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FEATURED ARTICLE

C-reactive protein levels and risk of dementia—Observational and genetic studies of 111,242 individuals from the general population

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Abstract

Introduction: Increased plasma levels of C-reactive protein (CRP) in midlife are associated with increased risk of Alzheimer's disease (AD), whereas in older age the opposite association is observed. Whether genetically determined CRP is associated with AD remains unclear.

Methods: A total of 111,242 White individuals from the Copenhagen General Population Study and the Copenhagen City Heart Study were included. Plasma levels of CRP and four regulatory genetic variants in the *CRP* gene were determined.

Results: For CRP percentile group 1 to 5 (lowest plasma CRP) versus the 50 to 75 group (reference), the hazard ratio for AD was 1.69 (95% confidence interval 1.29–2.16). Genetically low CRP was associated with increased risk of AD in individuals with body mass index ≤25 kg/m^2 ($P = 4 \times 10^{-6}$).

Discussion: Low plasma levels of CRP at baseline were associated with high risk of AD in individuals from the general population. These observational findings were supported by genetic studies.

KEYWORDS
Alzheimer's disease, body mass index, C-reactive protein, CRP, gene–environment interaction

1 | INTRODUCTION

Alzheimer's disease (AD) and other dementias are devastating neurodegenerative diseases affecting more than 47 million individuals. This number is estimated to increase 3-fold by 2050, mainly due to increased life expectancy.[1–3] At present there are no curative treatment options, and large fractions of the underlying biology are unknown. Interestingly, several discoveries have recently linked AD and inflammation.[4,5] Increased plasma levels of C-reactive protein (CRP) in midlife are associated with increased risk of AD,[6,7] whereas in older age the opposite association is observed.[8–10] Whether these associations are due to confounding and/or reverse causation, or whether CRP may be directly implicated in the development of AD is not known.

CRP is a well-known acute-phase reactant primarily produced in the liver, known to function as an opsonin, activate the complement system, and modulate leukocyte actions via Fc gamma receptor I and II (FCγRI and FCγRII).[11–13] Levels of CRP in cerebrospinal fluid (CSF) of individuals with intact blood–brain barrier (BBB) is highly correlated with plasma levels of CRP, albeit with lower concentrations as

would be anticipated,[14] and plasma levels of CRP may thus likely mimic brain CRP levels. CRP has been observed within neurofibrillary tangles and in amyloid plaques in brains of AD patients,[15,16] is produced locally in the brain, and is upregulated both at the mRNA and protein level in affected areas of AD brains.[17] Whether CRP may be directly implicated in the development and progression of AD or is a mere marker of underlying inflammatory processes remains to be established.

We tested the hypothesis that low plasma levels of CRP are associated with increased risk of AD and all-cause dementia. We further tested whether genetic variants in CRP—associated with low CRP levels—were associated with risk of dementia, thereby addressing whether a lifelong modest decrease in CRP contributes to the development of dementia.[18] For this purpose, we studied 111,242 individuals from the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS), all with baseline plasma CRP measurements. Of these individuals 104,672 were genotyped for rs3093077, rs1205, rs1130864, and rs3091244—four genetic variants within the *CRP* gene that compiled are reported to associate with up to a 64% change in plasma levels of CRP,[19] and that together describe full haplotype diversity in people of European descent.[20] Individuals were followed for up to 27 years for development of AD and all-cause dementia.

RESEARCH IN CONTEXT

1. **Systematic review**: We searched the PubMed database for articles published from January 1, 1980 to April 27, 2021 using the following search terms: "dementia," "Alzheimer," "C-reactive protein," and "CRP." Increased plasma levels of C-reactive protein (CRP) in midlife are associated with increased risk of Alzheimer's disease (AD), whereas in older age the opposite association is observed.
2. **Interpretation**: We found that low baseline CRP was associated with high risk of AD in a large prospective general population study. The observational findings were supported by genetic studies in individuals with body mass index (BMI) ≤25 kg/m^2—a situation not confounded by high BMI and related metabolic disturbances. These genetic findings are novel.
3. **Future directions**: Our study highlights the importance of a balanced inflammatory response in preventing AD, and suggests that increased knowledge of inflammatory pathways will improve our understanding of the underlying causes of AD and thus qualify pathways for drug targeting.

HIGHLIGHTS

- Low plasma levels of C-reactive protein (CRP) associate with high risk of Alzheimer's disease (AD).
- Genetically low CRP associates with high risk of AD in persons with body mass index (BMI) ≤25 kg/m^2.
- BMI interacts with genetically determined CRP in predicting AD.

2 | METHODS

2.1 | Participants

Studies were approved by institutional review boards and Danish ethical committees and were conducted according to the Declaration of Helsinki. Written informed consent was obtained from all study participants. All participants were White and of Danish descent. There was no overlap of individuals between studies. Data from two large independent studies of the Danish general population were included: the CGPS and the CCHS.[21–24] Study participants were randomly selected from the Danish Civil Registration System to reflect the adult population aged 20 to 100+ years. The combined studies included a total of 111,242 individuals of whom 2027 developed AD, and 3459 developed all-cause dementia. These 111,242 individuals were included in the observational analysis and the first consecutive 104,672 individuals were included in genetic analyses. Among genotyped individuals 1981 developed AD and 3396 developed all-cause dementia.

2.2 | The Copenhagen General Population Study

This prospective study of the Danish general population was initiated in 2003 with enrollment until 2015 and with ongoing follow-up examinations.[19,21–24] Data collection included a questionnaire, a physical examination, and blood sampling for biochemical analysis and DNA extraction. We included 101,292 consecutive individuals in the current analyses; among these 1570 developed AD and 2413 developed all-cause dementia.

2.3 | The Copenhagen City Heart Study

This prospective study of the Danish general population was initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003.[19,21–22] Participants were recruited and examined as in the CGPS. We included 9950 individuals who gave blood for CRP and other biochemical measurements as well as DNA analysis at the 1991 to 1994 or the 2001 to 2003 examinations; among these 457 developed AD and 1046 developed all-cause dementia.

2.4 | Endpoints

Information on diagnoses of AD and all-cause dementia was collected from the national Danish Patient Registry with data on all patient

contacts from all clinical departments in Denmark since 1977, including emergency wards and outpatient clinics since 1995. Data were also collected from the national Danish Causes of Death Registry, with data on all causes of deaths in Denmark, as reported by hospitals and general practitioners since 1977. AD was World Health Organization International Classification of Disease (ICD) 8 290.10 and ICD10 F00 and G30. All-cause dementia also included vascular dementia (ICD10 F01) and unspecified dementia (ICD8 290.18 ICD10 F03). The AD and all-cause dementia diagnoses from the Danish registry have high validity.[25,26] Follow-up began at time of blood sampling and ended at the occurrence of event, death, emigration, or on December 13, 2018 (the last update from the registries), whichever came first. Median follow-up for all-cause dementia and AD was 10 years (range: < 1–27) for both the observational and genetic analyses, with no losses to follow-up.

2.5 | Biochemical and genetic analyses

Plasma total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were determined as previously described.[24] Plasma CRP levels were determined using high-sensitivity turbidimetry (Dako) or nephelometry (Dade Behring) assays according to manufacturers' protocols as previously described.[19,27] Levels of CRP were measured in 9950 individuals from the CCHS 1991 to 1994 and 2001 to 2003 examinations, and in 101,292 individuals from the CGPS.

An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) was used to genotype four genetic variants in *CRP*, known to associate with CRP plasma levels: rs3093077, rs1205, rs1130864, and rs3091244.[19,20,27,28] Genotypes have been confirmed by DNA sequencing in more than 30 individuals with each genotype. Apolipoprotein E (*APOE*) was genotyped using rs429358 (c.388T > C) defining the $\varepsilon 4$ allele and rs7412 (c.526C > T) defining the $\varepsilon 2$ allele as previously described.[21,26] All four *CRP* variants were combined and used to generate two genetic instruments. The first genetic instrument was calculated for each individual using a weighted sum of *CRP* lowering alleles,[29,30] and divided into five reasonably sized groups. This genetic instrument was named the "weighted allele score groups" and coded 1 to 5 with 5 including most CRP-lowering alleles and as such the lowest CRP plasma level. The weights correspond to the sum of the individual β-coefficients for CRP-lowering alleles in each individual obtained from linear regression analysis accounting for the impact of the other *CRP* variants (Table S1 in supporting information). In this way we adjusted for linkage disequilibrium (LD) among the *CRP* variants, and at the same time captured the contributions beyond what was harbored by other variants due to LD. The second genetic instrument was a simple counting of CRP-lowering alleles in each individual; the instrument was divided into five reasonably sized groups and was named "simple allele score groups."

2.6 | Other covariates

Body mass index (BMI) was measured using weight in kilograms divided by height in meters squared. Hypertension was defined as use of antihypertensive medication, systolic blood pressure ≥140 mm Hg, and/or diastolic blood pressure of ≥90 mm Hg. Diabetes was defined as either self-reported disease, use of insulin, or oral hypoglycemic agents and/or non-fasting plasma glucose > 11 mmol/L (> 198 mg/dL). Smoking was defined as current smoking. Alcohol consumption was defined as > 14/21 U per week women/men (1 U = 12 g alcohol, equivalent to one glass of wine or spirit or one beer [33cl]). Physical inactivity was defined as ≤4 hours of light physical activity in leisure time per week. Menopausal status was self-reported, as was the use of hormonal replacement therapy. Lipid-lowering therapy was defined as use of lipid-lowering therapy (yes/no), and was primarily statins. Low education was defined as ≤8 years.

2.7 | Statistical analysis

We used Stata/S.E. version 15.1 (Stata Corp-). Missing data on continuous covariates were imputed from sex, age, and closely related continuous parameters; nonetheless, results were similar without imputed data. *P*-values < 0.0001 are given as powers of 10. Mann-Whitney U test and Pearson's χ^2 test were used in two-group comparisons of continuous and categorical variables. Subjects were coded by plasma CRP level into six groups with the group containing the median plasma CRP value serving as the reference. To account for age and sex differences, plasma CRP percentile groups were generated in groups stratified by sex and age (using age groups 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and ≥80 years) and combined into six percentile groups (1–5%, 5–25%, 25–50%, 50–75%, 75–95%, 95–100%). Other covariates were adjusted for in the Cox regression models detailed below.

First, in observational analyses between plasma levels of CRP and risk of AD and all-cause dementia we used Cox proportional hazards regression models with age as time-scale and delayed entry (left truncation) to estimate hazard ratios (HR) with 95% confidence intervals (CIs); with this approach age is automatically adjusted for. Models were multifactorially adjusted for known risk factors described in the previous paragraph. On a continuous scale, the associations between plasma levels of CRP and AD and all-cause dementia were evaluated using restricted cubic splines. Three knots were chosen to balance best fit and overfitting.[31] Second, to test whether genetic variants were associated with plasma levels of CRP we used Cuzik's extension of a Wilcoxon rank sum test. Third, we examined the association between the weighted/simple allele score groups and risk of AD and all-cause dementia, using multifactorially adjusted Cox proportional hazards regression models. Interaction between genetically determined CRP and covariates in predicting risk of AD or all-cause dementia were evaluated by inclusion of two-factor interaction terms in the

TABLE 1 Baseline characteristics of individuals in the Copenhagen General Population Study and the Copenhagen City Heart Study by percentiles of C-reactive protein

	C-reactive protein, percentiles					
	1–5	>5–25	>25–50	>50–75	>75–95	>95–100
CRP (mg/L)	0.21 (0.12–.31)	0.65 (0.49–.80)	1.18 (1.07–1.28)	1.69 (1.52–1.92)	3.27 (2.66–4.33)	10.08 (7.92–5.41)
No. of individuals	4456	22,198	27,746	27,716	22,188	6656
Age, years	51 (44–61)	56 (47–65)	56 (47–65)	59 (48–67)	61 (50–69)	62 (51–71)
Female (%)	55.2	55.6	55.6	55.6	55.6	55.6
Total cholesterol (mmol/L)	5.3 (4.6—6.0)	5.4 (4.8–6.2)	5.5 (4.8–6.3)	5.7 (5.0–6.4)	5.7 (5.0–6.5)	5.5 (4.8–6.3)
LDL cholesterol (mmol/L)	3 (2.4–3.6)	3.1 (2.5–3.7)	3.2 (2.6–3.8)	3.3 (2.7—4.0)	3.3 (2.7–4.1)	3.2 (2.6–3.9)
HDL cholesterol (mmol/L)	1.69 (1.39–2.05)	1.67 (1.34–.04)	1.61 (1.30–1.99)	1.52 (1.22–2.19)	1.44 (1.15–1.80)	1.40 (1.10–1.75)
Triglycerides (mmol/L)	1.0 (0.8–1.5)	1.2 (0.9–1.8)	1.3 (0.9–1.9)	1.5 (1.0–2.2)	1.7 (1.2–2.4)	1.6 (1.1–2.3)
ApoE (mg/dL)	3.7 (3.0–4.5)	3.9 (3.2–4.7)	4.0 (3.3–4.8)	4.2 (3.5–5.1)	4.3 (3.5–5.3)	4.2 (3.5–5.2)
Body mass index (kg/m^2)	23 (21.3–25)	24.3 (21.3–25)	24.7 (22.6–27.1)	26.2 (23.8–28.8)	27.5 (24.7–30.7)	27.6 (24.5–31.4)
Hypertension (%)	41.4	52.6	53.9	62.8	69.6	69.3
Diabetes (%)	2	2.9	3	3.7	5.6	7.8
Smoking (%)	14.5	14.2	17	20.6	26.6	29.7
Alcohol consumption (%)	14.1	15.1	16.5	18.7	18.8	17.7
Physical inactivity (%)	37.5	42.5	44.8	51.5	59.4	64.8
Lipid-lowering therapy (%)	6.9	11.1	10.3	11.2	11.9	11.5
Low education, ≤8 years (%)	4.9	7.4	8.6	12.1	17.6	20.4

Notes: Values are median (interquartile range) or percentage and are from the day of enrollment. Hypertension was defined as use of antihypertensive medication, systolic blood pressure of ≥140 mmHg, and/or diastolic blood pressure of ≥90 mmHg. Diabetes was defined as self-reported disease, use of insulin or oral hypoglycemic agents, and/or nonfasting plasma glucose level of > 11 mmol/L (> 198 mg/dL). Smoking was defined as current smoking. High alcohol consumption was defined as > 14/21 U per week for women/men (1U = 12 g alcohol, equivalent to 1 glass of wine or spirit or 1 beer [33cl]). Physical inactivity was defined as ≤4 hours per week of light physical activity in leisure time. Women reported menopausal status and use of hormonal replacement therapy. Lipid-lowering therapy was primarily statins (yes/no), and low education was defined as ≤8 years.
Abbreviations: CRP, C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein;

Cox regression model, using a likelihood ratio test between models excluding and including the interaction term.

3 | RESULTS

3.1 | Baseline characteristics

Baseline characteristics of the 111,242 study participants divided into six percentile groups of plasma CRP levels are shown in Table 1. Individuals in the lowest CRP percentile group were younger, had lower BMI, had higher education, and were less likely to be physically inactive, have diabetes, or to receive hormone replacement therapy. We found no interaction between plasma levels of CRP and study cohort in predicting AD (*P* for interaction = 0.92). Consequently, all further analyses were performed on the studies combined.

3.2 | Plasma levels of CRP and risk of dementia: Observational estimate

The distribution of plasma CRP, color coded by percentile groups, is shown in Figure S1A in supporting information and plasma CRP levels as a function of *APOE* genotypes are shown in Figure S1B.

Multifactorially adjusted restricted cubic spline Cox regression models evaluated risk of AD and all-cause dementia by plasma CRP levels, further adjusted for *APOE* genotype. Risk of AD and all-cause dementia was inversely associated with plasma levels of CRP (Figure 1). These associations remained after adjustment for plasma lipids (LDL cholesterol, HDL cholesterol, and triglycerides) and *APOE* genotype. For percentile group 1 to 5 (lowest plasma CRP) versus the 50 to 75 group (reference), HRs were 1.69 (95% CI 1.29–2.16) for AD and 1.60 (1.29–1.97) for all-cause dementia (Figure 2). When excluding the first 2, 5, and 10 years of follow-up, when stratifying on sex, when adjusting

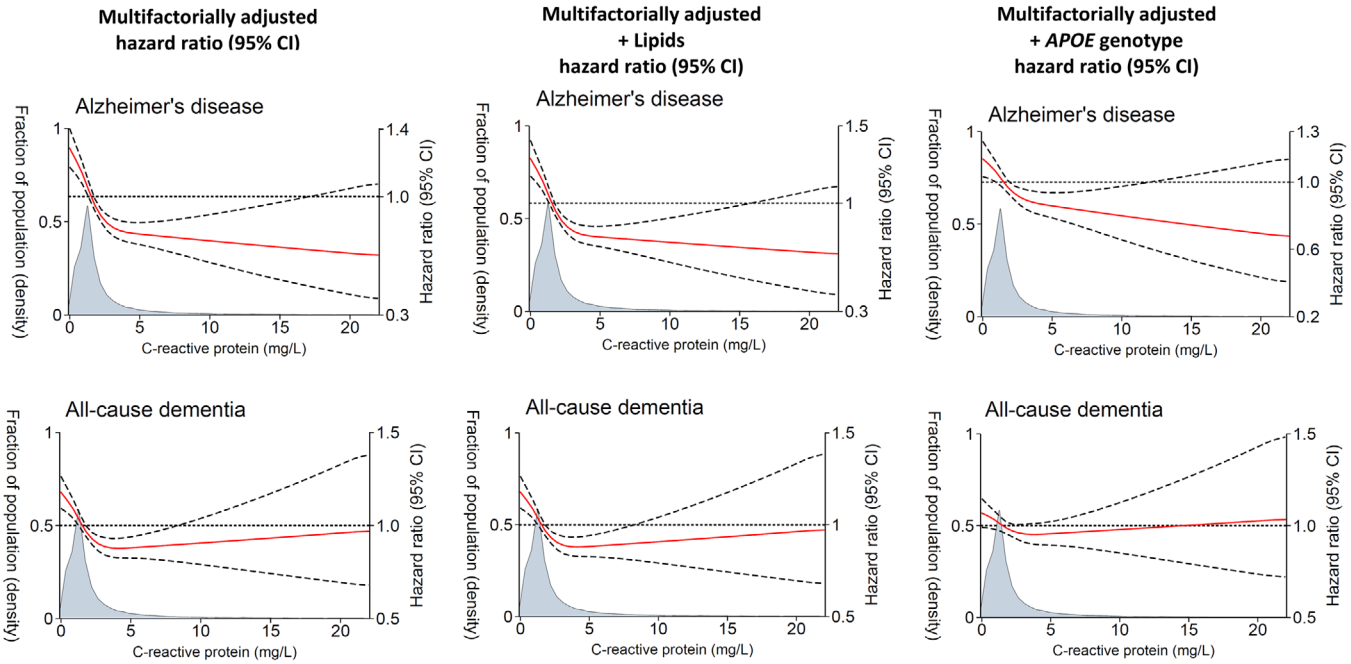

FIGURE 1 Restricted cubic splines illustrating risk of Alzheimer's disease and all-cause dementia as a function of plasma C-reactive protein (CRP) on a continuous scale. Solid lines are multifactorially adjusted hazard ratios, whereas dashed lines indicate 95% confidence intervals (CIs) derived from restricted cubic spline regressions with three knots. Graphs are truncated at 22.0 g/L, due to statistically unstable estimates at extremely high levels, thus including 109,926 individuals in these analyses. Cox regression models were adjusted for age (time scale), sex, body mass index, hypertension, diabetes, smoking, alcohol consumption, physical inactivity, menopausal status and hormonal replacement therapy (women only), lipid-lowering therapy, and education. *APOE*, apolipoprotein E

for exact measurement time of plasma CRP concentrations, or when adjusting for disease endpoints with inflammatory components, the inverse association between plasma CRP levels and risk of AD and all-cause dementia remained (Figures S2-S6 in supporting information).

3.3 | Genotype, plasma levels of CRP, and risk of dementia

Because weighted/simple allele score groups interacted with BMI in predicting risk of AD and all-cause dementia (*P* for interaction = 3×10^{-4} and 4×10^{-3}; Figures 3 and S7 in supporting information), we stratified all genetic analyses by BMI ≤25 kg/m^2 and BMI > 25 kg/m^2. CRP levels decreased with stepwise increasing weighted/simple allele score groups, with up to −31% from group 1 (highest CRP level) to group 5 (lowest CRP level; all *P* for trends < 8×10^{-138}; Figure 4). In individuals with BMI ≤25 kg/m^2 weighted/simple allele score groups were associated with increased risk of AD (*P* for trends = 4×10^{-6}; Figure 4), with similar results for all-cause dementia (Figure S8 in supporting information). In contrast, the associations disappeared in individuals with BMI > 25 kg/m^2 (Figures 4 and S8). In individuals with BMI ≤25 kg/m^2, HRs for weighted/simple allele score group 5 (lowest CRP) versus 1 (reference) were 1.93 (1.50–2.48) for AD and 1.43 (1.18–1.73) for all-cause dementia. Results remained after adjustment for *APOE* genotype (Figures 4 and S8).

4 | DISCUSSION

The principal findings of this study are that baseline low plasma CRP levels were associated with high risk of AD and all-cause dementia. A weighted score of CRP decreasing alleles interacted with BMI in predicting risk, and genetically low plasma CRP was associated with high risk of dementia in individuals with BMI ≤25 kg/m^2. The observation that the genetic associations were only present in individuals with BMI ≤25 kg/m^2—where the genetic association with CRP levels is not abolished by high BMI and related metabolic disturbances—suggests that lifelong moderate decreases in plasma levels of CRP may be implicated in the etiology of AD and all-cause dementia. These findings were observed in White individuals of Danish descent, and the observations should be interpreted with this homogeneity in mind. The findings are novel.

Several observational studies have investigated the association between plasma levels of CRP and risk of AD; however, the nature of these associations remains elusive.[6,7,9,10,32,33] Midlife elevated plasma CRP levels[6,7] as well as late-life decreased plasma CRP levels were reported to be associated with AD,[9,34] whereas other studies as well as meta-analyses found no association between plasma levels of CRP and risk of AD.[32,33] High CRP levels were reported to be associated with low cortical amyloid beta (Aβ) levels,[35] and CRP levels in CSF were lower in AD patients and in individuals with mild cognitive impairment that subsequently developed AD.[34,36] These studies are however

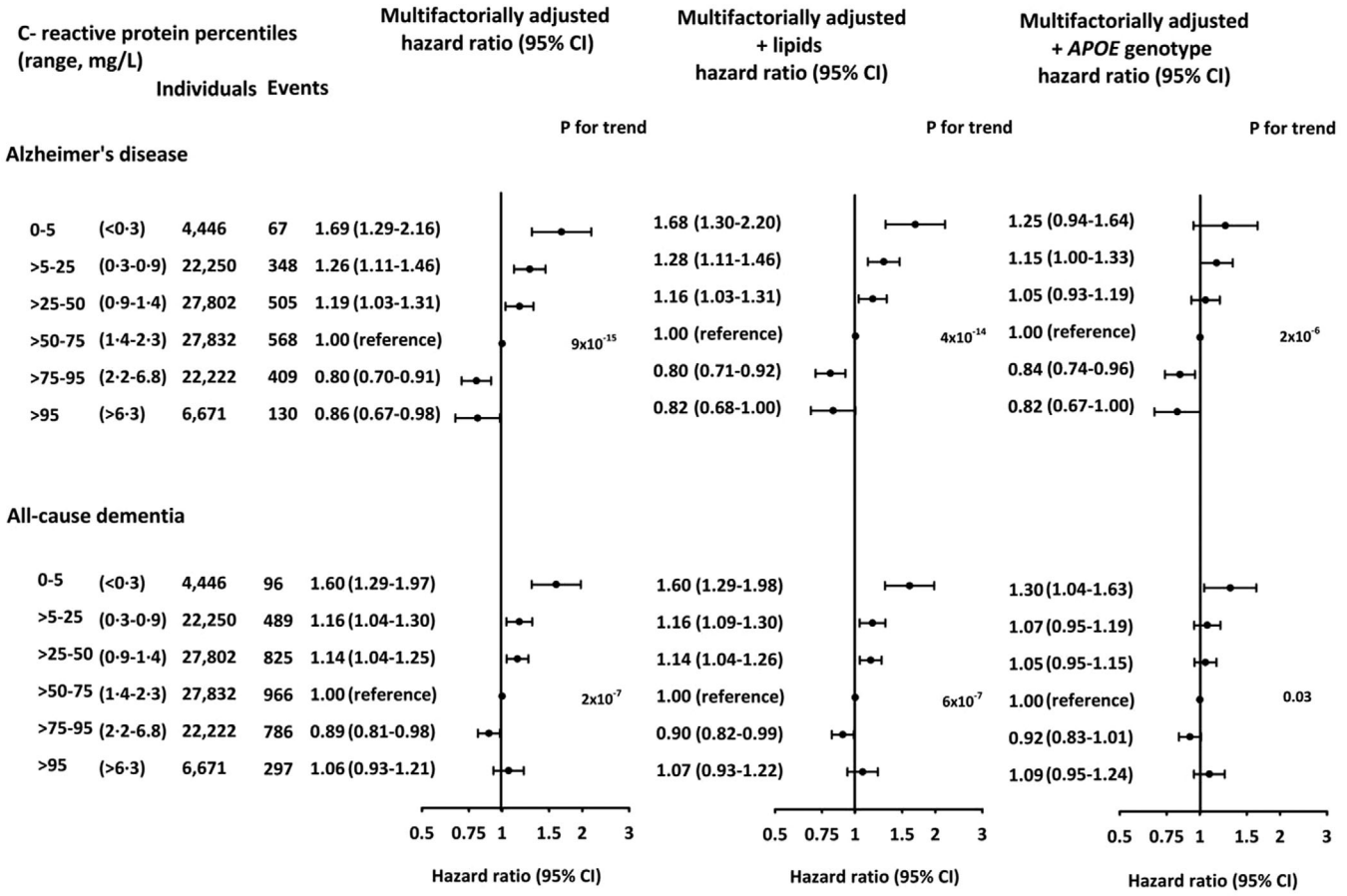

FIGURE 2 Plasma levels of C-reactive protein in percentile groups and risk of Alzheimer's disease and all-cause dementia. Hazard ratios were multifactorially adjusted for age (as time scale), sex, body mass index, hypertension, diabetes mellitus, smoking, alcohol consumption, physical inactivity, menopausal status and hormonal replacement therapy (women only), lipid-lowering therapy, and education. The middle column was additionally adjusted for total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides. The right column was additionally adjusted for *APOE* genotype. *APOE*, apolipoprotein E

moderate in size and do not have a long follow-up period, in contrast to the present study in which median follow-up was 10 years with up to 27 years of maximum follow-up time. Interestingly, our observational results remained after excluding up to 10 years of follow-up time, indicating that the present associations are not likely influenced by reverse causation. Recent large Mendelian randomization studies did not find associations between genetically determined CRP levels in plasma and risk of AD.[37,38] These studies were however based on summary level data without the possibility of testing for interaction with a spectrum of possible confounders, and thus unable to detect context-dependent effects, as in the present article with BMI. Three genetic variants used in the present study (rs3093077, rs1205, rs1130864) obtain full haplotype diversity in people of European descent.[20] We additionally included the triallelic variant (rs3091244)[19,28] to ensure the best possible coverage of the *CRP* locus. These common genetic variants are located in regulatory regions of the gene and have reliably been associated with differences in plasma CRP levels, and importantly not with any known change in CRP function.[20] As they only impact plasma CRP quantitatively, and not qualitatively, they are optimal to use as genetic instruments for plasma levels of CRP.

There is considerable evidence from the literature that BMI causally affects plasma levels of CRP.[39] We tested systematically for interaction between genetically determined CRP and 17 potential confounders and observed a statistically significant interaction for BMI in predicting dementia. Consequently, we stratified the analyses on BMI $\leq$ 25 kg/m^2 and > 25 kg/m^2. Interestingly, genetically low CRP was associated with increased risk only in individuals with BMI $\leq$ 25 kg/m^2. These findings suggest that in individuals with BMI $\leq$25 kg/m^2, plasma CRP is not elevated through low-grade inflammation caused by adiposity and related metabolic changes. Consequently, we observe the associations between the genetic variants and plasma levels of CRP more clearly, whereas in individuals with BMI > 25 kg/m^2 low-grade inflammation is present and tends to abolish the genetic associations.[40]

CRP is present in CSF of non-demented individuals with intact BBB, and CSF and plasma CRP levels are highly correlated. Thus a potential mechanistic role for CRP in AD pathogenesis is likely.[14] CRP is located within amyloid plaques and neurofibrillary tangles in brains of AD patients,[15,16] and seems to be produced locally by pyramidal neurons in affected areas.[17] CRP activates the classical complement pathway through binding with C1q,[41] and defects in the complement system are

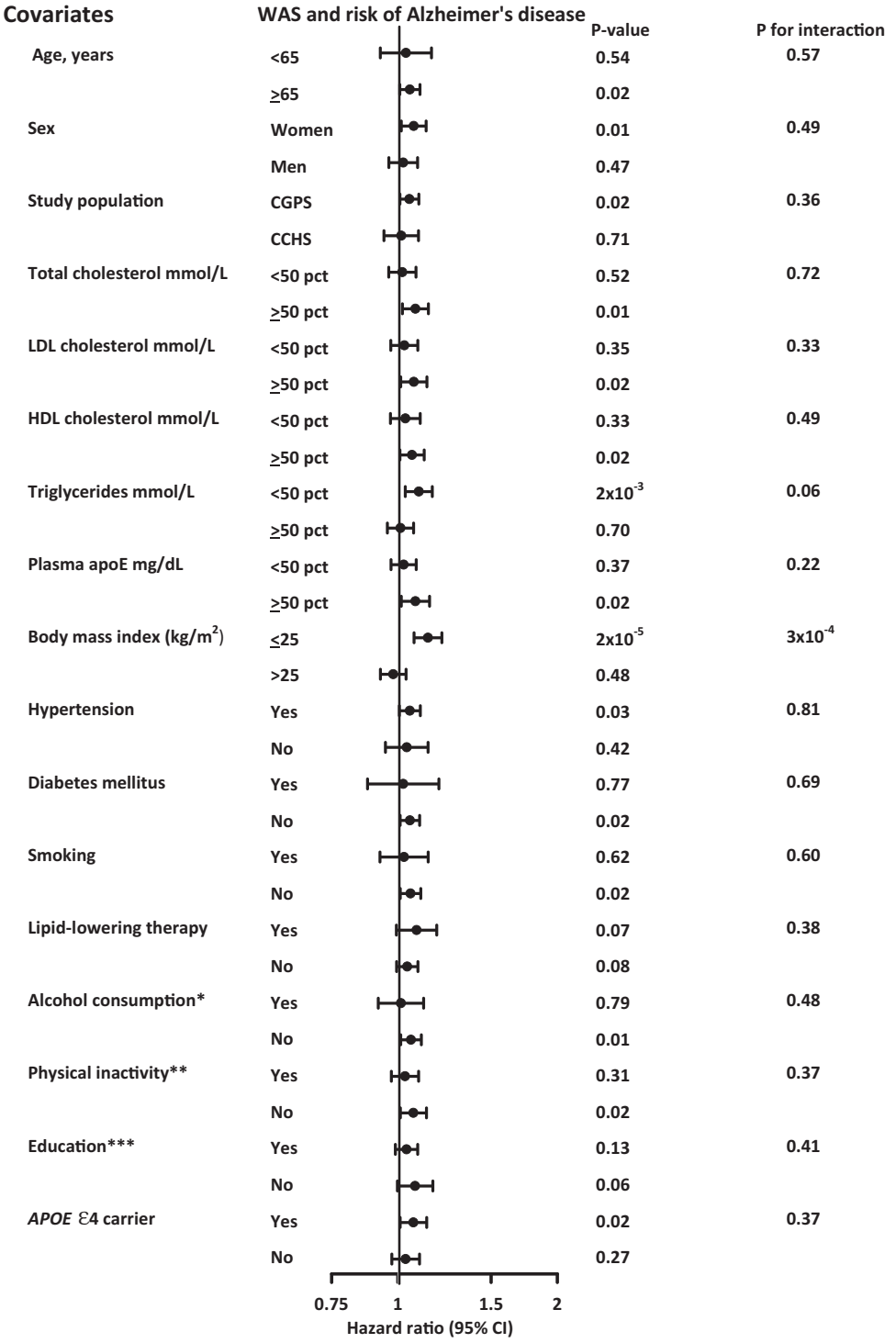

FIGURE 3 Interaction of potential confounders with weighted allele score groups in predicting risk of Alzheimer's disease. Potential confounders were dichotomized: Age (> 65 vs. ≤65 years); sex (men vs. women); study population (CGPS vs. CCHS); total cholesterol (≥ 50 pct vs. < 50 pct); LDL cholesterol (≥ 50 pct vs. < 50 pct); HDL cholesterol (≥ 50 pct vs. < 50 pct); triglycerides (≥ 50 pct vs. < 50 pct); plasma apoE (≥ 50 pct vs. < 50 pct); body mass index (> 25 vs ≤ 25); hypertension (use of antihypertensive medication, systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg vs. systolic blood pressure < 140 mm Hg and diastolic blood pressure < 90 mm Hg and no antihypertensive medication); diabetes (self-reported diabetes, use of insulin or oral hypoglycemic agents, and/or nonfasting plasma glucose level > 11 mmol/L vs. no diabetes); smoking (current smoking vs. no current smoking); lipid-lowering therapy (use of lipid-lowering therapy vs. no lipid-lowering therapy); alcohol consumption (> 14/21 vs. ≤14/21 U per week for women/men, with 1 U = 12 g alcohol, equivalent to 1 glass of wine or spirit or 1 beer [33 cl]); physical inactivity (≤4 vs. > 4 hours per week of light physical activity in leisure time); education (< 8 vs. ≥8 years), *APOE* ε4 carrier (yes vs. no). *APOE*, apolipoprotein E; CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CI, confidence interval; HDL, high density lipoprotein; LDL, low density lipoprotein; pct, percentile; WAS, weighted allele score

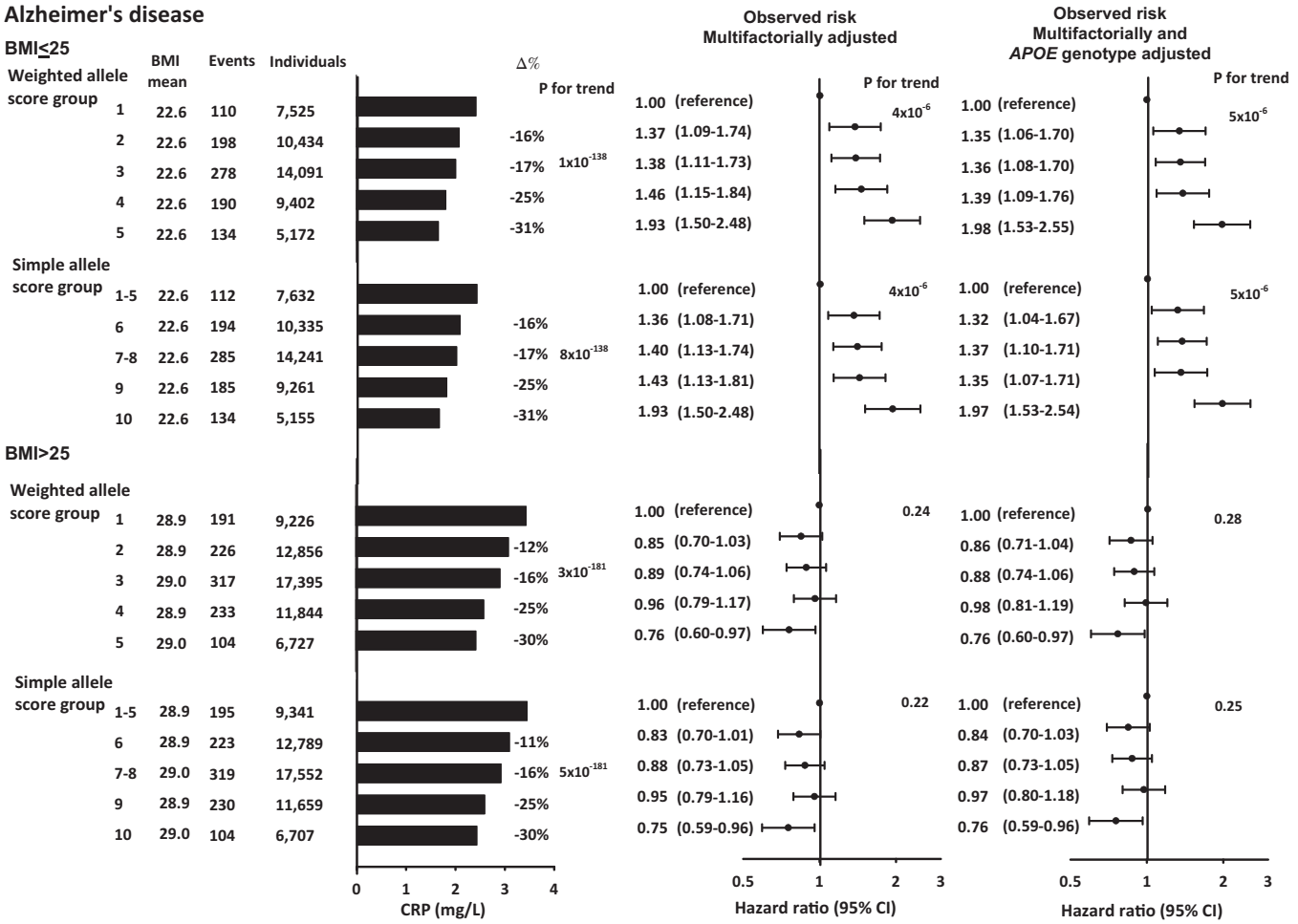

FIGURE 4 Plasma levels of C-reactive protein and hazard ratios of Alzheimer's disease as a function of weighted/simple allele score groups stratified by BMI at or below 25 kg/m^2 or BMI above 25 kg/m^2. Hazard ratios were multifactorially adjusted for age (as time scale), sex, hypertension, diabetes mellitus, smoking, alcohol consumption, physical inactivity, menopausal status and hormonal replacement therapy (women only), lipid-lowering therapy, and education. The right column was additionally adjusted for *APOE* genotype. *APOE*, apolipoprotein E; BMI, body mass index (kg/m^2); CI, confidence interval; CRP, C-reactive protein

reported to be associated with increased risk of AD in both genome-wide associations studies[5,42] and in population-based large prospective cohorts.[43] Mechanistically, we suggest that low CRP levels lead to decreased opsonization and decreased phagocytosis of Aβ by microglia as well as decreased activation of the complement system resulting in a less efficient clearance of Aβ. Several observational studies have found *APOE* genotype to be associated with plasma levels of CRP and the *APOE* ε4 carriers to have the lowest CRP levels among *APOE* genotypes.[44,45] This was replicated and extended in the present study, in which we found a gene dosage effect per *APOE* ε4 allele on lowering of CRP. Regardless of this, the association between low plasma CRP and risk of AD remained after adjusting for *APOE* genotype. Interestingly, apoE was recently reported to attenuate unresolvable inflammation by complex formation with activated C1q.[46] As CRP activates C1q and as apoE binds to C1q it may be these mutual interactions that somehow explain the known association between the ε4 allele and plasma CRP levels. Finally, Mendelian randomization studies have shown CRP to be causally associated with psychiatric disorders—low CRP with schizophrenia and high CRP with bipolar disorder—thus supporting that CRP may play a mechanistic role in disorders within the CNS.[37]

Strengths of our study include the large prospective general population design with no losses to follow-up and with plasma CRP measurements preceding the AD diagnosis. Furthermore, as the clinical diagnosis of AD is preceded by biomarker changes many years in advance, with Aβ marker being abnormal up to 20 years before onset of clinical symptoms, this study design using a general population cohort represents the most robust evaluation of the association between baseline measurement of CRP and development of AD without conditioning on the future event of AD onset. A potential limitation concerns the availability and completeness of the diagnostic information; however, the Danish Patient Registry includes all hospital visits as well as out-patient visits. It has previously been shown that the dementia diagnoses in the Danish Registries has high diagnostic validity with 85.8% of overall dementia and 81.0% of AD being correctly assigned, whereas other less frequent dementia subtypes had low kappa scores.[25,26] Further, the use of two different methods for analyzing plasma CRP

concentrations or the presence of disease endpoints with inflammatory components could have introduced some error. After adjusting for exact analysis time for each sample and for a range of inflammatory and infectious disease endpoints, findings were however similar to the main analysis, suggesting that bias due to these issues was not of major importance. Finally, as we studied White individuals of Danish descent only, these results may not be applicable to other ethnicities. We cannot dismiss the possibility that our results are chance findings; however, the strong observational results and the fact that the association remained significant after excluding up to 10 years of follow-up combined with corresponding genetic findings argues against this.

In conclusion, low baseline CRP was associated with high risk of AD and all-cause dementia in individuals from the general population, and the associations remained significant after excluding up to 10 years of follow-up. These observational findings were supported by genetic studies in individuals with BMI $\leq$25 kg/m^2—where the genetic associations with plasma levels of CRP are not confounded by high BMI and related metabolic disturbances—and as such may suggest that modest lifelong decreases in CRP may be implicated in AD etiology.

AUTHOR CONTRIBUTIONS

Sharif H. Hegazy: study concept and design, acquisition of data, statistical analysis, analysis and interpretation of data, validation of underlying data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, final approval for submission. Jesper Qvist Thomassen: statistical analysis, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, final approval for submission. Ida Juul Rasmussen: statistical analysis, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, final approval for submission. Børge G. Nordestgaard: acquisition of data; critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical, and material support; final approval for submission. Anne Tybjærg-Hansen: acquisition of data; critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical, and material support; final approval for submission. Ruth Frikke-Schmidt: study concept and design; acquisition of data; statistical analysis; analysis and interpretation of data; validation of the underlying data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical, and material support; study supervision; final approval for submission; accountable for all aspects of the work.

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CONFLICTS OF INTEREST

SHH, JQT, IJR, and ATH have nothing to disclose. BGN received consulting fees (personal fees) from AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Kowa, Denka, Amarin, Novartis, Novo Nordisk, Esperion, Silence Therapeutics. RFS received consulting fees (personal fees) from Novo Nordisk.

REFERENCES

1. Prince PM, Wimo A, Guerchet MM, Ali GC, Wu YT, Prina M, *World Alzheimer Report 2015 - the global impact of dementia. An analysis of prevalence, incidence, and cost and trends*. London: Alzheimer's Disease International 2015:84.
2. Prince M, Ali G, Guerchet M, Prina AM, Albanese E, Wu Y. Recent global trends in the prevalence and incidence of dementia, and survival with dementia. *Alzheimers Res Ther*. 2016;8:23.
3. Satizabal CL, Beiser AS, Chouraki V, Chêne G, Dufouil C, Seshadri S. Incidence of dementia over three decades in the Framingham Heart Study. *N Engl J Med*. 2016;374:523-532.
4. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet*. 2019;51:414-430.
5. Lambert J-C, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet*. 2013;45:1452-1458.
6. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. Early inflammation and Dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. *Ann Neurol*. 2002;52:168-174.
7. Tao Q, Ang TFA, DeCarli C, et al. Association of chronic low-grade inflammation with risk of Alzheimer's disease in *ApoE4* carriers. *JAMA Netw Open*. 2018;1:e183597.
8. Gong C, Wei D, Wang Y, et al. A meta-analysis of C-reactive protein in patients with Alzheimer's disease. *Am J Alzheimers Dis Other Demen*. 2016;31:194-200.
9. Haan MN, Aiello AE, West NA, Jagust WJ. C-reactive protein and rate of dementia in carriers and non carriers of apolipoprotein APOE4 genotype. *Neurobiol Aging*. 2008;29:1774-1782.
10. Gabin JM, Saltvedt I, Tambs K, Holmen J. The association of high sensitivity C- reactive protein and incident Alzheimer's disease in patients 60 years and older: the HUNT study, Norway. *Immun Ageing*. 2018;15:4.
11. Perea JR, Lorens-Martín M, Ávila J, Bolós M, Sergeant N. The role of microglia in the spread of tau: relevance for tauopathies. *Front Cell Neurosci*. 2018;12:172.
12. Jimenez RV, Wright TT, Jones NR, Wu J. C-reactive protein impairs dendritic cell development, maturation, and function: implications for peripheral tolerance. *Front Immunol*. 2018;9:372.
13. Tanigaki K, Sundgren N, Khera A, Vongpatanasin W, Mineo C, Shaul PW. Fcγ receptors and ligands and cardiovascular disease. *Circ Res*. 2015;116:368-384.
14. Coccaro EF, Lee R, Coussons-Read M. Cerebrospial fluid and plasma C-reactive protein and aggression in personality disordered subjects: a pilot study. *J Neural Transm*. 2016;122:321-326.
15. Iwamoto N, Nishiyama E, Ohwada J, Arai H. Demonstration of CRP immunoreactivity in brains of Alzheimer's disease: immunohistochemical study using formic acid pretreatment of tissue sections. *Neurosci Lett*. 1994;177:23-26.
16. Duong T, Nikolaeva M, Acton PJ. C-reactive protein-like immunoreactivity in the neurofibrillary tangles of Alzheimer's disease. *Brain Res*. 1997;749:152-156.
17. Yasojima K, Schwab C, Mcgeer EG, Mcgeer PL. Human neurons generate C-reactive protein and amyloid P: upregulation in Alzheimer's disease. *Brain Res*. 2000;887:80-89.

18. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;24:1133-1163.
19. Zacho J, Tybjærg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med*. 2008;359:1897-1908.
20. CRP CHD Genetics Collaboration. Collaborative pooled analysis of data on C-reactive protein gene variants and coronary disease: judging causality by Mendelian randomization. *Eur J Epidemiol* 2008; 23: 531-540.
21. Rasmussen KL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma levels of apolipoprotein E, APOE genotype, and all-cause and cause-specific mortality in 105,949 individuals from a white general population cohort. *Eur Heart J*. 2019;40:2813-2824.
22. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-function mutations in *APOC3* and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32-41.
23. Frikke-Schmidt R, Nordestgaard BG, Stene MCA, et al. Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. *JAMA*. 2008;299:2524-2532.
24. Rasmussen KL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma levels of apolipoprotein E and risk of dementia in the general population. *Ann Neurol*. 2015;77:301-311.
25. Phung TKT, Andersen BB, Høgh P, Kessing LV, Mortensen PB, Waldemar G. Validity of dementia diagnoses in the Danish hospital registers. *Dement Geriatr Cogn Disord*. 2007;24:220-228.
26. Rasmussen KL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R. Plasma apolipoprotein E levels and risk of dementia: a Mendelian randomization study of 106,562 individuals. *Alzheimers Dement*. 2018;14:71-80.
27. Allin KH, Nordestgaard BG, Zacho J, Tybjærg-Hansen A, Bojesen SE. C-reactive protein and the risk of cancer: a mendelian randomization study. *J Natl Cancer Inst*. 2010;102:202-206.
28. Kathiresan S, Larson MG, Vasan RS, et al. Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. *Circulation*. 2006;113:1415-1423.
29. Talmud PJ, Shah S, Whittall R, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. *Lancet*. 2013;381:1293-1301.
30. Haase CL, Tybjærg-Hansen A, Nordestgaard BG. Frikke-Schmidt R. HDL cholesterol and risk of type 2 diabetes: a mendelian randomization study. *Diabetes*. 2015;64:3328-3333.
31. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med*. 1989;8:551-561.
32. Tan ZS, Beiser AS, Vasan RS, et al. Inflammatory markers and the risk of Alzheimer's disease. *Neurology*. 2007;68:1902-1908.
33. Darweesh SKL, Wolters FJ, Ikram MA. Inflammatory markers and the risk of dementia and Alzheimer's disease: a meta-analysis. *Alzheimers Dement*. 2019;14:1450-1459.
34. Schuitemaker A, Dik MG, Veerhuis R, et al. Inflammatory markers in AD and MCI patients with different biomarker profiles. *Neurobiol Aging*. 2009;30:1885-1889.
35. Hooper C, De Souto P, Cantet C, et al. Chronically raised C-reactive protein is inversely associated with cortical β-amyloid in older adults with subjective memory complaints. *Exp Gerontol*. 2018;108:226-230.
36. Brosseron F, Traschütz A, Widmann CN, et al. Characterization and clinical use of inflammatory cerebrospinal fluid protein markers in Alzheimer's disease. *Alzheimers Res Ther*. 2018;10:25.
37. Ligthart S, Vaez A, Võsa U, et al. Genome Analyses of >200,000 individuals identify 58 loci for chronic inflammation and highlight pathways that link inflammation and complex disorders. *Am J Hum Genet*. 2018;103:691-706.
38. Larsson SC, Traylor M, Malik R, Dichgans M, Burgess S, Markus HS. Modifiable pathways in Alzheimer's disease: mendelian randomisation analysis. *BMJ*. 2017;359:j5375.
39. Timpson NJ, Nordestgaard BG, Harbord RM, et al. C-reactive protein levels and body mass index: elucidating direction of causation through reciprocal Mendelian randomization. *Int J Obes*. 2010;35:300-308.
40. Esposito K, Pontillo A, Di Palo C, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women. A randomized trial. *JAMA*. 2003;289:1799-1804.
41. Ma YJ, Garred P. Pentraxins in complement activation and regulation. *Front Immunol*. 2018;9:3046.
42. Lambert J-C, Heath S, Even G, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet*. 2009;41:1094-1099.
43. Rasmussen KL, Nordestgaard BG, Frikke-Schmidt R, Nielsen SF. An updated Alzheimer's hypothesis: complement C3 and risk of Alzheimer's disease–a cohort study of 95,442 individuals. *Alzheimers Dement*. 2018;12:1589-1601.
44. Chasman DI, Kozlowski P, Zee RY, Kwiatkowski DJ, Ridker PM. Qualitative and quantitative effects of APOE genetic variation on plasma C-reactive protein, LDL-cholesterol, and apoE protein. *Genes Immun*. 2006;7:211-219.
45. Marttinen M, Martiskainen H, Takalo M, et al. Decreased plasma C-reactive protein levels in APOE ε4 allele carriers. *Ann Clin Transl Neurol*. 2018;5:1229-1240.
46. Yin C, Ackermann S, Ma Z, et al. ApoE attenuates unresolvable inflammation by complex formation with activated C1q. *Nat Med*. 2019;25:496-506.

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