Association of prion protein with cognitive functioning in humans

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A R T I C L E  I N F O

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A B S T R A C T

Objectives: Recent animal studies have suggested a key role for cellular prion protein (PrPc) in the pathological consequences of amyloid-β formation, the hallmark of Alzheimer’s disease. This epidemiological study investigated whether serum concentrations of PrPc are associated with cognitive functioning in humans.

Design, Setting, Participants: Cross-sectional study of 1,322 participants from the elderly general population in Germany, aged 65+ years at baseline (2000–2002).

Measurements: Cognitive functioning was assessed by the COGTEL phone interview 5 years after baseline. Serum PrPc was determined by a commercial immunoassay.

Results: In multiple linear regression adjusted for important confounders, subjects in higher PrPc quintiles appeared to have lower cognitive functioning scores than those in the lowest PrPc quintile. Spline regression suggested pronounced non-linearity with an inverse association between PrPc and cognitive functioning levelling off beyond median PrPc. Cognitive subdomain-specific models produced somewhat heterogeneous results.

Conclusion: The findings are suggestive of an independent association of PrPc with cognitive functioning in humans. Confirmatory and longitudinal studies are needed to elucidate the potential of PrPc for applications in early risk stratification for cognitive impairment.

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1. Introduction

Dementias of the Alzheimer type and non-Alzheimer dementias are among the major challenges to aging societies in the early 21st century (Duron and Hanon, 2008). Cellular prion protein (PrPc) has recently been suggested to be involved in the deleterious consequences of amyloid-β build-up (Lauren et al., 2009), the hallmark of Alzheimer’s disease. The data primarily coming out of studies in PrPc knock-out mice suggest that PrPc is an important amyloid-β receptor relevant to the adverse effects of amyloid-β oligomers at the neural synapsis, and research into the clinical relevance of these findings must be considered rather urgent (Cisse and Mucke, 2009). Subsequent studies have shown that PrPc may also be involved in mediating neurotoxicity of various other β-sheet-rich molecules, suggesting a potential role in pathologies beyond prion disease and Alzheimer’s (Resenberger et al., 2011).

In the meantime, the PrPc mechanism in Alzheimer’s disease has been questioned by some investigators (Balducci et al., 2010; Kessels et al., 2010), but supported by others Bate and Williams (2011), who suggested differences in amyloid-β preparations and memory assessments as possible explanations for these discrepancies. Data on the potential association of PrPc with dementia or cognitive functioning in humans remains extremely scarce. One study revealed no association between brain expression of PrPc and Alzheimer’s disease (Saijo et al., 2011), whereas another study found such differences in sporadic cases of Alzheimer’s compared to controls (Whitehouse et al., 2010). Thus, although the role of PrPc in Alzheimer’s pathology remains controversial, it is warranted to consider PrPc a tentative candidate biomarker for studies of cognitive functioning in humans.

Interventions to maintain cognitive functioning are considered to have potential especially during the very early stages of cognitive decline. The present epidemiological study thus focussed on older subjects from the general population, who were characterised using the Cognitive Telephone Screening Instrument (COGTEL). COGTEL was purposely designed to assess cognition across the full range of adult functioning with a broad coverage of diverse cognitive domains (Breitling et al., 2010; Kliegel et al., 2007). The abundance of PrPc was determined in peripheral blood samples, the most relevant
sample matrix readily available in realistic screening settings in which persons at high risk of progressing to Alzheimer’s disease could eventually be identified and singled out for targeted prevention efforts.

The purpose of this study was to examine if PrP\(_c\) is associated with cognitive functioning in a screening setting, i.e. in the general population and making use of peripheral blood serum as a realistically accessible sample material. Such an association would provide first evidence for a potential utility of PrP\(_c\) for early risk stratification.

### 2. Subjects and methods

#### 2.1. Study design and participants

Participants were a subpopulation of the ESTHER study, a statewide epidemiological cohort study of the elderly population of the southwestern German state of Saarland (Low et al., 2004). In ESTHER, 9953 participants aged 50–74 years were recruited from 2000 to 2002 by their general practitioners during health screening visits. For maximum generalisability, no exclusion criteria except insufficient German language skills and unwillingness or inability to participate were applied. Socio-demographic and health-related data were collected according to a standardised protocol and self-administered questionnaires, and all covariable values refer to these baseline assessments. Glomerular filtration rate (eGFR) was estimated from creatinine values determined in blood samples drawn at the baseline health screening visit (mailed to a central laboratory and stored at −80 °C until analysis) according to the CKD-EPI equation (Levey et al., 2009).

The study was approved by the ethics committee of the University of Heidelberg and the medical board of the State of Saarland. Written informed consent was obtained before inclusion in ESTHER, and specific written informed consent was obtained before inclusion into the cognitive telephone instrument substudy, for which only the ten highest age groups (65–74 years at baseline) were eligible.

#### 2.2. Assessment of cognitive functioning

The cognitive telephone screening instrument (COGTEL) and its application in ESTHER have been described in detail elsewhere (Breitling et al., 2010; Kliegel et al., 2007). In brief, the interview consists of six components covering different cognitive domains (prospective memory, verbal short-/long-term memory, verbal fluency, working memory, inductive reasoning) and originating from well established standard instruments. The six subscores obtained are summed up to give a weighted total score, which is approximately normally distributed in the elderly general population with a mean ± standard deviation of 27.1 ± 8.7 points in ESTHER (Breitling et al., 2010). All interviews were conducted from May 2005 to July 2008 in the context of the 5-year follow-up of the ESTHER cohort by individuals trained in the application of COGTEL.

#### 2.3. Cellular prion protein (PrP\(_c\)) measurements

The concentration of PrP\(_c\) was determined in 30 µL of peripheral blood serum obtained during participants’ presentations to their general practitioner as part of the ESTHER year 5 follow-up. Samples had been mailed to a central laboratory and stored at −80 °C until analysis. PrP\(_c\) measurements were carried out at a commercial laboratory (Immundiagnostik, Bensheim, Germany) using the BetaPrion® HUMAN EIA Test Kit (cat. no. 0104000104) for the detection of human prion protein. Following the manufacturer’s advice, the standard protocol was refined by incorporating an incubation step with an unspecific-human-anti-mouse-antibodies-blocking buffer to increase assay specificity. The detection limit was 0.132 ng/mL. Pre-tests with blinded double measurements of 20 samples supported a high precision with a Spearman correlation of 0.97 between the measurements.

A total of 1952 COGTEL interviews had been conducted in the ten highest age groups of the ESTHER study population (51% of 3844 subjects aged 65+ years at baseline). After exclusion of 153 subjects because of hearing problems and 102 subjects because of other problems during interview conduct (e.g. help by a third person), 1697 interviewees were considered eligible for the present project. Of these, 1333 had provided blood samples as part of the ESTHER 5-year follow-up. Sufficient sample material for PrP\(_c\) measurements was available for 1322 subjects (99.2% of 1333). In 10 subjects with concentrations below the lower detection limit, the concentration was set to half the value of the detection limit (Lubin et al., 2004).

#### 2.4. Statistical analysis

Concentrations of PrP\(_c\) showed a right-skewed distribution and were analysed in quintiles or after applying a log\(_2\)-transformation. PrP\(_c\) concentrations and COGTEL total scores were first tabulated across major participant characteristics including sex, smoking status, education, and self-reported history of cerebrovascular disease (stroke or transient ischemic attacks, TIA) or depression. Correlations with age at interview, body mass index (BMI), intensity of regular alcohol consumption, and renal function (eGFR) were examined by Spearman coefficients. The associations between PrP\(_c\) quintiles and the covariables were also tested, using either chi-square (for categorical covariables) or Kruskal–Wallis tests (continuous covariables). To avoid excessive multiple testing issues in the present analyses, we explored only variables that we considered major epidemiological factors (e.g. sex, smoking) and/or important potential determinants of cognitive performance (e.g. education, history of cerebrovascular disease), or that were associated with PrPc levels according to the rare previous human studies on the subject (renal function (Starke et al., 2006)), history of hypertension, hypercholesterolaemia, and diabetes, were additionally included in order to assess the sensitivity of our analyses to further covariable adjustment.

The association of PrP\(_c\) with COGTEL total scores was analysed by linear regression. In addition to an unadjusted model, a multiple regression model was fitted controlling for all major participant characteristics listed above, which were considered to be potential confounding variables. As a sensitivity analysis, we fitted an additional model further adjusted for hypertension, hypercholesterolaemia, and diabetes; ultimately, we explored the impact of excluding subjects with diabetes, cerebrovascular disease, or depression from the main multiple regression model. As a secondary analysis, we analysed the association of PrP\(_c\) with overall cognitive performance in a dichotomised fashion, segregating subjects in a group with COGTEL total scores ≥ 18.5 points (reference group) vs. those with <18.5 points (cognitively impaired). The cutoff was chosen as the overall COGTEL total score mean minus 1 standard deviation, which is one approach used in the literature to identify subjects with mild cognitive impairment (Bischkopf et al., 2002).

Associations of PrP\(_c\) with COGTEL subdomain scores were examined by linear regression in the case of approximately normally distributed components (verbal short-term memory, verbal fluency, verbal long-term memory). The binary subscore for prospective memory was analysed by logistic regression. As in previous publications (Breitling et al., 2010), the pronouncedly skewed subscore for working memory and inductive reasoning were dichotomised at their medians (≥ 6 and ≥ 3, respectively) and analysed by logistic regression. In all these logistic models, the predicted variable was the odds of a favourable cognitive performance.

The dose–response relationship between continuous PrP\(_c\) and COGTEL scores was examined by restricted cubic spline analysis fully confounder-adjusted as described above, using the 10th, 50th, and 90th percentile as knots (Desquilbet and Mariotti, 2010). SAS 9.2 was used for all statistical analyses (SAS Institute, 2008).
3. Results

Table 1 presents major participant characteristics of the 1322 subjects ultimately contributing to the analyses. There were slightly more women than men, and the median age at interview was 74 years. Almost three quarters had obtained only the lowest school qualification.

The median PrPc concentration was 2.39 (interquartile range: 1.66–3.30) ng/mL, with a right-skewed distribution that could be reasonably normalised by logarithmic transformation (Fig. 1). As shown in Table 1, PrPc tended to be higher in women than in men, and subjects with vs. without hypertension or diabetes, and there was a positive correlation with age and a negative correlation with alcohol consumption and eGFR. Cognitive functioning as measured by the COGTEL total score increased with higher education and alcohol consumption, and decreased with higher age, higher BMI, GFR, and history of hypertension or diabetes (Table 1).

The bivariate distribution of COGTEL total scores with untransformed PrPc concentrations is shown in Fig. 2, including the corresponding linear regression line, which suggested an inverse association between the two variables. In further regression models predicting COGTEL total scores, there was evidence of pronounced non-linearity, and subjects with PrPc in the 2nd quintile showed a distinctly lower cognitive performance than those in the lowest quintile, regardless of confounder-adjustment (Table 2). In the higher quintiles, COGTEL scores were also lower, but this was somewhat less pronounced. The point estimates were altogether little altered by confounder adjustment, and the overall pattern of association remained similar (though somewhat attenuated) when additionally adjusting for hypertension, hypercholesterolaemia, and diabetes (Table 2). Excluding subjects with diabetes, depression, or cerebrovascular disease had no relevant impact on the estimates (2nd vs. lowest quintile: −1.98 [95% CI: −3.79 to −0.16]; details not shown). When modelling the presence of mild cognitive impairment (defined as a COGTEL score < 18.5) as the dependent variable using multiple logistic regression, again qualitatively similar results emerged: the confounder-adjusted odds ratios (95% confidence intervals) in reference to the lowest PrPc quintile were 1.84 (1.11–3.03), 1.37 (0.82–2.30), 1.03 (0.59–1.80), and 1.33 (0.79–2.26) for the 2nd, 3rd, 4th, and 5th quintile, respectively.

Fig. 3 shows the dose–response relationship between COGTEL total scores and PrPc as estimated by restricted cubic splines. In line with the quintile results, non-linearity was rather pronounced and suggested higher cognitive functioning in subjects with low PrPc concentrations (log2 of ng/mL).
and a levelling off of the negative association above median PrPc values.

The subdomain scores were distributed with a mean \(\pm\) standard deviation of 4.0 \(\pm\) 1.8 (verbal short-term memory), 21.4 \(\pm\) 6.3 (verbal fluency), and 4.7 \(\pm\) 1.7 (verbal long-term memory). The prospective memory task was successfully carried out by 812 subjects. For working memory, 759 participants had \(\geq\)median scores, and for inductive reasoning, 717 scored \(\geq\)median points. Confounder-adjusted associations with the various COGTEL subscores are shown by spline estimates in Fig. 4. Whereas the logistic regression for prospective memory appeared to deviate the most from the total score spline in Fig. 2, the results for most other subdomain scores were more in line with an inverse association. The confidence intervals around the spline estimates frequently were rather wide, cautioning that the differences in spline shapes should not be overinterpreted.

4. Discussion

In this epidemiological study on cellular prion protein and cognitive functioning in the elderly, an overall inverse and pronouncedly non-linear association of serum PrPc levels with a telephone-based cognitive functioning score covering multiple cognitive domains was found. Whereas most published evidence on PrPc and cognitive functioning still relies on mouse models and a few expression studies in human tissues accessible only in experimental settings, the present study took a complementary approach and directly attempted to assess associations of PrPc in a clinically relevant setting related to the early detection of impaired cognitive functioning in humans (Cisse and Mucke, 2009). The characterisation of serum biomarkers for cognitive disorders has been very challenging, and it thus would be relevant if PrPc were informative even to a moderate degree (Reitz and Mucke, 2009). The characterisation of serum biomarkers for cognitive disorders has been very challenging, and it thus would be relevant if PrPc were informative even to a moderate degree (Reitz and Mucke, 2009). The characterisation of serum biomarkers for cognitive disorders has been very challenging, and it thus would be relevant if PrPc were informative even to a moderate degree (Reitz and Mucke, 2009). The characterisation of serum biomarkers for cognitive disorders has been very challenging, and it thus would be relevant if PrPc were informative even to a moderate degree (Reitz and Mucke, 2009). The characterisation of serum biomarkers for cognitive disorders has been very challenging, and it thus would be relevant if PrPc were informative even to a moderate degree (Reitz and Mucke, 2009). The characterisation of serum biomarkers for cognitive disorders has been very challenging, and it thus would be relevant if PrPc were informative even to a moderate degree (Reitz and Mucke, 2009). The characterisation of serum biomarkers for cognitive disorders has been very challenging, and it thus would be relevant if PrPc were informative even to a moderate degree (Reitz and Mucke, 2009).

Relatively little is known about the physiological role of PrPc in humans. Mostly based on studies of (knock-out) mice, it has been suggested that PrPc might be neuroprotective, antiapoptotic, and antioxidative (Aguzzi et al., 2008; Roucou and LeBlanc, 2005). At the same time, one of the very few human investigations described higher PrPc concentrations in plasma of a small number of subjects with rather mixed neurologic diagnoses (Vollert et al., 2001). A crucial role of PrPc in the development of pathological consequences of amyloid-\(\beta\) accumulation has been described only very recently in the context of mouse studies, which were interpreted as suggesting that PrPc acts as an amyloid-\(\beta\) receptor initiating a cascade leading to detrimental changes of the nerve cell (Lauren et al., 2009). Where- as subsequent work produced inconsistent results (for an overview, see Ledford, 2010), other follow-up studies supported a "latent pro- pathogenic role" of PrPc depending on amyloid-\(\beta\) (Alier et al., 2011) and further suggested that PrPc also mediates memory impairment associated with amyloid-\(\beta\) accumulation (Cimbel et al., 2010), and even neuronal cell death (Kudo et al., 2012). These results are highly intriguing as they render PrPc a promising potential intervention target in Alzheimer disease (Nygaard and Strittmatter, 2009), and antibodies directed against PrPc do not only prevent some neurophysiological consequences of amyloid-\(\beta\) binding (Barry et al., 2011; Freir et al., 2011), but even have shown some effect in treating cognitive deficits in mice (Chung et al., 2010).

The findings in the present study suggested lowered overall cognitive performance in subjects with higher PrPc serum concentrations. This hardly concurs with predominantly beneficial, neuroprotective PrPc effects, and also is unlikely to be explained by increased susceptibility to effects of amyloid-\(\beta\) accumulation in this general population-based study cohort. Two very tentative hypotheses consistent with the pattern described would be that higher serum PrPc results from neurobiological changes associated with reduced cognitive functioning and featuring higher PrPc release due to reduced nerve cell integrity (essentially leading to a reverse causation phenomenon), or that serum PrPc actually reflects the abundance of PrPc in neuro-cellular membranes, where more abundant PrPc plausibly could be associated with higher unspecific (i.e., not triggered by amyloid-\(\beta\)-binding) activity of detrimental cascades downstream of PrPc. One could further speculate that the flat right end of the dose–response relationship

### Table 2

Linear regression analysis of cellular prion protein (PrPc) serum concentration as a predictor of COGTEL total scores.

<table>
<thead>
<tr>
<th>PrPc</th>
<th>Cutoff</th>
<th>(\beta) (95% CI)</th>
<th>(\beta^2) (95% CI)</th>
<th>(P_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quintile 1</td>
<td>(\leq 1.51) ng/mL</td>
<td>0.00</td>
<td>Ref</td>
<td>0.01</td>
</tr>
<tr>
<td>Quintile 2</td>
<td>(\leq 2.13) ng/mL</td>
<td>0.20</td>
<td>(0.29 to 0.62)</td>
<td>0.43</td>
</tr>
<tr>
<td>Quintile 3</td>
<td>(\leq 2.73) ng/mL</td>
<td>0.14</td>
<td>(0.24 to 0.60)</td>
<td>0.35</td>
</tr>
<tr>
<td>Quintile 4</td>
<td>(\leq 3.53) ng/mL</td>
<td>0.12</td>
<td>(0.27 to 0.52)</td>
<td>0.31</td>
</tr>
<tr>
<td>Quintile 5</td>
<td>(\geq 3.53) ng/mL</td>
<td>0.15</td>
<td>(0.23 to 0.50)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

\(P_a\) Adjusted model including sex, smoking status, education, history of cerebrovascular disease, history of depression, age, BMI, alcohol consumption, and estimated GFR.

\(\beta^2\) Sensitivity model additionally adjusted for hypertension, hypercholesterolaemia, and diabetes.
especially for the COGTEL total score—which might be a useful construct on statistical grounds, but admittedly could confound domain-specific associations—would argue against serum PrPc being merely a marker of neuron disintegration, as effects of higher and higher neuron loss on cognitive functioning probably would not be expected to level off in this way.

To provide a more complete picture of the potential association of PrPc with cognitive functioning in humans, results of domain-specific subscores of the COGTEL instrument also have been reported in the present work. Whereas one has to be cautious in the interpretation of such individual behavioural test items, pronounced differences in their association patterns with a marker such as PrPc could suggest which cerebral structures are involved/affected by the processes underlying the associations, e.g. hippocampal structures in the case of verbal long-term memory, or dorsolateral-prefrontal networks for verbal fluency (Crawford and Henry, 2005). For PrPc, additional studies are needed to draw reliable conclusions in this regard.

The observational nature of the present investigation limits the possibilities to draw causal conclusions. However, this does not compromise the objective of the study, which was to establish the presence or absence of an association per se. Residual confounding in particular due to unknown factors cannot be ruled out, especially given the scarcity of prior investigations in humans. The overall association nonetheless appeared rather stable in the multiple regression model adjusting for a variety of potential confounders (Starke et al., 2006; Volkel et al., 2001). Additional strengths included the large sample size, a well generalisable study setting, and the use of a cognitive functioning instrument covering multiple domains. In the present study, we also explored the use of a dichotomised version of this instrument, trying to approach the construct of mild cognitive impairment (Bischkopf et al., 2002). The results were rather similar to the main analysis, but ongoing work correlating COGTEL scores to more established cognitive instruments and in-depth geronto-psychiatric follow-up assessments might lead to refined pertinent analyses in the future.

The results presented in this report provide first evidence that readily accessible serum PrPc might have a relevant association with cognitive functioning in humans in the intermediate area encompassing low age-appropriate and mildly impaired cognitive performance. If replicated in additional studies and substantiated by longitudinal investigations, this could not only lead to prospects of early risk stratification, but also might hint to the possibility that knowledge derived from recent and future mouse studies could to some extent be generalisable to humans. It appears important in this regard to consider diverse cognitive domains, which might have heterogeneous associations with PrPc-related phenomena.

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The authors have no conflict of interest related to this work to declare.

Author contributions: LPB conceived the study question, analysed and interpreted data, and wrote the first manuscript draft. HM and CS conducted the study. MK and HB designed the study and interpreted results. HB served as principal investigator. All authors participated in manuscript revision and agreed to the submission.
References


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