commercial kits (QIAamp Blood Kit or QIAamp Tissue Kit; Qiagen, Hilden, Germany) according to the manufacturer's protocols. For screening for the pathogenic mutations, the coding region of *PRNP* was amplified by PCR and subjected to single strand conformational polymorphism (SSCP) analysis or direct sequence analysis.[1] Genomic DNA from the KORA probands was extracted from blood leukocytes (Puregene DNA Isolation Kit; Gentra Systems, Minnesota 55441, USA), following the manufacturer's instructions.

MALDI-TOF MS genotyping

Genotyping of single nucleotide polymorphisms was performed using matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) (Mass Array; Sequenom, San Diego, CA, USA). Genomic DNA (5 ng) was amplified by PCR using 0.1 U HotStar *Taq* DNA polymerase (Qiagen). PCR conditions were 95℃ for 15 minutes, followed by 44 cycles of 95℃ for 30 seconds, 56℃ for 30 seconds and 72℃ for 1 minute, with a final cycle of 72℃ for 10 minutes. (Primer information is given in supplementary table 1, available online at http://www.jmedgenet.com/supplemental.) PCR products were treated with shrimp alkaline phosphatase (SAP; Amersham, Freiburg, Germany) for 20 minutes at 37℃ to remove excess dNTPs, followed by 10 minutes at 85℃ to inactivate SAP. Base extension (homogenous MassEXTEND; Sequenom) reactions in a final volume of 10 µl contained extension primers (supplementary table 2, available online at http://www.jmedgenet.com/supplemental) at a final concentration of 0.54 µmol/l and 0.6 U ThermoSequenase (Amersham, Freiburg, Germany). Base extension reaction conditions were 94℃ for 2 minutes, followed by 40 cycles of 94℃ for 5 seconds, 52℃ for 5 seconds, and 72℃ for 5 seconds. The final base extension products were treated with SpectroCLEAN resin (Sequenom). Aliquots (10 nl) of the reaction solution were dispensed onto a 384 format microarray (SpectroCHIP; Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid. A modified Bruker Biflex MALDI-TOF MS was used for data acquisitions from the SpectroCHIP. Genotype calling was performed in real time with MassARRAY RT software (version 3.0.0.4; Sequenom). For quality control, negative controls were included in all assays. To establish reproducibility of genotyping data, 10% of randomly selected samples were genotyped in duplicate. Genotype frequencies at all loci were subjected to Hardy-Weinberg equilibrium analysis.

Statistical analysis

Hardy-Weinberg equilibrium of all SNPs was determined individually in controls using log likelihood tests. To evaluate the non-random association of SNPs, pairwise linkage disequilibrium (LD) statistics D′ and correlation coefficient r^2 were calculated using Haploview software (version 3.2; Whitehead Institute for Biomedical Research; http://www.broad.mit.edu/mpg/haploview/index.php). D′ explains the difference in frequency between the observed and expected number of SNP pairs. Being scaled to D_{max}, it spans the range −1 to 1, and r^2 is the squared correlation coefficient between the markers.[23]

Differences in genotype distribution between cases and controls were tested by the robust version of Cochran-Armitage trend test[24], adjusting for multiple testing according to the Sidak's method.[25] Genetic association was expressed as odds ratios (OR) for heterozygotes and homozygotes of the rare allele versus homozygotes of the common allele, and estimated within logistic regression models. These estimations were repeated within subgroups defined by the genotype of marker *PRNP* M129V (that is, homozygous MM and VV, and heterozygous MV).

Haplotype frequencies for all observed markers were estimated applying the EM algorithm of Excoffier and Slatkin,[26] using Arlequin software (version 2.000). Statistical analysis was performed using SAS (version 8.2; SAS Institute, Cary, NC, USA). The project was accomplished according the Guter Epidemiologischer Praxis (GEP) recommendations.[27]

RESULTS

Association study

To reveal the association of *PRNP* and *PRND* with sCJD, we genotyped nine SNPs in the *PRNP* locus and one SNP in the *PRND* locus in 593 sporadic CJD patients and 748 healthy

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controls (fig 1). In addition to the SNPs in the coding region of *PRNP* (*PRNP* A117A and *PRNP* M129V) and *PRND* (*PRND* T174M), other SNPs were identified by Mead *et al*.[10] The SNP panel was chosen for genotyping because they are evenly distributed across the region of interest and have been shown to be highly polymorphic in the UK population and the families registered with CEPH.[10]

The genotype frequencies of all SNPs studied achieved the criteria of Hardy-Weinberg equilibrium in the healthy control group (data not shown). As shown in table 1, we observed a significant association between sCJD and the allele frequency of the *PRNP* 129, confirming the predisposing role of the *PRNP* 129 polymorphism in sCJD. Additionally, we detected a highly significant association between sCJD and six other SNPs of the *PRNP* locus: apart from the rare SNPs *PRNP* 12533 and *PRNP* A117A, all eight *PRNP* SNPs with a minor allele frequency >15% showed significant differences in allele frequencies between sCJD and controls. The allele frequency of *PRND* T174M did not show any association with sCJD.

To define more precisely the role of the SNPs in risk of developing sCJD, we further investigated the association of various genotypes and sCJD (fig 1 and supplementary table 3). Apart from *PRNP* 12533 and *PRND* T174M, we observed a significant association with the genotypes of all other *PRNP* SNPs including the rare SNP *PRNP* A117A. While *PRNP* 1368 and *PRNP* 34296 showed increased ORs in a minor allele dose dependent manner, heterozygosity for all other *PRNP* SNPs significantly decreased the risk of sCJD. The homozygous common alleles of these SNPs (including PRNP 13436, 16987, 22976, M129V, and 28878) were associated with higher risk of developing sCJD compared with the homozygous minor alleles.

Linkage disequilibrium analysis

We observed strong LD in controls within eight adjacent SNPs, from *PRNP* 1368 to *PRNP* 28878 ($D' = 0.78$ to 1.00, $r^2 = 0.5$ to 0.9), but markedly less LD to *PRNP* 34296 ($D' = 0.33$ to 0.86, $r^2 = 0.12$ to 0.53) (fig 2). Owing to the lower frequency of the minor alleles in *PRNP* 12533 (13%) and *PRNP* A117A (3%), the r^2 values of these less frequent SNPs were markedly lower ($r^2<0.3$). There was no LD between the *PRNP* SNPs and *PRND* T174M ($D' = 0.01$ to 0.38, $r^2 = 0.00$ to 0.15).

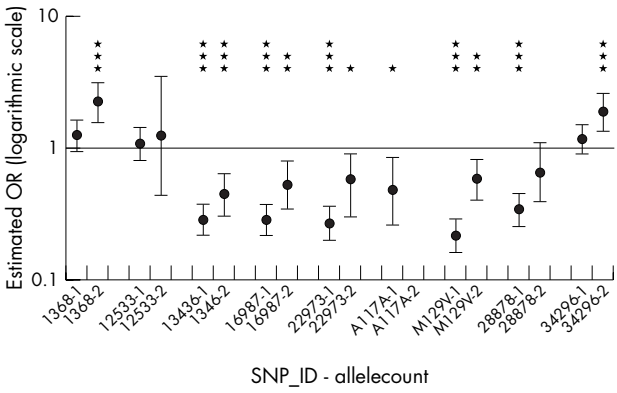

Figure 1 Association of genotypes of *PRNP* SNPs with sCJD. Estimated odds ratios of heterozygous (SNP ID-1) and homozygous (SNP ID-2) minor alleles versus the homozygous common allele, and 95% confidence intervals are shown. No homozygous minor allele was observed in *PRNP* A117A. Detailed information is given in supplementary table 3 (available online at http://www.jmedgenet.com/supplemental). * p=0.01, ** p=0.001, *** p<0.0001.

Additional *PRNP* risk factors aside of the *PRNP* M129V polymorphism

Given the strong LD between SNPs in the *PRNP* gene, the significant association of *PRNP* SNPs with sCJD may be due to hitchhiking effects with the known predisposing *PRNP* M129V polymorphism. To identify possible risk factors additional to the *PRNP* M129V, we performed analysis in the *PRNP* M129V subgroups. This revealed a significantly higher risk for minor allele homozygotes of *PRNP* 1368 in the subgroup of *PRNP* codon 129 MM homozygotes (OR = 1.7, 95% confidence interval (CI) = 1.002 to 2.931) (table 2). In the same subgroup, only the *PRNP* 34296 minor allele showed a trend for association with sCJD (OR = 1.5, 95% CI = 0.94 to 2.45), independent of the *PRNP* M129V polymorphism. All other SNPs did not show any association with sCJD independent of the genotype of marker *PRNP* M129V.

SNP haplotypes

In the region between *PRNP* 1368 and *PRNP* 34296, we revealed the existence of 188 complete and incomplete haplotypes. As shown in table 3, five major *PRNP* haplotypes showed a frequency >5% in all cases and controls, representing 62% of all estimated haplotypes. Two of these five haplotypes (haplotypes 4 and 5 in table 3), which only differed by the allele of the marker *PRNP* 34296 at the far end of the screen region, were significantly over-represented in sCJD patients. The remaining three haplotypes (haplotypes 1–3 in table 3) , which differed again at marker *PRNP* 34296 and additionally at *PRNP* 28878, were significantly under-represented in patients.

DISCUSSION

The identification of genetic risk factors in sporadic CJD is important for our understanding of possible pathogenic mechanisms and the susceptibility to the disease. Efforts have been made to identify genetic risk factors other than the well described *PRNP* M129V polymorphism. Several previous studies focused on the characterisation of SNPs in *PRNP* and *PRND* genes, generating contradictory results on relatively small sample sets. This led us to evaluate a panel of nine SNPs in the *PRNP* locus and one SNP in the *PRND* gene in a large German sCJD cohort and population based healthy controls.

We found a significant association of sCJD with seven of nine *PRNP* SNPs studied, including *PRNP* 1368, 13436, 16987, 22976, M129V, 28878, and 34296. In agreement with previous studies, individuals with homozygosity of both methionine and valine for *PRNP* M129V showed an increased risk in sCJD. We further found that the association of *PRNP* 1368, located ~24 kb upstream of the coding region *PRNP*, was independent of the genotype of *PRNP* M129V. This result confirmed a previous study that compared sCJD cases in the UK with either the CEPH families from France or healthy UK population.[10] However, this finding disagrees with the result from an earlier study based on a Dutch population.[11] The difference between these studies and the Dutch study may be due to differences in the ethnic populations, or more likely, may be influenced by the small sample size in the Dutch study (sCJD n = 23; controls n = 83). In addition to *PRNP* 1368, we also found that the homozygous minor allele of *PRNP* 34296, an SNP located ~8 kb downstream of the 3′ coding region of *PRNP*, tended to increase the susceptibility to sCJD of individuals homozygous for methionine at *PRNP* M129V. It should be noted that the power of this study is limited owing to lower sample size within the heterozygous *PRNP* M129V group (96 patients versus 324 control probands) and the homozygous valine group (78 patients versus 99 control probands). The power to detect genotype related risks in these two *PRNP* M129V subgroups for a marker with,

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Table 1 SNP allele frequencies in sCJD cases and healthy controls

	Sample size (n)		MAF (%)		p	
SNP ID	sCJD	Controls	sCJD	Controls	Marker	Family
PRNP 1368 (T/C)	507	740	50	40	<0.001	<0.001
PRNP 12533 (C/G)	534	740	14	13	0.651	1.000
PRNP 13436 (T/C)	542	726	24	39	<0.001	<0.001
PRNP 16987 (T/G)	588	746	18	32	<0.001	<0.001
PRNP 22976 (A/G)	570	697	15	28	<0.001	<0.001
PRNP A117A (A/G)	575	744	1	3	0.035	0.454
PRNP M129V (A/G)	582	722	22	36	<0.001	<0.001
PRNP 28878 (T/C)	589	742	13	23	<0.001	<0.001
PRNP 34296 (A/G)	585	741	45	37	<0.001	<0.001
PRND T174M (C/T)	582	740	49	50	0.662	1.000

The significance of association results was examined by the Cochran-Armitrage trend test (marker wise) and the p values adjusted for multiple testing (family wise) according to Sidak.[25]

for example, an allele frequency of 0.3, is only 44% and 28%, respectively. Therefore, larger studies are needed for further evaluation of the association of *PRNP* SNPs with sCJD, independent of *PRNP* M129V.

Attention has been paid to the genetic variances in the regulatory region of *PRNP*, as there is evidence in animal models that PrP gene expression levels influence the susceptibility to prion diseases and the incubation time of disease. Overexpression of the PrP gene in transgenic animals demonstrated shortening of incubation time of animals inoculated with prions,[28] while mice heterozygous for a disrupted PrP gene and expressing only half of the normal PrP have prolonged incubation times following infection, whereas *PRNP* knockout mice are completely resistant to prion disease. Moreover, Hardy *et al* stated that those who express high levels of prion protein and are homozygous at codon 129 are most susceptible to disease, and that in diseases where protein deposition is part of the process,

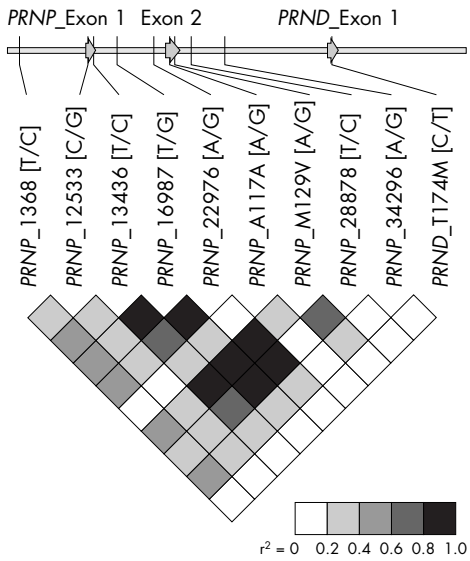

Figure 2 SNP location and pairwise linkage disequilibrium between genotyped variants in *PRNP* and *PRND*. The top indicates the physical SNP locations relative to the exons of the *PRNP* and *PRND* locus on chromosome 20p12. SNPs in coding regions are indicated by the location of the codons and exchange of amino acids. All other SNPs are designated by their location on clone U29185. The base exchange in each SNP is given in parentheses. The first letter represents the major allele and the second the minor allele. Exons of *PRNP* and *PRND* genes are highlighted by arrows. Shading of the diamonds represents magnitude and significance of the pairwise r^2 between the two SNPs, defined by the top left and the top right sides of the diamond, with black reflecting high r^2 ($r^2>0.8$) and white reflecting low r^2 ($r^2<0.2$).

genetic variability in the promoter of the gene should be considered as a factor influencing risk of sporadic diseases.[29] Therefore, the underlying mechanism of association of SNP 1368, located in the promoter region of *PRNP*, with sporadic prion diseases may possibly originate in high expression of the prion protein gene. However, this hypothesis has to be proven by functional analyses.

In addition, our data clearly show the independence of association of SNP1368 with sCJD from that of M129V with sCJD. This is in line with Hardy's ''law of mass action'', which states that the concentration of the pathogenic protein might only cause the initiation of the disease through the formation of a pathogenic template.[30] After initiation, the proteins adopt the same conformation of the template. This process is probably facilitated by homozygosity at codon 129, due to symmetry considerations in prion-prion interactions. This process becomes self propagating. Thus, the latter pathogenesis may be largely independent of prion protein concentration.

Previous work has identified several regulatory regions of human *PRNP*, located about 273 bp from exon 1,[12 31] and in the intronic region between 292 and 625 bp downstream of exon 1.[12] McCormack *et al* evaluated three SNPs in these regions in an association study with small sample sizes (controls n = 100, CJDs n = 25), and suggested that these SNPs, including an SNP corresponding to *PRNP* 12533 in the present study may be risk factors for CJD. However, we could not confirm a significant association between *PRNP* 12533 and sCJD. In addition, *PRNP* 13436, an SNP in the intronic regulatory region, did not show a significant association with sCJD, independent of *PRNP* M129V. Our data suggest that the SNPs in these previously defined regulatory regions may not function as additional risk factors to sCJD.

In the present study, we demonstrated the existence of five major haplotypes of *PRNP*, determined by eight SNPs. It should be noted that the markers *PRNP* 12533 and *PRNP* A117A showed the same allele in all five major haplotypes, while markers *PRNP* 28878 and *PRNP* 34296 showed different alleles within the under-represented and over-represented haplotypes. The remaining five markers (*PRNP* 1368, 13436, 16987, 22976 and M129V) distinguished the under-represented and over-represented haplotypes. Therefore, a predisposing role of the region spanned by these five SNPs is highly probable. This region is 24 491 bp in length, and shows strong LD as described above. The identified major haplotypes showed strong consistency with the findings in the UK study,[10] both in genotypes and in their distribution in sCJD and controls. For example, the two most common haplotypes in the UK study (haplotypes A and B; table 3) coincide with the two most common haplotypes in the present study (haplotypes 1 and 5), suggesting that German *PRNP*

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Table 2 Odds ratios of examined SNPs in *PRNP* and *PRND* in the subgroup of *PRNP* 129 methionine homozygote patients

SNP ID	Genotypes compared	OR	95% CI	p
PRNP 1368 (T/C)	C/T *v* T/T	1.498	0.890 to 2.521	0.1281
	C/C *v* T/T	1.714	1.002 to 2.931	0.0491
PRNP 12533 (C/G)	C/G *v* G/G	0.828	0.576 to 1.191	0.3084
	C/C *v* G/G	0.572	0.162 to 1.935	0.3689
PRNP 13436 (T/C)	C/T *v* T/T	0.767	0.480 to 1.227	0.2684
	C/C *v* T/T	ND	—	—
PRNP 16987 (T/G)	G/T *v* T/T	0.756	0.304 to 1.880	0.5476
	G/G *v* T/T	n.d	—	—
PRNP 22976 (A/G)	A/G *v* A/A	0.540	0.049 to 5.990	0.6157
	G/G *v* A/A	ND	—	—
PRNP A117A (A/G)	A/G *v* A/A	ND	—	—
	G/G *v* A/A	ND	—	—
PRNP M129V (A/G)	A/G *v* A/A	ND	—	—
	G/G *v* A/A	ND	—	—
PRNP 288778 (T/C)	C/T *v* T/T	0.567	0.051 to 6.293	0.6443
	C/C *v* T/T	ND	—	—
PRNP 34296 (A/G)	A/G *v* A/A	1.073	0.705 to 1.632	0.7435
	G/G *v* A/A	1.519	0.942 to 2.447	0.0862
PRND T174M (C/T)	C/T *v* C/C	0.921	0.616 to 1.379	0.6903
	T/T *v* C/C	0.886	0.554 to 1.417	0.6125

Odds ratios of heterozygous and homozygous minor allele versus homozygous common allele, and 95% confidence intervals (CI) are given. ND, genotypes were not detectable in the subgroup of *PRNP* 129 MM homozygotes.

genealogy can also be characterised by these two proposed major haplotype branches.

Recently, attention has also been focused on *PRND* and the association of SNPs at the *PRND* locus with CJD. However, the results of earlier studies are contradictory. Studies on *PRND* T174M either showed no significant relationship,[10 16 17] or a significantly increased risk for those heterozygous[15] or homozygous for methionine.[11] The present work showed neither significant association of different genotypes of *PRND* T174M with sCJD, nor strong linkage disequilibrium between the *PRNP* SNPs and *PRND* T174M, confirming the finding of a previous study based on UK and French populations.[10] This result suggests a less important role of *PRND* T174M in prion disease pathogenesis. However, *PRND* cannot be excluded as a possible candidate gene. According to our study and a previous study,[10] *PRNP* 34296 demonstrated a significant association with sCJD and was located in a region of strong LD, also including SNPs upstream of *PRND*.[10] Thus, associations of other genetic variants of *PRND* with sCJD, especially those upstream of *PRND*, independent of *PRND* T174M, may be possible.

In conclusion, we identified a significant association of seven *PRNP* SNPs with sCJD. Most of these associations are hitchhiking effects with the known predisposing effect of the *PRNP* M129V polymorphism. We confirmed the results of a previous study indicating that *PRNP* 1368 is associated with sCJD, independent of *PRNP* M129V. In addition, our results suggest that *PRNP* 34296 may also be an additional independent risk factor. Furthermore, we detected a strong LD region from *PRNP* 1368 to *PRNP* 28878, and identified the major haplotypes of *PRNP* SNPs that were over-represented or under-represented in sCJD cases. The cases and controls analysed in this study are the largest cohorts for association study in sCJD to date. These cohorts provide a basis for the association studies of further genetic variants in candidate genes involved in the susceptibility and pathogenesis of sCJD.

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ELECTRONIC-DATABASE INFORMATION

Accession numbers and URLs for data in this article are as follows:

NCBI Entrez Gene, for clones U29185 and AL133396; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=gene

Online Mendelian Inheritance in Man (OMIM) for PRNP (MIM 176640), CJD (MIM 123400), and PRND (MIM604263); http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=omim

Table 3 Major haplotypes of *PRNP* SNPs and their frequencies in cases and controls

Over-represented in:		Haplotypes of *PRNP* 1368	12533	13436	16987	22976	A117A	M129V	28878	34296	Frequency Cases	Controls	p
Controls	1	T	C	C	G	G	A	G	C	A	9.08%	16.46%	<0.00001
	2	T	C	C	G	G	A	G	T	A	2.36%	5.16%	0.0002
	3	T	C	C	G	G	A	G	C	G	1.69%	4.17%	0.0002
		T	C	C	G	G	A	G	C	—	Haplotype A in Mead *et al*[10]		
Cases	4	C	C	T	T	A	A	A	T	A	13.99%	10.56%	0.007
	5	C	C	T	T	A	A	A	T	G	33.03%	26.26%	0.0001
		C	C	T	T	A	A	A	T	—	Haplotype B in Mead *et al*[10]		

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