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Association of prion protein with cognitive functioning in humans ☆

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ABSTRACT

Objectives: Recent animal studies have suggested a key role for cellular prion protein (PrPc) in the pathological consequences of amyloid plaque 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.

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 (PrP^c) has recently been suggested to be involved in the deleterious consequences of amyloid-β build-up (Lauren et al., 2009), the hall-mark of Alzheimer's disease. The data primarily coming out of studies in PrP^c knock-out mice suggest that PrP^c 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 PrP^c 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 PrP^c 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 PrP^c with dementia or cognitive functioning in humans remains extremely scarce. One study revealed no association between brain expression of PrP^c 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 PrP^c in Alzheimer's pathology remains controversial, it is warranted to consider PrP^c 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 PrP^c was determined in peripheral blood samples, the most relevant

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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 PrPc 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 subscores 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).