Creutzfeldt–Jakob disease is a rare, fatal, neurodegenerative disease caused by the accumulation of abnormally folded prion proteins. The common polymorphism at codon 129 (methionine/valine) in the prion protein (PRNP) gene is the most important determinant of genetic susceptibility. Homozygotes of either allele have a higher risk of sporadic Creutzfeldt–Jakob disease. Various studies suggest that this polymorphism is also involved in other forms of dementia. We studied the association between the codon 129 polymorphism of the PRNP gene and mild cognitive impairment in 3605 participants from the Rotterdam Study using logistic regression analyses. Subsequently, we studied the association between this polymorphism and incident dementia, including Alzheimer’s disease, in 11 070 participants using Cox proportional hazard models. Analyses were adjusted for age and sex. We found the prevalence of mild cognitive impairment to be higher for carriers of the methionine/methionine genotype (odds ratio, 1.40; 95% confidence interval, 1.11–1.78; $P = 0.005$) as well as for carriers of the valine/valine genotype (odds ratio, 1.37; 95% confidence interval, 0.96–1.97; $P = 0.08$). The codon 129 polymorphism was not associated with the risk of incident dementia or Alzheimer’s disease. In conclusion, we found a statistically significant higher prevalence of mild cognitive impairment in carriers of the methionine/methionine genotype in the codon 129 polymorphism of the PRNP gene within this population-based study. No associations were found between the codon 129 polymorphism and dementia or Alzheimer’s disease in the general population.
Introduction

Creutzfeldt–Jakob disease is a rare, fatal, neurodegenerative prion disease caused by the accumulation of abnormally folded prion proteins. These proteins are the product of the prion protein (PRNP) gene. In prion diseases, the cellular prion protein (PrP^C) converts into a misfolded infectious state, the pathological prion protein (PrP^Sc) (Prusiner, 1982). Creutzfeldt–Jakob disease occurs in different forms: sporadic, genetic, iatrogenic, and variant. The sporadic form of Creutzfeldt–Jakob disease is the most common from. A major driver of the development and course of sporadic Creutzfeldt–Jakob disease is the genetic variant at codon 129 (methionine/valine) of the PRNP gene (Parchi et al., 2012). Persons homozygous for either the methionine or valine allele are at increased risk of sporadic Creutzfeldt–Jakob disease (Alperovitch et al., 1999; Parchi et al., 2009). It has been speculated that homozygosity for either allele results in the synthesis of identical proteins, enabling the propagation and aggregation of PRNP in the brain, thus influencing both the risk and progression of sporadic Creutzfeldt–Jakob disease (Alperovitch et al., 1999; Parchi et al., 2009). Clinically, sporadic Creutzfeldt–Jakob disease is characterized by rapidly progressive dementia, and ultimately death on average 6 months after diagnosis. The variant form of Creutzfeldt–Jakob disease is the zoonotic form of the disease, which is transmitted through meat and other food products contaminated with material from cattle with bovine spongiform encephalopathy (Brandel et al., 2009). Up to 2017, all patients diagnosed with variant Creutzfeldt–Jakob disease had the methionine/methionine (MM) genotype on PRNP codon 129. Recently, the first patient with the methionine/valine (MV) genotype on PRNP codon 129 with the variant form was reported (Mok et al., 2017).

While the dementia seen in Creutzfeldt–Jakob disease patients is likely a result of prion protein aggregation, various studies suggest that the PRNP gene is also involved in other forms of dementia, but results are inconsistent (Rujescu et al., 2003; Del Bo et al., 2006; Jeong et al., 2007; Poleggi et al., 2008; Choi et al., 2010; Golanska et al., 2013; He et al., 2013; Zhang et al., 2016). Furthermore, studies performed on early cognitive decline and early-onset Alzheimer’s disease also show inconsistency regarding which of the homozygous carriers have a higher risk (Croes et al., 2003; Dermaut et al., 2003). In contrast, a meta-analysis on the role of the M129V polymorphism in Alzheimer’s disease suggests that individuals with at least one valine allele have a lower risk of Alzheimer’s disease (He et al., 2013), while the International Genetics of Alzheimer Disease Project did not find an association (Kunkle et al., 2019).

In this study, we report on the association between the PRNP M129V polymorphism and mild cognitive impairment and dementia, including Alzheimer’s disease, within the population-based Rotterdam Study.

Materials and methods

Setting and study population

The Rotterdam Study is a prospective population-based middle-aged and elderly cohort that started in 1990 in the district of Ommoord, in Rotterdam, The Netherlands. The study includes 14 926 participants and has three
Loh formed using the Haplotype Reference Consortium panel and genotype dosage values (Niemeijer et al., 2015). Imputation quality for this single-nucleotide polymorphism was high ($r^2 = 0.97$). We rounded the imputed single-nucleotide polymorphism dosages to 0 (MM), 1 (MV) and 2 (valine/valine (VV)).

**Genotyping**

A total of 11 496 participants who were genotyped passed genotyping quality control (92% of all subjects with genotyping) (Niemeijer et al., 2015). Exclusion criteria were a call rate <98%, Hardy–Weinberg $P$-value $<10^{-6}$, minor allele frequency $<0.01\%$, excess autosomal heterozygosity $>0.336$, sex mismatch and outlying identity-by-state clustering estimates. Imputations were performed using the Haplotype Reference Consortium panel (Loh et al., 2016). We selected one single-nucleotide polymorphism within the PRNP gene: M129V. Imputation quality for this single-nucleotide polymorphism was high ($r^2 = 0.97$). We rounded the imputed single-nucleotide polymorphism dosages to 0 (MM), 1 (MV) and 2 (valine/valine (VV)).

**Assessment of mild cognitive impairment**

We defined mild cognitive impairment using the following criteria: (i) presence of subjective cognitive complaints, (ii) presence of objective cognitive impairment and (iii) absence of dementia, as previously described (de Bruijn et al., 2014). The first criterion, presence of subjective cognitive complaints, was evaluated by an interview, which consisted three questions on memory complaints and three questions on impaired daily functioning. If the participant confirmed the presence of one of these six complaints or impairments, subjective cognitive complaints were seen as present. The second criterion, presence of objective cognitive impairment, was assessed using a cognitive test battery comprising letter digit test, Stroop test, Purdue Pegboard test, fluency task and 15-word verbal learning test based on Rey’s recall of words. Participants were classified as objectively cognitive impaired if they scored <1.5 standard deviation of the age-adjusted and education-adjusted mean of the study population. This has previously been explained in more detail (de Bruijn et al., 2014). For this study, we included participants with genetic data available and mild cognitive impairment assessment during the implementation period of extensive neuropsychological tests, as a large number of participants were then screened for mild cognitive impairment for the first time.

**Assessment of dementia**

Dementia ascertainment involved cognitive screening at the study research centre. We further assessed individuals with a Mini-Mental State Examination score of <26 or a Geriatric Mental State Schedule organic level of >0 (de Bruijn et al., 2015), by administering the Cambridge Mental Disorders of the Elderly Examination by a research physician. We also interviewed spouses or informants. A consensus panel headed by a consultant neurologist established the final diagnosis according to the standard criteria. We studied the outcomes of all-cause dementia (DSM-III-R) and Alzheimer’s disease (NINCDS–ADRDA). For the assessment of dementia, and type of dementia, the latest follow-up information with available data was used to determine the disease state. Follow-up for dementia was near complete until 1 January 2015. Within this period, participants were censored at the date of dementia diagnosis, death or loss to follow-up.

**Brain MRI measurements**

Brain MRI scanning was performed using a 1.5-T scanner (General Electric Healthcare, Milwaukee, WI, USA), and it included T1-weighted sequence, proton-density-weighted sequence, fluid-attenuated inversion recovery-weighted sequence and T2-weighted sequences (Ikram et al., 2015). Details on methods for the measurement of brain volume and hippocampal volume have been described earlier (Ikram et al., 2015).

**Statistical analysis**

Logistic regression analyses were used to study the association between the M129V polymorphism and mild cognitive impairment. For mild cognitive impairment as outcome of interest, analyses were conducted in all
participants who had mild cognitive impairment assessment at the implementation of neuropsychological tests. Subsequently, the association between the M129V polymorphism and incident dementia, including incident Alzheimer’s disease, was studied using Cox proportional hazard models. For these two outcomes of interest, analyses were conducted in all participants who had dementia status and dementia type assessed. We ran the analyses unadjusted (Model 1) and also while adjusting for age and sex (Model 2). As secondary analyses, we ran linear regression analyses to study the association between the M129V polymorphism and MRI-derived brain volume and hippocampal volume, while adjusting for age, sex and intracranial volume.

Data availability statement

Because of restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository. Data can be obtained upon reasonable request. Requests for the Rotterdam Study data should be directed towards the management team (secretariat.epi@erasasmusmc.nl).

Results

Descriptive statistics

We examined the 11,496 participants in the Rotterdam Study who had genotyping data available from the M129V polymorphism from the PRNP gene (Fig. 1). Of these participants, 4991 (43.4%) had the MM genotype, 5209 (45.3%) had the MV genotype and the remaining 1295 (11.3%) had the VV genotype. The carriers of the MM genotype tended to be slightly older than the two other genotype groups. In the overall population, the distribution of this genotype was in Hardy–Weinberg equilibrium. The general characteristics of the study population are shown in Table 1.

PRNP M129V and the prevalence of mild cognitive impairment

We found that carriers of the MM genotype have a higher prevalence of mild cognitive impairment than those who are heterozygous (odds ratio, 1.40; 95% confidence interval, 1.11–1.78; \( P = 0.005 \)), as shown in Table 2. Carriers of the VV genotype showed a borderline significant higher prevalence of mild cognitive impairment than heterozygous carriers (odds ratio, 1.37; 95% confidence interval, 0.96–1.97; \( P = 0.08 \)). Combining homozygous carriers (MM and VV) resulted in an odds ratio of 1.40 (95% confidence interval, 1.11–1.73; \( P = 0.004 \)). The allele and genotype distribution of the M129V polymorphism was not in Hardy–Weinberg equilibrium in mild cognitive impairment cases. There was an excess of homozygotes (both MM and VV carriers) in the mild cognitive impairment cases.

PRNP M129V and other outcomes

We did not find significant evidence for the association of PRNP M129V polymorphism with incident dementia, including Alzheimer’s disease, as shown in Table 3. In dementia and Alzheimer’s disease patients and controls, the M129V polymorphism was in Hardy–Weinberg equilibrium. We also did not find a significant association of the M129V polymorphism with brain volume and hippocampal volume.

Discussion

In this study, we investigated the association of the M129V polymorphism of the PRNP gene with mild cognitive impairment and dementia. We found that homozygous carriers had more often mild cognitive impairment than heterozygous carriers. We did not find an association between this polymorphism and dementia. We found a significantly higher prevalence of mild cognitive impairment in carriers of the MM genotype than in carriers of the MV genotype and a non-significant higher prevalence of mild cognitive impairment in carriers of the VV genotype than in carriers of the MV genotype. In contrast, our results do not indicate a role of the M129V polymorphism of the PRNP gene in dementia, including...
Alzheimer’s disease, nor in brain volume and hippocampal volume. The mechanism underlying the association between the M129V polymorphism of the PRNP gene and neurodegeneration is thought to be caused by the effect the M129V polymorphism has on the aggregation of pathological prion proteins in the brain. The aggregation of pathological prion proteins is a biological process similar to the aggregation of tau protein and amyloid accumulation (Walker, 2018), which are key features in Alzheimer’s disease (Bloom, 2014). While most neurodegenerative diseases are known to involve more than one misfolded or abnormally aggregated protein, a recent study demonstrated prion protein aggregation to be an independent pathogenic mechanism, with no cross-seeding between prion protein and misfolded amyloid beta (Rossi et al., 2019), although prion protein is a stress protein that is elevated in (amyloid beta) plaques (Kellett and Hooper, 2009). This suggests that the neurodegenerative changes due to prion protein accumulation could occur regardless of other ongoing neurodegenerative processes.

This could be a possible explanation for our finding that the M129V polymorphism of the PRNP gene plays a role in mild cognitive impairment, and not in dementia. Our study showed that the M129V polymorphism of the PRNP gene may increase the prevalence of mild cognitive impairment. Mild cognitive impairment is often seen as pre-stage of Alzheimer’s disease, related to apolipoprotein E. This study showed the heterogeneity of mild cognitive impairment as also other genes and other non-Alzheimer’s disease dementing disorders are included in the mild cognitive impairment population.

Several limitations in this study should be addressed. Our analyses of the association of the M129V polymorphism with mild cognitive impairment have been performed cross-sectionally. We were not able to calculate the life-time risk of the M129V polymorphism on mild cognitive impairment in the participants from our population-based cohort study. Another potential limitation is the generalizability of our findings, as our study was performed in mainly Caucasians. Previous studies have

| Table 1 Characteristics of the study population |
|-----------------------------------------------|
| Genotype at codon 129 of the prion protein gene |
| P |
| MM N = 4991 | MV N = 5209 | VV N = 1296 |
| Age at baseline, median (range) | 63.2 (45.6–99.1) | 62.6 (45.5–99.2) | 62.6 (45.7–99.2) |
| Sex, female, n (%) | 2878 (58) | 3068 (59) | 726 (56) |
| APOE/e4 carrier, n (%) | 1377 (29) | 1451 (29) | 369 (30) |

*Four percent of missings in each genotype group.

APOE/e4, apolipoprotein E epsilon 4; n, number of people; N, number of people at risk; M, methionine; V, valine.

| Table 2 Association of PRNP M129V polymorphism with mild cognitive impairment |
|-----------------------------------------------|
| Genotype | Cases, n (%) | Controls, n (%) | Model 1 OR (95% CI) | P | Model 2 OR (95% CI) | P |
| MM | 182 (51) | 1414 (44) | 1.42 (1.13–1.80) | 0.003 | 1.40 (1.11–1.78) | 0.005 |
| MV | 133 (37) | 1471 (45) | 1.00 (reference) | 1.00 (reference) |
| VV | 45 (13) | 360 (11) | 1.38 (0.97–1.98) | 0.08 | 1.37 (0.96–1.97) | 0.08 |

Hardy–Weinberg equilibrium P: overall: 0.95, cases: 0.0099, controls: 0.44.

CI, confidence interval; MM, methionine/methionine; MV, methionine/valine; n, number of people; OR, odds ratio; VV, valine/valine.

| Table 3 Risk of incident dementia, including Alzheimer’s disease, stratified on PRNP M129V polymorphism |
|-----------------------------------------------|
| Genotype | Cases, n (%) | Cohort at risk, n (%) | Model 1 HR (95% CI) | P | Model 2 HR (95% CI) | P |
| Dementia | | | | | | |
| MM | 602 (43) | 4802 (43) | 1.01 (0.90–1.13) | 0.89 | 1.01 (0.90–1.13) | 0.90 |
| MV | 631 (44) | 5015 (45) | 1.00 (reference) | 1.00 (reference) |
| VV | 176 (13) | 1253 (11) | 1.12 (0.95–1.32) | 0.18 | 1.16 (0.98–1.37) | 0.09 |
| Alzheimer’s disease | | | | | | |
| MM | 480 (43) | 4680 (44) | 1.01 (0.89–1.15) | 0.84 | 1.01 (0.87–1.15) | 0.82 |
| MV | 500 (45) | 4884 (45) | 1.00 (reference) | 1.00 (reference) |
| VV | 125 (11) | 1202 (11) | 1.02 (0.84–1.24) | 0.87 | 1.06 (0.87–1.29) | 0.56 |

Hardy–Weinberg equilibrium P for dementia: overall: 0.30, cases: 0.59, controls: 0.19. Hardy–Weinberg equilibrium P for Alzheimer’s disease: overall: 0.18, cases: 0.76, controls: 0.19.

CI, confidence interval; HR, hazard ratio; MM, methionine/methionine; MV, methionine/valine; n, number of people; VV, valine/valine.
studied the effect of the M129V polymorphism of the PRNP gene in mild cognitive impairment and different types of dementia in Asians (Jeong et al., 2007; Choi et al., 2010; Zhang et al., 2016). No association was found between the M129V polymorphism and mild cognitive impairment in a Korean population (Choi et al., 2010). Our findings are therefore not generalizable to other ethnicities.

Our study also had several strengths. We used a population-based cohort study, and our study had a relatively large sample size. Although the sample size of the participants who underwent initial mild cognitive impairment assessment was smaller than the overall sample with available genotyping data and dementia assessment, we had sufficient power to demonstrate the association between the M129V polymorphism and mild cognitive impairment.

In conclusion, we found a statistically significant higher prevalence of mild cognitive impairment in carriers of the MM genotype in the M129V polymorphism of the PRNP gene in the Rotterdam Study, but no associations were found between this polymorphism and incidence of dementia, including Alzheimer’s disease. Future studies should further elucidate the role of the M129V polymorphism of the PRNP gene in cognitive function, dementia and other neurodegenerative traits.

Acknowledgements

We are thankful to the study participants, the staff from the Rotterdam Study, the participating general practitioners and the participating pharmacists. We acknowledge F.J.A. van Rooij as data manager, B.C.T. Leening-Kieboom as study coordinator and J. Verkroost-van Heemst for her invaluable contribution to data collection. Furthermore, we acknowledge Prof. Dr. R.G. Will from the University of Edinburgh for sharing his invaluable expertise on prion diseases.

Funding

This work is funded by the European Union’s Horizon 2020 research and innovation programme as part of the Common mechanisms and pathways in Stroke and Alzheimer’s disease (CoSTREAM) project. The current study is supported by Memorabel supported by The Netherlands Organization for the Health Research and Development (ZonMw). The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, ZonMw, the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII) and the Municipality of Rotterdam.

Prof. Dr. Cornelia M. van Duijn is funded by the National Institute of Health (NIH), National Institute on Aging (NIA) with the following grants: Gut Liver Brain Biochemical Axis in Alzheimer’s Disease – R01 (Grant Project No. 1RF1AG058942-01) and Metabolomic Signatures for Disease Sub-classification and Target Prioritization in AMP-AD – U01 (Grant Project No. 5U01AG061359-02).

Competing interests

The authors report no competing interests.

References

Alperovitch A, Zerr I, Pocchiari M, Mitrova E, de Pedro Cuesta J, Hegyi I, et al. Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. Lancet 1999; 353: 1673–4.
Bloom GS. Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol 2014; 71: 505–8.
Brandel JP, Heath CA, Head MW, Levavasseur E, Knight R, Laplanche JL, et al. Variant Creutzfeldt-Jakob disease in France and the United Kingdom: evidence for the same agent strain. Ann Neurol 2009; 65: 249–56.
Choi IG, Woo SI, Kim HJ, Kim DJ, Park BL, Cheong HS, et al. Lack of association between PRNP M129V polymorphism and multiple sclerosis, mild cognitive impairment, alcoholism and schizophrenia in a Korean population. Dis Markers 2010; 28: 315–21.
Croes EA, Dermaut B, Hoving-Duistermaat JJ, Van den Broeck M, Cruts M, Breteler MM, et al. Early cognitive decline is associated with prion protein codon 129 polymorphism. Ann Neurol 2003; 54: 275–6.
de Bruijn RF, Akoudad S, Cremers LG, Hofman A, Niessen WJ, van der Lugt A, et al. Determinants, MRI correlates, and prognosis of mild cognitive impairment: the Rotterdam Study. J Alzheimers Dis 2014; 42: S239–49. Suppl.de Bruijn RF, Bos MJ, Portegies ML, Hofman A, Franco OH, Koudstaal PJ, et al. The potential for prevention of dementia across two decades: the prospective, population-based Rotterdam Study. BMC Med 2015; 13: 132.
Del Bo R, Scarlato M, Ghezzi S, Martinelli-Boneschi F, Fenoglio C, Galimberti G, et al. Is M129V of PRNP gene associated with Alzheimer’s disease? A case-control study and a meta-analysis. Neurobiol Aging 2006; 27: 770.e1–5.
Dermaut B, Croes EA, Rademakers R, Van den Broeck M, Cruts M, Hofman A, et al. PRNP Val129 homozygosity increases risk for early-onset Alzheimer’s disease. Ann Neurol 2003; 53: 409–12.
Golanska E, Sieruta M, Corder E, Gresner SM, Pfeffer A, Chodakowska-Zebrowska M, et al. The prion protein M129V polymorphism: longevity and cognitive impairment among Polish centenarians. Prion 2013; 7: 244–7.
He J, Li X, Yang J, Huang J, Fu X, Zhang Y, et al. The association between the methionine/valine (M/V) polymorphism (rs1799990) in the PRNP gene and the risk of Alzheimer disease: an update by meta-analysis. J Neurol Sci 2013; 326: 89–95.
Ikram MA, Brusselle GGO, Murad SD, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2018 update on objectives, design and main results. Eur J Epidemiol 2017; 32: 807–50.
Ikram MA, van der Lugt A, Niessen WJ, Koudstaal PJ, Krestin GP, Hofman A, et al. The Rotterdam Scan Study: design update 2016 and main findings. Eur J Epidemiol 2015; 30: 1299–315.
Jeong BH, Lee KH, Jeong YE, Huang KA, Lee YJ, Carp RI, et al. Polymorphisms at codons 129 and 219 of the prion protein gene (PRNP) are not associated with sporadic Alzheimer’s disease in the Korean population. Eur J Neurol 2007; 14: 621–6.
Kellett KA, Hooper NM. Prion protein and Alzheimer disease. Prion 2009; 3: 190–4.
Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al.; Alzheimer Disease Genetics Consortium (ADGC). Genetic meta-analysis of diagnosed Alzheimer’s disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. Nat Genet 2019; 51: 414–30.

Loh PR, Danecek P, Palamara PF, Fuchsberger C, Ar Y, H KF, et al. Reference-based phasing using the Haplotype Reference Consortium panel. Nat Genet 2016; 48: 1443–8.

Mok T, Jaunmuktane Z, Joiner S, Campbell T, Morgan C, Wakerley B, et al. Variant Creutzfeldt-Jakob disease in a patient with heterozygosity at PRNP codon 129. N Engl J Med 2017; 376: 292–4.

Niemeijer MN, van den Berg ME, Deckers JW, Aarnoudse AL, Hofman A, Franco OH, et al. ABCB1 gene variants, digoxin and risk of sudden cardiac death in a general population. Heart 2015; 101: 1973–9.

Parchi P, de Boni L, Saverioni D, Cohen ML, Ferrer I, Gambetti P, et al. Consensus classification of human prion disease histotypes allows reliable identification of molecular subtypes: an inter-rater study among surveillance centres in Europe and USA. Acta Neuropathol 2012; 124: 517–29.

Parchi P, Strammiello R, Notari S, Giese A, Langeveld JP, Ladogana A, et al. Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrPSc types: an updated classification. Acta Neuropathol 2009; 118: 659–71.

Poleggi A, Bizzarro A, Acciarri A, Antuono P, Bagnoli S, Cellini E, et al. Codon 129 polymorphism of prion protein gene in sporadic Alzheimer’s disease. Eur J Neurol 2008; 15: 173–8.

Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216: 136–44.

Rossi M, Kai H, Biaardi S, Bartoletti-Stella A, Carla B, Zenesini C, et al. The characterization of AD/PART co-pathology in CJD suggests independent pathogenic mechanisms and no cross-seeding between misfolded Abeta and prion proteins. Acta Neuropathol Commun 2019; 7: 53.

Rujescu D, Hartmann AM, Gonnermann C, Moller HJ, Giegling I. M129V variation in the prion protein may influence cognitive performance. Mol Psychiatry 2003; 8: 937–41.

Walker LC. Prion-like mechanisms in Alzheimer disease. Handb Clin Neurol 2018; 153: 303–19.

Zhang W, Jiao B, Xiao T, Pan C, Liu X, Zhou L, et al. Mutational analysis of PRNP in Alzheimer’s disease and frontotemporal dementia in China. Sci Rep 2016; 6: 38435.