NULL HYPOTHESIS

Autoantibodies against the prion protein in individuals with *PRNP* mutations

Karl Frontzek, MD, PhD, Manfredi Carta, MD, Marco Losa, MD, Mirka Epskamp, MSc, Georg Meisl, PhD, Alice Anane, ND, Jean-Philippe Brandel, MD, Ulrike Camenisch, PhD, Joaquín Castilla, PhD, Stéphane Haïk, MD, PhD, Tuomas Knowles, PhD, Ewald Lindner, MD, Andreas Lutterotti, MD, Eric Vallabh Minikel, PhD, Ignazio Roiter, MD, Jiri G. Safar, MD, Raquel Sanchez-Valle, MD, PhD, Dana Žáková, PhD, Simone Hornemann, PhD, and Adriano Aguzzi, MD, PhD, for the THAUTAN-MC Study Group

Correspondence
Dr. Frontzek
karl.frontzek@usz.ch
or Dr. Aguzzi
adriano.aguzzi@usz.ch

Neurology® 2020;95:e2028-e2037. doi:10.1212/WNL.0000000000009183

Abstract

Objective

To determine whether naturally occurring autoantibodies against the prion protein are present in individuals with genetic prion disease mutations and controls, and if so, whether they are protective against prion disease.

Methods

In this case–control study, we collected 124 blood samples from individuals with a variety of pathogenic *PRNP* mutations and 78 control individuals with a positive family history of genetic prion disease but lacking disease-associated *PRNP* mutations. Antibody reactivity was measured using an indirect ELISA for the detection of human immunoglobulin $G_{1–4}$ antibodies against wild-type human prion protein. Multivariate linear regression models were constructed to analyze differences in autoantibody reactivity between (1) *PRNP* mutation carriers vs controls and (2) asymptomatic vs symptomatic *PRNP* mutation carriers. Robustness of results was examined in matched cohorts.

Results

We found that antibody reactivity was present in a subset of both *PRNP* mutation carriers and controls. Autoantibody levels were not influenced by *PRNP* mutation status or clinical manifestation of prion disease. Post hoc analyses showed anti-PrP^C autoantibody titers to be independent of personal history of autoimmune disease and other immunologic disorders, as well as *PRNP* codon 129 polymorphism.

Conclusions

Pathogenic *PRNP* variants do not notably stimulate antibody-mediated anti-PrP^C immunity. Anti-PrP^C immunoglobulin G autoantibodies are not associated with the onset of prion disease. The presence of anti-PrP^C autoantibodies in the general population without any disease-specific association suggests that relatively high titers of naturally occurring antibodies are well-tolerated.

Clinicaltrials.gov identifier

NCT02837705.

From the Institute of Neuropathology (K.F., M.C., M.L., M.E., S. Hornemann, A.A.), Institute of Surgical Pathology (U.C.), and Department of Neurology, Neuroimmunology and MS Research (NIMS) (A.L.), University of Zurich, Switzerland; Department of Chemistry (G.M., T.K.), University of Cambridge, UK; CJD Foundation Israel (A.A.), Pardes Hanna; ICM (J.-P.B.), Salpêtrière Hospital, Sorbonne University, Paris, France; CIC bioGUNE and IKERBASQUE (J.C.), Basque Foundation for Science, Bizkaia, Spain; Sorbonne University (S. Haïk), ICM, Salpêtrière Hospital, Paris, France; Ophthalmology Division (E.L.), University of Graz, Austria; Broad Institute (E.V.M.), Cambridge, MA; Treviso Hospital (I.R.), Italy; Department of Pathology, Neurology, and National Prion Disease Pathology Surveillance Center (J.G.S.), Case Western Reserve University, Cleveland, OH; Alzheimer's Disease and Other Cognitive Disorders Unit (R.S.-V.), Hospital Clinic, IDIBAPS, University of Barcelona, Spain; and Department of Prion Diseases (D.Ž.), Slovak Medical University, Bratislava, Slovakia.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

THAUTAN-MC Study Group coinvestigators are listed in the appendix at the end of the article.

This Null Hypothesis article is published as part of a collaborative effort, between *Neurology* and CBMRT, and the article has been made freely available through funds provided by the CBMRT.

This article published online ahead of print on February 26, 2020. doi:10.1212/WNL.0000000000009183

Glossary

CJD = Creutzfeldt-Jakob disease; **EBNA** = Epstein-Barr nuclear antigen; **FT** = flexible tail; **GD** = globular domain; **gPrD** = genetic prion diseases; **GSS** = Gerstmann-Sträussler-Scheinker; **sCJD** = sporadic Creutzfeldt-Jakob disease.

Prion diseases are diseases of the CNS that not only occur as sporadic and transmissible forms, but can also be transmitted through the germ line as autosomal dominant traits.[1] Genetic prion diseases (gPrDs) account for ~10%–15% of all prion diseases and are characterized by pathogenic, nonsynonymous mutations of the human prion protein gene *PRNP*.[2] The most prevalent human prion disease, sporadic Creutzfeldt-Jakob disease (sCJD), is characterized by a rapidly progressive dementia and a short survival time (usually <1 year) from clinical onset.[3] In contrast, *PRNP* mutation carriers often present with atypical phenotypes; for example, long survival rates can be observed in Gerstmann-Sträussler-Scheinker (GSS) disease.[4]

The cellular prion protein PrP^C consists of an unstructured flexible tail (FT) on its N-terminal end and a C-terminal globular domain (GD).[5] We showed in 2001 that humoral immunity against PrP^C can protect against prion neuroinvasion.[6] Antibodies against the FT of PrP^C, or removal of amino acid residues from the FT, abrogate the neurotoxic effects of anti-PrP^C-GD antibodies and reduce the toxicity of bona fide prions.[7,8] Naturally occurring PrP antibodies may exist in the general population: for instance, reactivity against a 21-residue PrP peptide was observed in commercial pooled immunoglobulin,[9] and a unique blood group has been observed in individuals homozygous for the E219K polymorphism.[10]

Clinical trials have yet to deliver an effective antiprion agent.[11–14] An ongoing clinical study involves the administration of PRN100, a humanized antibody against PrP^C-GD, to individuals with Creutzfeldt-Jakob disease (CJD).[15] There is much hope that this trial will be successful, but the murine counterpart of PRN100, ICSM18, exhibits an on-target, dose-dependent toxicity, and whether a therapeutic window exists has not yet been established.[16–18]

The frequency of *PRNP* missense variants exceeds the reported gPrD prevalence, suggesting a spectrum of disease penetrance in gPrDs rather than complete penetrance of nonsynonymous *PRNP* mutations.[19] The mechanisms by which these mutations induce disease are largely unclear. The majority of structural studies on human PrP^C variants linked to gPrD failed to identify consistent effects on global protein stability.[20] Age at onset in gPrD is highly variable, and typically middle age or older, which might suggest that a protective mechanism guards some individuals against the prion protein–induced toxicity.[2] We hypothesized that subtle conformational alterations of pathogenic PrP^C variants could stochastically generate immunogenic neoepitopes, which in turn might elicit a protective humoral anti-PrP^C immune response. We therefore conducted an extensive search for such autoantibodies in individuals carrying pathogenic *PRNP* mutations, and in unaffected relatives as controls.

Methods

Standard protocol approvals, registrations, and patient consents

The Cantonal Ethics Committee of the Canton of Zurich approved this study (permit no. KEK-ZH Nr.2015-0514). This trial was registered at clinicaltrials.gov (no. NCT02837705). The protocol for this study was approved by the institutional review board at each participating institution with the University of Zurich being the lead regulatory site. Written informed patient consent was received by all individuals participating in this study.

Human participants and study design

We defined *PRNP* mutation carriers as individuals with a nonsynonymous mutation in the open reading frame of the *PRNP* gene that was previously reported to be pathogenic.[2] Between September 2015 and October 2018, we contacted both international patient organizations as well as national prion disease reference centers for further reuse of existing blood samples. Individuals at any age with a confirmed *PRNP* mutation were considered eligible for this study. Individuals with a confirmed *PRNP* mutation in a blood relative who did not undergo *PRNP* sequencing prior to enrollment in this study were also considered eligible if they gave consent for *PRNP* sequencing. Blood samples without information on age or sex were excluded from further analysis. *PRNP* wild-type individuals with neurologic or psychiatric symptoms indicative of gPrD were excluded from the study.[21] Clinical manifestation of gPrD was defined as presence of both a pathogenic *PRNP* mutation and PrD-typical symptoms.[21] The latter were assessed by clinical examination and neuropsychological assessment, in some cases complemented by ancillary tests such as presence of 14-3-3 proteins in CSF, real-time quaking-induced conversion assays, EEG, and MRI.[22] Personal history of autoimmune disease and other immunologic disorders could be obtained in 141 participants. A detailed description of the patient cohort is given in table 1. For sensitivity analysis, cases and controls were matched on age (±5 years), sex, and blood sample type (i.e., serum or plasma).

PRNP genotyping

PRNP genotyping was performed using a modified version of the DNeasy Blood & Tissue Kit (Qiagen, Venlo, the Netherlands). Twenty microliters of proteinase K (600 mAU/mL) and 200 μL of 5 M guanidine hydrochloride with 1% Triton-

Table 1 Baseline characteristics of the unmatched cohort

	PRNP mutation carriers, n (%)	*PRNP* wild-type, n (%)	Missing data, n (%)	*p* Value
Individuals enrolled, n	124	78		
Age, y				
Mean	49.3	42.8		0.004
SD	16.5	13.9		
Autoimmune disease[a]	8/141 (5.7)		61 (30.2)	0.49
Female sex	80 (64.5)	37 (47.4)		0.02
14-3-3 protein in CSF			80 (39.6)[b]	NA
Test performed	17/63 (27.0)	0/59 (0.0)		
Positive 14-3-3	8/17 (47.0)	NA		
Codon 129 polymorphism			20 (9.9)[c]	<0.0001
Met/Met	69/121 (57.0)	15/61 (24.6)		
Met/Val	50/121 (41.3)	37/61 (60.7)		
Val/Val	2/121 (1.7)	9/61 (14.8)		
Pathogenic *PRNP* mutation				
P102L	3 (2.4)	NA		
D178N	37 (29.8)	NA		
E200K	77 (62.1)	NA		
V210I	2 (1.6)	NA		
Unique	5 (4.0)	NA		
Blood storage[d]				
Plasma	98 (79.0)	70 (89.7)		
Serum	26 (21.0)	8 (10.3)		

[a] Due to few events of autoimmune disease, we pooled genotypes to eliminate possible identification. *p* Value compares numbers of individuals with or without autoimmune disease in *PRNP* mutation carriers and wild-type *PRNP*.
[b] Missing values: 61 *PRNP* mutation carriers, 19 *PRNP* wild-type.
[c] Missing values: 3 *PRNP* mutation carriers, 17 *PRNP* wild-type.
[d] Unique (n = 1) mutations: D178N/N171S, V180I, T183A, F198S, E200G.

X100 at pH = 5.0 were added to 200 μL of anticoagulated blood, vortexed thoroughly, and incubated for 24 hours at room temperature. Two hundred microliters EtOH (96%–100%) was added to the reaction and the rest of the DNA purification was performed according to the manufacturer's guidelines. The primer pair PRNP_up and PRNP_low (table e-1, doi.org/10.5061/dryad.08kprr4xk) was used in combination with Q5 high-fidelity DNA polymerase (New England Biolabs, Ipswich, MA) to amplify the open reading frame from exon 2 of *PRNP*. Sanger sequencing was performed at the Department of Molecular Pathology (Institute of Surgical Pathology, University Hospital Zurich) using 4 different sequencing primers (PRNP_up, PRNP_up2, PRNP_low, PRNP_low2; table e-1, doi.org/10.5061/dryad.08kprr4xk). Sequencing traces were aligned to reference DNA from the Reference Sequence Database (RefSeq at National Center of Biotechnology Information, Bethesda, MD) using CLC Main Workbench (Qiagen) and packages sangerseqr[23] and DECIPHER[24] for Bioconductor[25] in R.

Statistical analyses

We performed a priori testing of anti-PrP^C autoantibody reactivity for the following hypotheses: (1) differences in anti-PrP^C autoantibody reactivity between *PRNP* mutation carriers and *PRNP* wild-type individuals and (2) differences in anti-PrP^C autoantibody reactivity between *PRNP* mutation carriers showing clinical signs of prion diseases and those without. All other analyses were performed post hoc. We used already established predictors of autoimmune disease such as age[26] and sex[27] as well as storage conditions known to affect antibody responses such as presence of coagulation factors[28] as covariates in our multivariate regression model. Using the purposeful selection of covariates method as described previously,[29] effects of covariates on autoantibody titers were

tested by bivariate linear regression analyses using the Wald test and included for multivariate testing at a p value cutoff point of 0.25. In the multivariate model, covariates were removed if they were nonsignificant at the 0.1 α level or not a confounder, as determined by a change in the remaining parameter estimate greater than 20% as compared to the full model. *PRNP* mutation status, clinical signs of prion disease, and *PRNP* codon 129 polymorphism were added after establishment of significant confounders. In matched cohorts, multivariate models were adjusted for matching factors.

All values are given as average ± SD unless mentioned otherwise. For analysis, autoantibody titers were $\log_{10}$-transformed, and reported β coefficients and confidence intervals represent back-transformed values. Normality was tested using the D'Agostino-Pearson normality test. For values following a Gaussian distribution, differences between 2 groups were compared using 2-tailed Student t test. For not normally distributed values, Mann-Whitney U test was used for comparison of 2 groups. For comparison of categorical variables, Fisher exact test and χ^2 test were used for comparison of 2 and more than 2 groups, respectively. Pearson correlation coefficient was computed for data sampled from Gaussian distributions and Spearman ρ for those sampled from non-Gaussian distributions. Matching of cases and controls was done using the *find.matches* function from the *Hmisc* package in R. We used lm for R for linear regression analysis. Python and R were used for statistical analysis and data visualization was performed using Prism 7 (GraphPad).

Data availability

The study participants, if they have not undergone predictive testing themselves, participated under the condition of not knowing their *PRNP* genotype. Due to the relatively small sample size and risk of de-identification, all raw study data involving human participants were made available to the editors and reviewers but will not be made available publicly. Supplementary data, as well as DNA sequences of gene blocks used for construction of humanized antibodies and human PrP^C-AviTag, are available at Dryad (doi.org/10.5061/dryad.08kprr4xk).

Results

Description of the cohort

We received blood samples and clinical information from a total of 241 individuals and selected 202 unmatched cases and controls for this analysis (figure 1). To test the robustness of our results, we matched 64 cases on 64 controls based on age (±5 years), sex, and blood storage conditions (i.e., serum/plasma; table e-2, doi.org/10.5061/dryad.08kprr4xk). Anti-PrP^C autoantibody reactivity was measured by a sandwich ELISA; a description of the assay is provided in extended text and figures e-1 and e-2 (doi.org/10.5061/dryad.08kprr4xk). Briefly, blood samples were diluted over a range of >2 logs and bound autoantibodies were detected with anti-human IgG antibodies. Antibody titers are expressed as negative common logarithm of the half-maximal effective concentration (figure e-1E). Anti-PrP^C antibody reactivity was independent of serum IgG levels (Spearman $\rho = 0.07$, $p = 0.69$; figure 2A). The age of probands did not influence the IgG levels (Pearson $r = 0.33$, $p = 0.16$). To confirm our ability to detect human antibodies against specific targets, we tested a subset of individuals for the presence of IgG against EBNA. A total of 4/5 $PRNP^{WT}$ and 16/16 $PRNP^{Mut}$ individuals tested positive (corresponding to 95% positive individuals), in line with anti-EBNA IgG seroprevalence in the general population (figure 2B).[30]

Prevalence of anti-PrP^C autoantibodies in *PRNP* mutation carriers

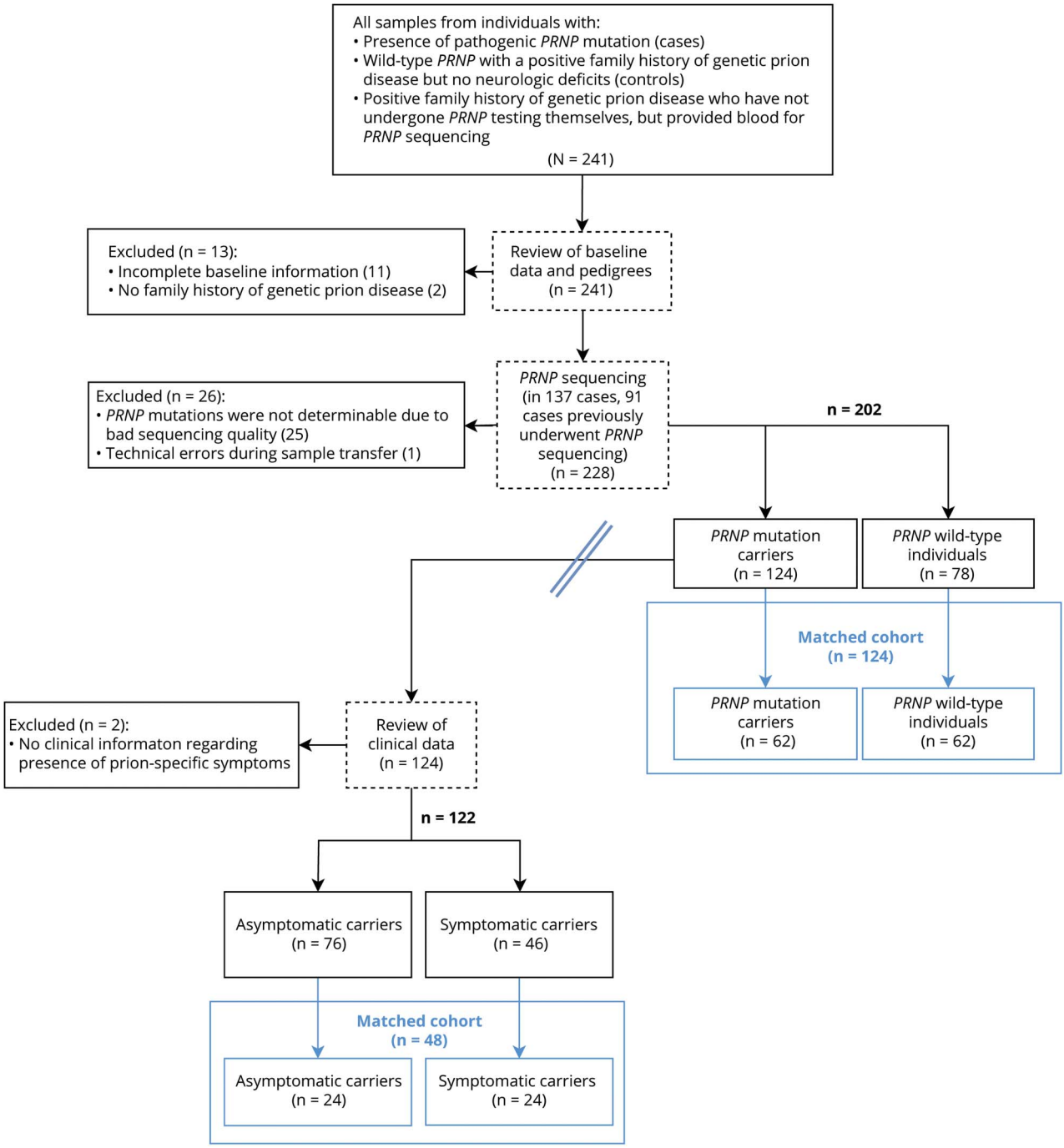
The presence of coagulation factors (e.g., plasma instead of serum), and possibly age, but not female sex were associated with anti-PrP^C autoantibody reactivity in bivariate and multivariate analyses (table 2).[29] We henceforth adjusted all analyses for age and presence of coagulation factors. Presence or absence of a pathogenic *PRNP* mutation was not associated with significant changes in anti-PrP^C autoantibody reactivity (table 3). In addition, we matched 62 cases and controls on age (±5 years), sex, and blood sample type[26–28] (table e-2, doi.org/10.5061/dryad.08kprr4xk). As with the unmatched cohort, *PRNP* mutation did not significantly influence anti-PrP^C autoantibody titers in multivariate linear regression adjusted for matching factors (table e-2).

We then tested whether anti-PrP^C autoantibody response was associated with symptoms of prion disease. Presence or absence of clinical signs was reported by 122 *PRNP* mutation carriers (out of a total of 124 enrolled): 76 (62.3%) were asymptomatic carriers whereas 46 (37.7%) presented with clinically apparent disease. Detailed clinical data were available in 14 cases. The most common clinical presentations entailed cerebellar signs (n = 12 [85.7%]) and dementia (n = 11 [78.6%]). Status of 14-3-3 protein in CSF, albeit a poor predictor of gPrD,[31] was provided by 121 study participants. Seventeen individuals (all *PRNP* mutation carriers with clinically apparent disease) were tested, with 8 (47.1%) testing positive, in line with previous findings.[31] Presence of prion-specific symptoms was not associated with alterations in anti-PrP^C autoantibodies in an unmatched cohort (table 3). This was confirmed in an analysis of a cohort consisting of 24 symptomatic *PRNP* mutation carriers and 24 asymptomatic *PRNP* mutation carriers matched on *PRNP* mutation, age, and sample type (table e-2, doi.org/10.5061/dryad.08kprr4xk).

Post hoc subgroup analyses on the association of anti-PrP^C autoantibodies with specific PrP^C mutations, PrP^C p.129 polymorphism, and autoimmune disease and other immunologic disorders

We analyzed the effects of *PRNP* mutations that were present at least 5 times in the study population, namely D178N and

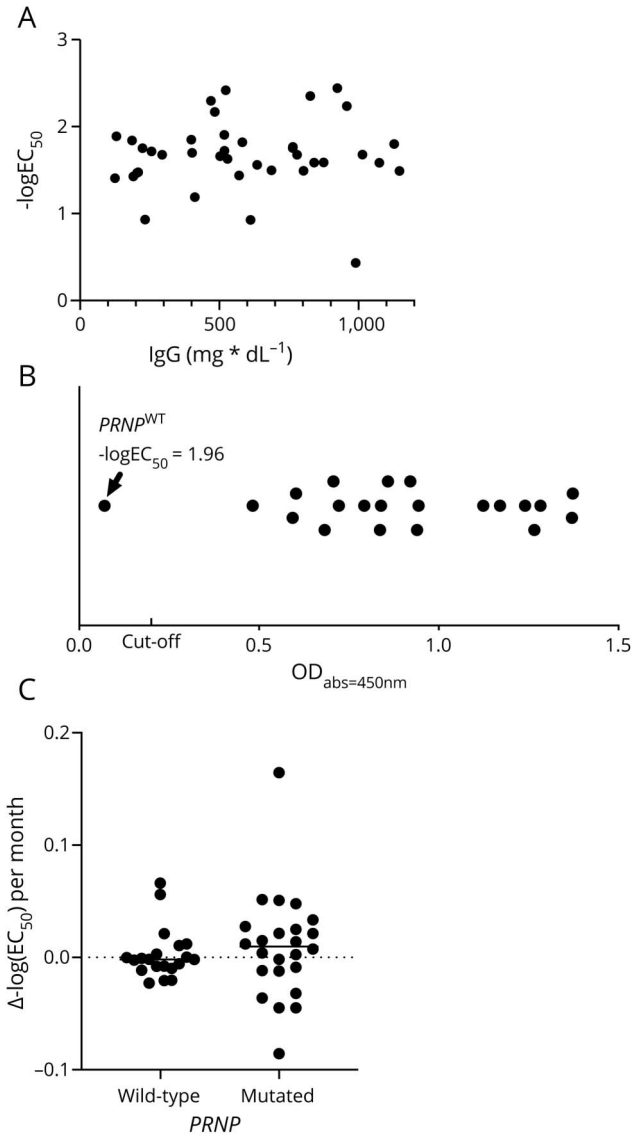
Figure 1 Flowchart of patient selection



Double line indicates cohorts selected for comparison of anti-PrP^C autoantibody titers from individuals carrying wild-type or mutated *PRNP* alleles (right of double line) and cohort selected for comparing anti-PrP^C autoantibody titers of symptomatic vs asymptomatic mutation carriers (left of double line). Blue boxes indicate matched cohorts.

E200K, on anti-PrP^C autoantibody titers: individuals with D178N mutations showed a significant trend towards lower autoantibody titers in bivariate analysis (table 3). This finding, however, was not significant after adjusting for age and sample type (table 3). E200K mutation carriers did not show significant changes in autoantibody reactivity (table 3). The methionine/valine polymorphism at codon 129 of the human *PRNP* gene was reported to affect the susceptibility to prion diseases.[32] Information on p.129 polymorphism was available in 182 study participants: 84 (46.2%) were homozygous for methionine (p.129MM), 87 (47.8%) p.129MV, and 11 (6.0%) p.129VV. None of the polymorphisms significantly altered autoantibody response to PrP^C in a post hoc analysis (table 4). In D178N carriers, the clinical phenotype was suggested to be dependent on the *PRNP* cis c.129 polymorphism: methionine was associated with fatal familial

Figure 2 Correlation of anti-PrP^C autoantibody reactivity with total immunoglobulin G (IgG) levels, IgG anti–Epstein-Barr virus (EBV) autoantibodies, and change of autoantibody titers over time



(A) Correlation of total IgG with anti-PrP^C autoantibody titers. (B) Qualitative assessment of anti–Epstein-Barr nuclear antigen (EBNA) IgG antibodies in blood shows 1 *PRNP*^{WT} individual without detectable anti-EBNA IgG antibodies. Cutoff: OD_{abs} = 450 nm (optical density at absorbance λ = 450 nm) = 0.2 according to the manufacturer's guidelines. (C) In 2 subsequent blood drawings, mean change in antibody titers per year is stable and similar between *PRNP* mutation and wild-type carriers, but variance is larger in *PRNP* mutation carriers.

Table 2 Age and lack of coagulation factors in blood (e.g., serum probes), but not sex, are significantly associated with anti-PrP^C autoantibody reactivity

Risk factor	β coefficient	95% confidence interval	*p* Value
Bivariate analyses			
Age	0.990	0.982–0.998	0.05
Female sex	1.14	0.86–1.48	0.42
Plasma instead of serum	1.84	1.31–2.58	0.004
Multivariate analysis: model A			
Age	0.990	0.982–0.997	0.032
Plasma instead of serum	1.88	1.34–2.63	0.002
Multivariate analysis: model B			
Age	0.989	0.981–0.997	0.026
Female sex	1.16	0.90–1.50	0.35
Plasma instead of serum	1.86	1.33–2.61	0.003

Due to lack of confounding effects of sex in multivariate model B, all further analyses were adjusted for blood sample type (serum/plasma) and age.

insomnia and valine with familial CJD,[2] although this association may not be universal.[33,34] In our cohort, 28 patients could be unambiguously identified as D178N_cis129M and 5 patients as D178N_cis129V. No differences in mean antibody reactivity were seen between those 2 groups (table 4).

Co-occurrence of multiple autoimmune diseases is a commonly observed phenomenon.[35] In order to test the influence of preexisting autoimmune diseases on anti-PrP^C autoantibody titers, we searched clinical reports of study participants for presence of autoimmune disease and other immunologic disorders. We were able to retrieve this information in 141 (69.8%) cases: 8 individuals were diagnosed with autoimmune disease, namely Hashimoto thyroiditis (n = 3), Graves disease (n = 1), monoclonal gammopathy of unknown significance (n = 1), multiple sclerosis (n = 1), psoriasis (n = 1), and rheumatoid arthritis (n = 1). Multivariate linear regression analysis adjusted for age, sex, and type of blood sample did not show a significant association of autoimmune disease with anti-PrP^C autoantibody titers (table 4).

Temporal evolution of anti-PrP^C autoantibodies

Forty-four individuals (21.8%) donated blood multiple times, several months apart, on which we performed a post hoc time course analysis. *PRNP* wild-type individuals were observed over a longer time period compared to *PRNP* mutation carriers (17 ± 1.78 months vs 10 ± 6.21 months, $p = 1.42 \times 10^{-5}$). *PRNP* mutation carriers showed larger variability in autoantibody titers; mean proportional change per year, however, was similar across groups ($p = 0.23$) and was overall negligible between 2 blood drawings (113.2 ± 61.44% per year in *PRNP* mutation carriers vs 99.95 ± 17.22% per year in *PRNP* wild-type individuals, figure 2C). None of the *PRNP* mutation carriers tested in this time course analysis exhibited clinical signs of prion disease.

Table 3 Effect of *PRNP* mutation status on anti-PrP^C autoantibody reactivity

Risk factor	β coefficient	95% confidence interval	*p* Value
***PRNP* mutation (all)**			
Crude	0.81	0.62–1.05	0.19
Adjusted	0.92	0.70–1.20	0.61
***PRNP* D178N mutation**			
Crude	0.61	0.44–0.86	0.02
Adjusted	0.75	0.53–1.06	0.17
***PRNP* E200K mutation**			
Crude	1.16	0.89–1.16	0.36
Adjusted	1.18	0.91–1.54	0.30
Clinical signs of prion disease			
Crude	0.90	0.59–1.38	0.64
Adjusted	0.94	0.61–1.46	0.79

Table 4 Effect of *PRNP* codon 129 polymorphism and history of autoimmune disease on anti-PrP^C autoantibody reactivity

Risk factor	β coefficient	95% confidence interval	*p* Value
p.129MM			
Crude	0.83	0.63–1.10	0.28
Adjusted	0.88	0.67–1.15	0.43
p.129MV			
Crude	1.23	0.94–1.62	0.21
Adjusted	1.13	0.86–1.48	0.46
p.129VV			
Crude	0.88	0.49–1.56	0.71
Adjusted	1.04	0.59–1.83	0.90
D178N/cis-129M			
Crude	1.18	0.44–3.15	0.78
Adjusted	1.09	0.37–3.22	0.90
History of autoimmune disease			
Crude	0.96	0.47–1.96	0.92
Adjusted	1.12	0.55–2.26	0.76

Discussion

The diagnosis of a disease-associated *PRNP* mutation is a fateful and often devastating event for individuals carrying such mutations. The clinical penetrance of *PRNP* mutations can be very high, and no disease-modifying therapy is available.[2] Clinical signs of familial prion disease typically erupt in late adulthood, although carriers arguably produce the mutated protein from the first day of their life.[5] There are at least 2 scenarios that may account for this phenomenon: (1) the pathogenic mutations may slightly destabilize PrP^C, thereby infinitesimally increasing the probability of pathologic aggregation; or (2) the pathogenic conformation of PrP^C is attained early on, but the body's defenses stave off its consequences for decades.

In the case of scenario 1, extensive structural studies on pathogenic PrP^C variants failed to reveal major structural alterations.[20] We hypothesized that under scenario 2, the stochastic generation of PrP^{Sc} in mutation carriers might engender neoantigens, which in turn might lead to protective humoral responses. Remarkably, however, we found no evidence of induction of humoral antibody-mediated immunity against PrP^C by pathogenic *PRNP* variants. Instead, our study suggests the prevalence of naturally occurring anti-PrP^C antibodies in the general population independent of clinical signs of prion disease, *PRNP* variant, or *PRNP* p.129 polymorphism. Although reactivity to wild-type PrP has been reported in the serum of E219K homozygotes,[10] and reactivity to a non-naturally occurring PrP peptide was reported in commercial IgG,[9] the present report is to our knowledge the first observation of the *PRNP* genotype-independent presence of autoantibodies to full-length, wild-type PrP in humans. Without disease-specific antibodies, one might speculate that *PRNP* mutations accumulate subclinical levels of prions to a point when clinical symptoms become evident.

In a subset of individuals, anti-PrP^C autoantibody reactivity was tested in multiple blood drawings up to 1.5 years apart: the mean change of autoantibody titers was similar across *PRNP* genotypes, in line with previous reports that showed stable autoantibody levels at least over several years.[36,37]

Matching in case–control studies is a controversial topic.[38] In our study, initial analyses were performed on unmatched cohorts adjusted for known confounders of blood autoantibody levels; this approach was described to increase statistical power.[39] To strengthen our arguments, we compared anti-PrP^C autoantibody levels in cases and matched controls. These results are in line with findings from the unmatched cohorts.

An increasing number of autoantibodies against neurodegenerative targets are being explored as biomarkers and as potential therapeutics. Naturally occurring autoantibodies against hyperphosphorylated tau protein have been isolated from several asymptomatic blood donors.[40] Researchers from Neurimmune (Schlieren, Switzerland) recently reported the development of a fully human antibody against amyotrophic lateral sclerosis targeting pathologically misfolded SOD1, α-miSOD1, from a memory B-cell library from healthy elderly

individuals.[41] Phase III trials involving aducanumab, a bona fide human antibody with potent β-amyloid clearing capabilities, were stopped prematurely.[42]

In previous works, we found that anti-PrP^C antibodies can efficaciously counteract prions,[6] a finding later confirmed by several other researchers.[43] We speculated that anti-PrP^C autoantibodies from the general population could represent a reservoir of potential therapeutic agents against prion diseases. We find, however, that the distribution of titers appears similar between mutation carriers and controls, and between symptomatic and presymptomatic mutation carriers, arguing against the possibility that these autoantibodies are broadly beneficial. This is at variance with a previous preclinical report claiming neuroprotective effects for naturally occurring antibodies to a PrP peptide.[9] Similarly, naturally occurring anti-β-amyloid autoantibodies with neuroprotective effects were reported in mice, but did not meet primary cognitive endpoints when tested in a phase III clinical trial.[44]

Nonetheless, our work does not rule out the possibility of protective anti-PrP autoantibodies in the general population or in *PRNP* mutation carriers specifically. Our study was restricted to the assessment of autoantibody levels against full-length, wild-type, recombinant human PrP^C. We did not evaluate the presence of antibodies specific to pathogenic *PRNP* mutations or to neoepitopes created by those mutations. Moreover, it is possible that humans develop antibodies specific to PrP^{Sc}, the aggregated form of the prion protein. In our experience, such anti-PrP^{Sc} antibodies tend to cross-react, at least to some level, with PrP^C.[45] Another difficulty is that PrP^{Sc} structure is very heterogenous in gPrDs: while brains from patients with genetic CJD and sCJD show similar patterns of PrP^{Sc}, PrP^{Sc} is fragmented and of low molecular weight in brains from patients with GSS and can show marked variation in individuals with the D178N mutation.[2,46] Future studies will focus on the detection of rare, low-titer anti-PrP^{Sc} antibodies, which may possess unique prion-clearing properties.

Acknowledgment

The authors thank the individuals who participated in this study, the participating patients, the patients' families, the CJD Foundation, referring clinicians, all the members of the National Prion Disease Pathology Surveillance Center for technical help, and Anne Kerschenmeyer, Tina Kottarathil, and Rita Moos at the University Hospital of Zurich for technical assistance.

Study funding

The authors received financial support from the EPSRC, BBSRC, ERC, and the Frances and Augustus Newman Foundation. This work was supported by the programs "Investissements d'avenir" ANR-10-IAIHU-06, "Santé Publique France," and grants from NIH (R01NS103848) and CDC (UR8/CCU515004). Collection of samples at Massachusetts General Hospital was funded by Prion Alliance. The funders played no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure

K. Frontzek received an unrestricted grant by Ono Pharmaceuticals and was funded by the Theodor Ida Herzog-Egli Stiftung. M. Carta, M. Losa, and M. Epskamp report no disclosures. G. Meisl is funded by a Ramon Jenkins Research Fellowship at Sidney Sussex College. A. Anane, J.-P. Brandel, U. Camenisch, J. Castillas, and S. Haïk report no disclosures. T. Knowles received financial support by the EPSRC, BBSRC, ERC, and the Frances and Augustus Newman Foundation. E. Lindner was funded by the National Organization for Rare Diseases. A. Lutterotti reports no disclosures. E.V. Minikel has received research support in the form of charitable contributions from Charles River Laboratories and Ionis Pharmaceuticals and has consulted for Deerfield Management. I. Roiter, J.G. Safar, R. Sanchez-Valle, and D. Žáková report no disclosures. S. Hornemann is the recipient of grants from SystemsX.ch (SynucleiX) and the innovations commission of the University Hospital of Zurich. A. Aguzzi is the recipient of an Advanced Grant of the European Research Council (ERC 250356) and is supported by grants from the Swiss National Foundation (SNF, including a Sinergia grant), the Swiss Initiative in Systems Biology, SystemsX.ch (PrionX, SynucleiX), the Klinische Forschungsschwerpunkte (KFSPs) "small RNAs" and "Human Hemato-Lymphatic Diseases," and a Distinguished Investigator Award of the Nomis Foundation. Go to Neurology.org/N for full disclosures.

Publication history

Received by *Neurology* March 24, 2019. Accepted in final form December 4, 2019.

Appendix 1 Authors

Name	Location	Contribution
Karl Frontzek, MD, PhD	Institute of Neuropathology, University of Zurich, Switzerland	Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, software, supervision, validation, visualization, writing of original draft
Manfredi Carta, MD	Institute of Neuropathology, University of Zurich, Switzerland	Investigation, validation, writing of original draft
Marco Losa, MD	Institute of Neuropathology, University of Zurich, Switzerland	Investigation, validation, writing of original draft
Mirka Epskamp, MSc	Institute of Neuropathology, University of Zurich, Switzerland	Investigation, methodology, validation, writing of original draft

Continued

Appendix 1 *(continued)*

Name	Location	Contribution
Georg Meisl, PhD	Department of Chemistry, University of Cambridge, UK	Data curation, formal analysis, software, writing of original draft
Alice Anane, ND	CJD Foundation Israel	Data curation, investigation, resources
Jean-Philippe Brandel, MD	Sorbonne University, Paris, France	Data curation, investigation, resources
Ulrike Camenisch, PhD	Institute of Surgical Pathology, University of Zurich, Switzerland	Methodology, resources, manuscript writing, review, and editing
Joaquín Castilla, PhD	CIC bioGUNE and IKERBASQUE, Basque Foundation for Science, Bizkaia, Spain	Data curation, investigation, resources
Stéphane Haïk, MD, PhD	Sorbonne University, Paris, France	Data curation, investigation, resources
Tuomas Knowles, PhD	Department of Chemistry, University of Cambridge, UK	Data curation, formal analysis, software, manuscript writing, review, and editing
Ewald Lindner, MD	Ophthalmology Division, University of Graz, Austria	Data curation, investigation, resources
Andreas Lutterotti, MD	Department of Neurology, Neuroimmunology and MS Research (NIMS), University of Zurich, Switzerland	Methodology, manuscript writing, review, and editing
Eric Vallabh Minikel, PhD	Broad Institute, Cambridge, MA	Data curation, investigation, resources, manuscript writing, review, and editing
Ignazio Roiter, MD	Treviso Hospital, Italy	Data curation, investigation, resources
Jiri G. Safar, MD	Department of Pathology, Neurology, and National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, OH	Data curation, investigation, resources
Raquel Sanchez-Valle, MD, PhD	Alzheimer's Disease and Other Cognitive Disorders Unit, Hospital Clinic, IDIBAPS, University of Barcelona, Spain	Data curation, investigation, resources
Dana Žáková, PhD	Department of Prion Diseases, Slovak Medical University, Bratislava, Slovakia	Data curation, investigation, resources
Simone Hornemann, PhD	Institute of Neuropathology, University of Zurich, Switzerland	Conceptualization, supervision, writing of original draft
Adriano Aguzzi, MD, PhD	Institute of Neuropathology, University of Zurich, Switzerland	Conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing of original draft

Appendix 2 Coinvestigators

Name	Location	Role	Contribution
Marc L. Cohen	Department of Pathology and National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, OH	Center codirector	Diagnostic neuropathology
Hasier Eraña, PhD	Atlas Molecular Pharma SL, Derio, Bizkaia, Spain	Head of project: prion diseases	Laboratory research responsible for blood sample extraction from patients/families with a genetic prion disease
Sonia M. Vallabh, JD	Broad Institute, Cambridge, MA	Site investigator	Provided samples and clinical data
Chloe Nobuhara, BS	Massachusetts General Hospital, Boston	Site coordinator	Provided samples and clinical data
Chase Wennick, BS	Massachusetts General Hospital, Boston	Site coordinator	Provided samples and clinical data
Steven E. Arnold, MD	Massachusetts General Hospital, Boston	Site investigator	Provided samples and clinical data
Gianluigi Forloni, PhD	Mario Negri Institute for Pharmacologic Research, Italy	Department head	Provided samples and clinical data

References

1. Aguzzi A, Lakkaraju AKK, Frontzek K. Toward therapy of human prion diseases. Annu Rev Pharmacol Toxicol 2018;58:331–351.
2. Kim MO, Takada LT, Wong K, Forner SA, Geschwind MD. Genetic PrP prion diseases. Cold Spring Harbor Perspect Biol 2018;10:pii: a033134.
3. Will RG, Ironside JW. Sporadic and infectious human prion diseases. Cold Spring Harb Perspect Med 2017;7:pii: a024364.
4. Minikel EV, Vallabh SM, Orseth MC, et al. Age at onset in genetic prion disease and the design of preventive clinical trials. Neurology 2019;93:e125–e134.
5. Scheckel C, Aguzzi A. Prions, prionoids and protein misfolding disorders. Nat Rev Genet 2018;19:405–418.
6. Heppner FL, Musahl C, Arrighi I, et al. Prevention of scrapie pathogenesis by transgenic expression of anti-prion protein antibodies. Science 2001;294:178–182.
7. Sonati T, Reimann RR, Falsig J, et al. The toxicity of antiprion antibodies is mediated by the flexible tail of the prion protein. Nature 2013;501:102–106.
8. Herrmann US, Sonati T, Falsig J, et al. Prion infections and anti-PrP antibodies trigger converging neurotoxic pathways. PLoS Pathog 2015;11:e1004662.
9. Wei X, Roettger Y, Tan B, et al. Human anti-prion antibodies block prion peptide fibril formation and neurotoxicity. J Biol Chem 2012;287:12858–12866.
10. Omae Y, Ito S, Takeuchi M, et al. Integrative genome analysis identified the KANNO blood group antigen as prion protein. Transfusion 2019;59:2429–2435.
11. Haik S, Marcon G, Mallet A, et al. Doxycycline in Creutzfeldt-Jakob disease: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2014;13:150–158.
12. Haïk S, Brandel JP, Salomon D, et al. Compassionate use of quinacrine in Creutzfeldt–Jakob disease fails to show significant effects. Neurology 2004;63:2413–2415.
13. Collinge J, Gorham M, Hudson F, et al. Safety and efficacy of quinacrine in human prion disease (PRION-1 study): a patient-preference trial. Lancet Neurol 2009;8:334–344.
14. Otto M, Cepek L, Ratzka P, et al. Efficacy of flupirtine on cognitive function in patients with CJD: a double-blind study. Neurology 2004;62:714–718.
15. Killworth H. Fourth patient to be given innovative treatment for CJD [online]. Available at: ucl.ac.uk/news/2019/jan/fourth-patient-be-given-innovative-treatment-cjd. Accessed September 13, 2019.

16. Reimann RR, Aguzzi A. Intrinsic toxicity of antibodies to the globular domain of the prion protein. Biol Psychiatry 2018;84:e51–e52.
17. Reimann RR, Sonati T, Hornemann S, et al. Differential toxicity of antibodies to the prion protein. PLoS Pathog 2016;12:e1005401.
18. Wu B, McDonald AJ, Markham K, et al. The N-terminus of the prion protein is a toxic effector regulated by the C-terminus. Elife 2017;6:pii: e23473.
19. Minikel EV, Vallabh SM, Lek M, et al. Quantifying prion disease penetrance using large population control cohorts. Sci Transl Med 2016;8:322ra329.
20. Biljan I, Ilc G, Plavec J. Understanding the effect of disease-related mutations on human prion protein structure: insights from NMR spectroscopy. Prog Mol Biol Transl Sci 2017;150:83–103.
21. WHO Manual for Surveillance of Human Transmissible Spongiform Encephalopathies, Including Variant Creutzfeldt-Jakob Disease. Geneva: World Health Organization; 2003.
22. Ironside JW, Ritchie DL, Head MW. Prion diseases. Handb Clin Neurol 2017;145:393–403.
23. Hill JT, Demarest BL, Bisgrove BW, Su YC, Smith M, Yost HJ. Poly peak parser: method and software for identification of unknown indels using sanger sequencing of polymerase chain reaction products. Dev Dyn 2014;243:1632–1636.
24. Wright ES. Using DECIPHER v2.0 to analyze big biological sequence data in R. R J 2016;8:352–359.
25. Huber W, Carey VJ, Gentleman R, et al. Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods 2015;12:115–121.
26. Watad A, Bragazzi NL, Adawi M, et al. Autoimmunity in the elderly: insights from basic science and clinics: a mini-review. Gerontology 2017;63:515–523.
27. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol 2014;35:347–369.
28. Kifude CM, Rajasekariah HG, Sullivan DJ Jr, et al. Enzyme-linked immunosorbent assay for detection of *Plasmodium falciparum* histidine-rich protein 2 in blood, plasma, and serum. Clin Vaccin Immunol 2008;15:1012–1018.
29. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol Med 2008;3:17.
30. Hess RD. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. J Clin Microbiol 2004;42:3381–3387.
31. Zerr I, Bodemer M, Gefeller O, et al. Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. Ann Neurol 1998;43:32–40.
32. Kobayashi A, Teruya K, Matsuura Y, et al. The influence of PRNP polymorphisms on human prion disease susceptibility: an update. Acta Neuropathol 2015;130:159–170.
33. Sun L, Li X, Lin X, Yan F, Chen K, Xiao S. Familial fatal insomnia with atypical clinical features in a patient with D178N mutation and homozygosity for Met at codon 129 of the prion protein gene. Prion 2015;9:228–235.
34. Chen S, He S, Shi XH, et al. The clinical features in Chinese patients with PRNP D178N mutation. Acta Neurol Scand 2018;138:151–155.
35. Matusiewicz A, Strozynska-Byrska J, Olesinska M. Polyautoimmunity in rheumatological conditions. Int J Rheum Dis 2019;22:386–391.
36. Nagele EP, Han M, Acharya NK, DeMarshall C, Kosciuk MC, Nagele RG. Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. PLoS One 2013;8:e60726.
37. Lacroix-Desmazes S, Mouthon L, Kaveri SV, Kazatchkine MD, Weksler ME. Stability of natural self-reactive antibody repertoires during aging. J Clin Immunol 1999;19:26–34.
38. Mansournia MA, Jewell NP, Greenland S. Case-control matching: effects, misconceptions, and recommendations. Eur J Epidemiol 2018;33:5–14.
39. Faresjo T, Faresjo A. To match or not to match in epidemiological studies: same outcome but less power. Int J Environ Res Public Health 2010;7:325–332.
40. Pascual G, Wadia JS, Zhu X, et al. Immunological memory to hyperphosphorylated tau in asymptomatic individuals. Acta Neuropathol 2017;133:767–783.
41. Maier M, Welt T, Wirth F, et al. A human-derived antibody targets misfolded SOD1 and ameliorates motor symptoms in mouse models of amyotrophic lateral sclerosis. Sci Transl Med 2018;10.
42. Selkoe DJ. Alzheimer disease and aducanumab: adjusting our approach. Nat Rev Neurol 2019;15:365–366.
43. White AR, Enever P, Tayebi M, et al. Monoclonal antibodies inhibit prion replication and delay the development of prion disease. Nature 2003;422:80–83.
44. Relkin NR, Thomas RG, Rissman RA, et al. A phase 3 trial of IV immunoglobulin for Alzheimer disease. Neurology 2017;88:1768–1775.
45. Polymenidou M, Stoeck K, Glatzel M, Vey M, Bellon A, Aguzzi A. Coexistence of multiple PrPSc types in individuals with Creutzfeldt-Jakob disease. Lancet Neurol 2005;4:805–814.
46. Haik S, Peoc'h K, Brandel JP, et al. Striking PrPsc heterogeneity in inherited prion diseases with the D178N mutation. Ann Neurol 2004;56:909–910; author reply 910–911.

Autoantibodies against the prion protein in individuals with *PRNP* mutations

Karl Frontzek, Manfredi Carta, Marco Losa, et al.

Neurology 2020;95;e2028-e2037 Published Online before print February 25, 2020
DOI 10.1212/WNL.0000000000009183