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Associations of gene sequence variation and serum levels of C-reactive protein and Interleukin-6 with Alzheimer's disease and dementia

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Abstract

Inflammatory mechanisms have been implicated in Alzheimer's disease (AD) and dementia. We therefore sought to study DNA sequence variation and serum levels of the potent inflammatory mediators Interleukin-6 (IL6) and C-reactive protein (CRP) in relation to AD and dementia.

Tagging single nucleotide polymorphisms (tagSNPs) were chosen to capture most variation in and around *CRP* and *IL6* in 3937 elderly Swedish men and women (1,265 AD cases). A subset of the population (N=723) with serum measurements of CRP and IL6 was included in A) a nested case-control study of incident dementia cases, and B) a case-control study of prevalent dementia cases. None of the SNPs or haplotypes was significantly associated with AD or dementia after correcting for multiple testing nor were elevated baseline levels of hsCRP or IL6 (measured on average 4.3 years before dementia onset) significantly associated with risk of future AD or dementia. However, prevalent AD cases had higher levels of IL6 (measured on average 5.5 years after dementia onset) than age- and sex-matched controls, OR 2.24 (95% CI 1.27–3.95), p-value 0.006.

In summary, this data suggests that AD patients have an altered immune profile with higher circulating levels of IL6 than age-and sex-matched controls. However, neither variation in the CRP and IL6 genes nor circulating levels of their respective protein products were associated with an increased risk of developing late-life dementias.

Keywords

Alzheimer disease; dementia; inflammation; interleukin-6; C-reactive protein; biological markers; candidate gene analysis; matched case-control studies; nested case-control studies

INTRODUCTION

Alzheimer's disease (AD) is a dementing disorder with a life-time risk of 10–20%, affecting one in eight individuals over 65 years of age [1]. The etiology of AD is still subject to debate but multiple findings have implicated inflammatory mechanisms as possible contributors to the development of the disease [2].

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Interleukin-6 (IL6) and C-reactive protein (CRP) are inflammatory proteins that are secreted upon infection or tissue injury. IL6 is a key regulatory cytokine [2] and is also the main regulator of the acute-phase reactant CRP. CRP and IL6 have been found in association with senile plaques and neurofibrillary tangles in subjects with AD [2] and to be elevated in temporal cortex [3]. The degree to which inflammation in general, or levels of circulating markers of inflammation in particular, is a causal agent in the development of AD or simply reflects a response to the developing disease remains an issue of current research.

Twin studies have shown that genetic variation is of considerable importance for the development of AD. Heritability ranges between approximately 60 and 80% [4] indicating that genes account for the majority of the variation in the liability to AD in the population. Whereas early-onset familial AD (which represents less than 1% of all AD cases) is caused in any individual by one of several autosomal dominant genes, sporadic AD is assumed to be caused by many common variations in a large number of genes with low penetrance [5]. Among this presumed large number of genes, the ε4 variant of the gene coding for apolipoprotein E, *APOE*, is the only susceptibility gene consistently linked to AD [6], and a considerable proportion of the genetic risk remains unaccounted for. IL6 and CRP are good candidates for involvement in AD and dementia development. Besides biological plausibility, circulating levels of IL6 and CRP are under strong genetic influence [7], augmenting the rationale for a genetic association with AD.

In this population-based study we sought to perform a comprehensive survey of IL6 and CRP in relation to AD and dementia in a sample of elderly Swedish men and women by examining both genes and circulating proteins. We therefore systematically examined genetic sequence variation in and around the *IL6* and *CRP* genes in almost 4000 individuals (of whom 1,265 with AD). We also measured serum levels of IL6 and CRP in a sub-sample of over 700 individuals, including future dementia cases, current dementia cases and non-demented controls.

MATERIALS and METHODS

Study populations

In total, DNA was available from 3,937 individuals; 1,567 incident and prevalent dementia cases (of whom 1,265 with AD) and 2,370 controls. Samples used in the present study were derived from the population-based Swedish Twin Registry (STR) (described in detail previously [8]) and an independent non-twin case–control Swedish AD samples. Fifty-eight percent of the sample was female, 71.5% of the sample was twins. Average age at sampling (±SD) for controls was 77.7±8.7 years and age at onset for dementia cases was 75.3±8.2 years.

Twin sample—All twins in the sample were participants of cohorts aimed to study processes of ageing and dementia collected within the Swedish twin registry [9–12]. The participants were aged 50 or older at baseline and all participants underwent cognitive testing, clinical dementia evaluation and blood sampling and anthropometric measurements were collected. Information on pharmaceutical use, smoking and education is based on self reported questionnaires. Data on cardiovascular disease and diabetes were collected until time of blood sampling through linkage to the national Cause of death and National Patient Register (NPR) which contains information on all hospital admissions and discharges in Sweden. In the twin samples, dementia was ascertained through a two-step procedure which entailed, first, a cognitive screening and, second, diagnostic assessment of each suspected case. In brief, the Mini-Mental State Examination (MMSE) [13] or, if by telephone, the TELE [14] was used to screen for cognitive dysfunction. Twins who screened positive for suspicion of dementia (and their twin partners) were further evaluated through cognitive

testing, physical and neurological examinations, informant interviews, reviews of medical records and laboratory tests. Final diagnoses of dementia were set at a multidisciplinary consensus conference. Dementia was diagnosed according to the criteria in the Diagnostic and Statistical Manual of Mental Disorders versions III-R [15] and IV [16] and differentially diagnosed as possible or probable (71%) AD (NINCDS/ADRDA criteria) [17], vascular dementia (NINDS-AIREN criteria) [18], mixed dementia (VaD+AD), other specified dementia, or unspecified dementia.

LOAD-cohort—The Swedish non-twin case–control sample consists of 896 late-onset probable Alzheimer's disease (LOAD) patients and 248 controls recruited from prospective longitudinal studies of dementia patients in Sweden [19]. In the LOAD cohort, 803 had a clinical and 93 a neuropathological diagnosis. All clinically diagnosed AD patients underwent a thorough investigation, which included a medical history, physical, neurological and psychiatric examination, screening laboratory tests, ECG, X-ray of the chest, EEG, and computerized tomography (CT) of the brain. MMSE was administered by a trained nurse and recorded in journals. Clinical AD diagnoses were made according to the NINCDS-ADRDA criteria. All neuropathologically diagnosed AD patients also fulfilled the clinical NINCDS-criteria for probable AD and met the neuropathological CERAD criteria for definitive AD. Among controls, 140 were healthy volunteers without history, symptoms or signs of psychiatric or neurological disease, malignant disease, or systemic disorders. There were 108 autopsy controls consisting of patients who had died from cardiac disease or malignant disease and whose medical records revealed no history of dementia or other psychiatric or neurological diseases. Post mortem examination revealed no macroscopic infarcts. All autopsy individuals (AD and control) were matched by age at death and all clinically diagnosed AD cases and healthy volunteers were matched by age at onset/age at exam, respectively.

Marker Selection and genotyping—Genotyping procedures have been described in detail previously [19]. In brief, genotyping was performed on 3937 individuals. This sample consisted of 71% twins, of which 29% were twin pairs discordant for dementia. Genotyping was done using the Illumina GoldenGate assay system on Illumina BeadStation 500GX equipment at the Uppsala University SNP Technology Platform. All samples were subjected to Whole Genome Amplification (WGA) using standard kits involving Phi29 DNA polymerase (Amersham, Arlington Heights, IL). Genotype-tagging single-nucleotide polymorphisms (tagSNPs) around *CRP* (including 17 kbp upstream of the transcription start site and 6 kbp downstream of the transcription end site) and *IL6* (including 10 kbp upstream and 6 kbp downstream) were selected with HaploView 4.1 Tagger [20] using Linkage Disequilibrium (LD) and the CEU HapMap population. The tagSNPs included in the study were selected to capture as much genetic variation in the region as possible on the following criteria: Hardy-Weinberg Equilibrium (HWE) p-value cutoff= 0.05, minimum genotyping success rate= 90%, maximum numbers of Mendelian inheritance errors=1, Minor Allele Frequency (MAF)>=0.05, r^2>=0.95). Markers with previously documented associations to cognitive functioning and related outcomes were included even if they did not match these criteria. Illumina SNP design scores were calculated by an algorithm that predicts success of the assay for the marker. Marker rs1800797 in *IL6* did not satisfy the criteria for Illumina probe chemistry and was not genotyped and had no SNP in perfect LD for replacement. The minimum detectable relative risk (calculated *a posteriori*) ranged from 1.2 to 1.6 depending on the disease associated allele frequency [21] where we had an 83% power to detect effects at 1.2. The 22 SNPs included in this study are listed in supplement 1.

Serum samples—Serum levels of CRP and IL6 were measured in 993 and 964 twins, respectively. The association between serum levels and AD/dementia were investigated in

two different study settings: A) a nested case-control study of 179 incident dementia cases (serum collected before onset of dementia) matched to 364 controls and, B) 97 prevalent dementia cases (serum collected after onset of dementia) matched to 205 controls. Serum fractions were prepared from venous blood according to standard procedures and stored at −70°C. CRP levels were determined with a high sensitivity near infrared particle immunoassay rate (NIPIA rate) method (measurement interval 0.2–380 mg/L) using Beckman reagents on Synchron LX20 automated equipment (Beckman Coulter, Fullerton, CA USA). IL-6 levels were analyzed using the Quantikine high-sensitvity ELISA commercial kit by R&D systems (Minneapolis, MN USA) with a mean minimum detectable dose of 0.039 ng/L.

Statistical analysis—HWE for individual loci was assessed using the Pearson $\chi 2$ statistic. Alternating logistic regression (ALR) was used to account for relatedness in the twin sample and generate odds ratios (OR) and 95% confidence intervals (CI). Tests of genotypes versus quantitative traits (e.g. age of dementia onset) were conducted using analysis of variance (ANOVA). Haplotypes were estimated after LD block definition [22] in individual blocks using Haploview v4.1 which was also used for statistical analyses. Statistical significance was considered at an overall $\alpha = 0.05$. Adjustments for multiple testing included the Bonferroni correction with a study specific significance threshold at 0.002 and permutation tests for haplotype data.

All serum analyses were done separately on incident and prevalent case data. Quantile cutoffs were based on the marker distribution in the control groups. Associations were determined by conditional logistic regression models. Linear trend was assessed by including ordinal variables as continuous variables in the logistic regression model. Age and gender were matched for by design of the study. Non-normally distributed variables were log-transformed (ln) for a better normal approximation. SAS was used for the statistical analyses (release 9.2, SAS Institutet Inc.Cary,NC).

RESULTS

Genetic variation in CRP and IL6

The observed genotype distributions were consistent with HWE among the controls except for SNPs rs1417938 (p=0.031) and rs1800796 (p=0.017) that deviated significantly before, but not after, accounting for multiple tests (Bonferroni significance threshold p=0.002). Key findings concerning the association between *CRP and IL6* genotypes and AD are summarized in Table 1 and shown in whole in Supplementary table 1. For alleles, the strongest findings were with rs1800947 and rs1417938. At rs1800947 (an exon synonymous SNP), minor allele C was more common in AD cases (9.2%) than in controls (7.8%) (p=0.028). At rs1417938 (an intron SNP), minor allele T was more common in controls (31.3%) than in AD cases (29.2%) (p=0.072). None of the analyzed tagSNPs were significant at an overall $\alpha = 0.05$. There were no significant associations between genotypes and age of dementia onset (data not shown). There were four common haplotypes in the *CRP* gene (Supplementary table 2). One haplotype (C4) was significantly more common in controls (9.3%) than cases (7.8%) but did not permutation corrections for multiple testing (p-value=0.099).

In *IL6* the most significant association was found with rs2069861 (located near the 3′ of the IL6 gene) where minor allele A was more common in controls (8.5%) than cases (6.8%), p=0.011. There were no significant associations between genotypes and age of dementia onset. There were two LD-blocks covering the *IL6* gene region (Supplementary table 2). None of the investigated haplotypes were significantly different comparing AD cases to controls.

Two SNPs in the *CRP* region were significantly associated with circulating CRP levels, minor allele C at rs1800947 was associated with lower geometric mean levels (± geometric standard deviation) of hsCRP (mg/L) (G/G = 2.27 ± 3.26, G/C = 1.74 ± 3.17, C/C = 0.64 ± 1.92) (ANOVA F =5.65, p=0.004); and minor allele T at rs1417938 was associated with increased levels (A/A = 1.91 ± 3.20, A/T = 2.43 ± 3.30, T/T = 2.34 ± 3.23) (F =4.13, p=0.016). Percent of total variation in serum levels explained by SNPs rs1800947 and rs1417938 was 1.3% and 1.0%, respectively. For *IL6*, minor allele A of rs2056576, was associated with decreased levels of circulating IL6 (ng/L) (G/G = 2.56 ± 1.95, G/A = 2.40 ± 1.92, A/A = 1.97 ± 2.10) (F =4.20, p=0.015). Percent of total variation in serum levels explained by SNP was 1.0%.

Investigating subgroups of the non-demented population (*APOE4+/E4−*, men/women, high education/low education, CHD+/CHD−) revealed that *APOE4* carriers had lower circulating hsCRP (but not IL6) than APOE4 non-carriers (1.89 and 2.28 mg/L, respectively. Age-adjusted ANOVA, p=0.059). There were no differences in CRP or IL6 levels between the other subgroups analyzed.

Serum levels of hsCRP and IL6 were moderately correlated, Rho=0.50 (p=<0.0001) in the non-demented control population and adjusted for age at blood draw. hsCRP and IL6 correlated significantly with BMI (Pearson 0.24, p<0.0001 and 0.15, p<0.0001, respectively). IL6 also correlated significantly with total serum cholesterol (Pearson −0.16, p<0.0001).

Circulating levels of CRP and IL6 and the association with AD and dementia

Detailed characteristics of the study population included in the analysis of circulating hsCRP and IL6 are shown in Table 2. ApoE4 genotype was more common among both incident and prevalent cases than controls and low education and history of myocardial infarction and stroke was more common among prevalent cases than controls. The distribution of CRP and IL6 is shown in Table 3. IL-6 (but not CRP) was significantly elevated in prevalent AD and dementia cases compared to controls whereas vascular or mixed AD/vascular dementia cases had significantly higher geometric mean levels of hsCRP (but not IL6) than controls (4.9 and 2.6 mg/L respectively, p=0.011). The association between hsCRP and IL6 levels and dementia and AD are shown in Table 4. There was no association between hsCRP or IL6 levels (measured on average 4.3 years before dementia onset) and the risk of developing dementia or AD. Additional adjustment for *APOE4*, BMI, smoking, blood pressure, education, diabetes, CHD and stroke did not change this finding.

The study of prevalent dementia cases (blood drawn on average 5.5 years *after* dementia onset) revealed that AD patients were more likely to belong to the highest IL6 (but not CRP) quartile than were controls, OR 2.24, 95% CI 1.27–3.95. There was also a positive linear trend of increasing odds ratios with each increase in IL6 tertile (p=0.055). Results were similar for all dementias, age- and sex-matched OR 2.30 (95% CI 1.43–3.73), and VaD/Mixed dementia OR 2.48 (95% CI 1.01-6-07). The association between IL6 and AD was stronger in those who had lived longer with their dementia diagnosis. The association between IL6 and AD in those below the median years survived from dementia to blood draw (<4.8 years) was OR 1.55 (0.78–3.08), in those above the median (>=4.8 years), OR 2.99 (1.47–6.08).

DISCUSSION

We have performed gene-wide analysis of the inflammatory genes *CRP* and *IL6* genes in an Alzheimer's disease case-control study of almost 4000 individuals (1,265 AD cases). SNPs were selected to capture more than 95% of the common genetic variation in the gene regions

but we did not find an association with AD or all-cause dementia. We also examined the association between circulating levels of CRP and IL6 and the risk of developing AD/dementia in incident cases with prospectively measured cytokine levels but, again, found no increased risk of dementing disease. However, we did find that patients with prevalent AD have higher levels of circulating IL6, but not hsCRP, than their controls of similar age.

There are a few previously published studies based on gene-wide analysis of all common sequence variation in *CRP* and IL6 and the association with AD or dementia. The Rotterdam study [23] reported the CRP marker rs1205 (also referred to as 2042C>T) to induce lower levels of CRP as well as a lower risk of dementia. Marker rs1800947 (referred to as 1059G>C) [24] was investigated in an Italian outpatient study but without revealing any associations with AD. For IL6, most reports of sequence variation in relation to AD have focused on the promoter variant rs1800795 (−174 G>C). In the AlzGene database [6], meta-analysis of all 16 published AD case-control association studies show a small and non-significant reduced OR of AD in carriers of the C-allele (OR 0.91, 0.79–1.06). We included the above mentioned markers in our study but could not corroborate the positive finding in the Rotterdam study at the present power level. In recently published genome-wide association studies, identifying variants at CLU, CR1 and PICALM as possible risk loci for AD, failed to find significant signals in the regions of CRP and IL6 [25–27]. The closest signals to CRP and IL6 with $p<=1*10^{-3}$ were more than 1 million base-pairs away from the actual genes and did not reach genome wide significance with $p=1*10^{-4}$.

We found no significant association between baseline circulating hsCRP or IL6 and the risk of future AD or dementia in this elderly population of women and men despite assessment of levels of CRP and IL6 with highly sensitive methods to capture variation even in the lower ranges. This is in line with several studies[28–30] including findings from the Framingham study [31] as well as with a recent study on CRP and IL6 levels and the future development of AD/dementia in a cohort of 70-year old men [32]. Our lack of findings are however also in contrast with other cohorts[33, 34] including the all-male Honolulu-Asia Aging Study (HAAS) [35]. In HAAS, high CRP levels measured in midlife were associated with a three-fold increased risk of developing AD or dementia later in life. It is possible that increased inflammation in midlife is either more detrimental than increased levels later in life or that measuring CRP in midlife better captures lifelong inflammatory exposure than measuring inflammatory markers in an aged population with a high burden of comorbidity.

When comparing dementia cases to non-demented controls, we found a difference in circulating inflammatory levels, especially for IL6. High levels of IL6 were significantly more common in all types of dementia cases, including AD. Mean hsCRP was significantly higher in cases with vascular/mixed dementia compared to both controls and cases with AD. It is possible that this is a reflection of their higher proportion of vascular comorbidities. We also observed an interesting link between CRP and AD in that variation in the major susceptibility gene for AD, *APOE*, contributes to the variation in circulating CRP levels. It is estimated that 2.4–4.1% of the variation in CRP in Caucasians is accounted for by *APOE* [36]. Nonetheless, we found no interaction between CRP levels and *APOE4* status on the likelihood of having AD/VaD + mixed/any dementia.

Increased circulating levels of IL6 in AD patients could reflect neurodegeneration or compensatory neuroprotection in the central-nervous system. One proposed mechanism for a direct influence of IL6 is through induction of AD-type phosphorylation of tau protein by deregulating the cdk5/p53 pathway [37]. Another possibility is that increased circulating levels of inflammatory molecules in AD patients compared to healthy controls is due to impaired function of parasympathetic nerves that inhibit macrophage activation through cholinergic signaling via the vagus nerve [38]. The vagus nerve originates in the brain stem

and affects systemic inflammation (by regulating TNF-transcription), heart rate and blood pressure, all of which can be affected in dementia disorders.

Serum hsCRP and IL6 levels were correlated and one might therefore expect to find similar results for the association between these markers and AD. However, in our study we found no significant differences in circulating CRP levels between AD patients and non-demented controls, whereas we did find an association between IL6 levels and prevalent AD. This could indicate that IL6 captures some part of the inflammatory process that is altered in AD but that is not associated with altered CRP levels. It is also possible that CRP is too sensitive to environmental stimuli, or has too high inter-individual variability, to give a reliable measurement of low-grade systemic inflammation at these ages.

In conclusion, this study does not generally support a strong influence of the actions of CRP or IL6 in the events in later life leading up to the onset of AD. Our data do however suggest that elderly AD and dementia patients have an altered immune profile with higher circulating levels of IL6 than their non-demented peers. Whether this is a reflection of inflammatory processes in the brain or a consequence of peripheral immunological alterations due to disease remains to be established.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Key findings on the associations between CRP and IL6 SNPs and risk of Alzheimer's disease. The full data is presented in supplementary table 1.

Gene/Marker		Controls N=2370 (%)	AD cases N=1265 (%)	OR (95% CI)	P_{trend}
CRP/rs1800947	GG	85.2	82.7	1	
	GC	14.1	16.1	1.09 (0.90–1.31)	
	CC	0.7	1.2	1.57 (0.79–3.74)	0.22
CRP/rs1417938	AA	46.3	49.3	1	
	AT	44.9	43.0	0.90 (0.79–1.04)	
	TT	8.8	7.7	0.80 (0.62–1.03)	0.04
IL6/rs2069861	GG	84	87	1	
	GA	16	11	0.74 (0.60–0.90)	
	AA	0.6	1	1.50 (0.76–2.99)	0.04
IL6/rs12700386	CC	69	67	1	
	CG	28	30	1.09 (0.94–1.27)	
	GG	3	4	1.39 (0.96–2.01)	0.08
IL6/rs2056576	GG	54	52	1	
	GA	39	39	1.09 (0.94–1.25)	
	AA	7	9	1.25 (0.97–1.61)	0.07
IL6/rs1546762	GG	29	27	1	
	AG	49	47	1.02 (0.88–1.20)	
	AA	22	25	1.17 (0.97–1.43)	0.12

Odds Ratios (OR) with 95% Confidence Intervals (CI) from Alternating Logistic Regression with the most common homozygote genotype as the reference level. Adjusted for age and sex.

Table 2

Characteristics of the study population included in the analysis of hsCRP and IL6 in serum

	Nested case-control (incident) sample			Patient case-control (prevalent) sample		
Characteristic[†]	***Dementia* (N=179)**	***AD* (N=91)**	***Controls* (N=364)**	***Dementia* (N=97)**	***AD* (N=71)**	***Controls* (N=205)**
Age (years), mean±SD	78.5 ± 6.3	79.5 ± 6.2	78.2 ± 6.6	81.7 ± 5.2	81.6 ± 5.3	81.3 ± 5.6
Sex (women), N (%)	110 (61.5)	62 (68.1)	219 (60.2)	64 (66.0)	52 (73.2)	127 (62.0)
Low education, N (%)	127 (71.0)	69 (75.8)	242 (66.5)	76 (80.0)*	54 (78.3)*	128 (62.4)
Ever smokers, N (%)	73 (40.8)	38 (41.8)	147 (40.4)	39 (40.2)	24 (33.8)	93 (45.4)
Myocardial infarction, N (%)	15 (8.4)	4 (4.4)	25 (6.9)	9 (9.3)*	3 (4.2)	7 (3.4)
Stroke, N (%)	8 (4.5)	1 (1.1)	18 (5.0)	22 (22.7)**	5 (7.0)	14 (6.8)
Diabetes, N (%)	10 (5.6)	4 (4.4)	9 (2.5)	16 (16.5)**	11 (15.5)**	6 (2.9)
APOE4, N (%)	70 (39.8)**	36 (39.6)*	87 (24.3)	41 (43.2)**	33 (47.2)**	44 (22.3)
BMI (kg/m^2), mean±SD	25.5 ± 3.9	25.3 ± 4.0	25.5 ± 3.8	24.3 ± 3.5	24.0 ± 3.1*	25.2 ± 4.3
SBP (mmHg), mean±SD	159.1 ± 23.2	158.1 ± 24.3	155.8 ± 22.0	146.3 ± 25.8*	146.7 ± 25.2	153.0 ± 20.2

[†]N based on all individuals with a hsCRP or IL6 measurement;

*p<0.05,

**p<0.001 comparing cases to controls Low education: elementary school or less;

BMI: Body Mass Index; SBP: Systolic Blood Pressure

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Table 3

Distribution of hsCRP and IL6 levels in serum by study sample and dementia status.

	Nested case-control (incident) sample			**Patient case-control (prevalent) sample**		
	Dementia	***AD***	***Controls***	***Dementia***	***AD***	***Controls***
hsCRP (mg/L)	*N=179*	*N=91*	*N=358*	*N=93*	*N=67*	*N=205*
Geometric mean±SD	2.0 ± 3.1	1.8 ± 3.0	2.1 ± 3.1	3.1 ± 3.7	2.5 ± 3.9	2.6 ± 3.4
Median	2.1	1.9	2.0	2.8	2.4	2.6
25th/75th percentile	1.0/4.3	0.9/3.7	1.0/4.3	1.4/5.1	1.2/4.4	1.2/5.5
Interquartile range	3.3	2.8	3.3	3.7	3.2	4.3
Bottom Tertile, N (%)	60 (33.5)	32 (35.2)	121 (33.8)	27 (29.0)	23 (34.3)	68 (33.2)
Middle Tertile, N (%)	60 (33.5)	32 (35.2)	119 (33.2)	36 (38.7)	27 (40.3)	69 (33.7)
Top Tertile, N (%)	59 (33.3)	27 (29.7)	118 (33.0)	30 (32.3)	17 (25.4)	68 (33.2)
Ptrend (tertiles)	*ns*	*ns*	*ref*	*ns*	*ns*	*ref*
IL6 (ng/L)	*N=164*	*N=82*	*N=352*	*N=97*	*N=71*	*N=198*
Geometric mean±SD	2.4 ± 1.8	2.5 ± 1.8	2.3 ± 1.9	4.3 ± 1.9**	4.1 ± 2.0*	3.0 ± 1.9
Median	2.4	2.4	2.3	4.1**	3.7*	2.8
25th/75th percentile	1.7/3.4	1.6/3.4	1.6/3.3	2.7/7.1	2.4/6.9	2.0/4.6
Interquartile range	1.7	1.8	1.7	4.4	4.5	2.6
Bottom Tertile, N (%)	47 (28.7)	25 (30.5)	118 (33.5)	17 (17.5)	16 (22.5)	66 (33.3)
Middle Tertile, N (%)	55 (33.5)	26 (31.7)	117 (33.2)	28 (28.9)	21 (29.6)	66 (33.3)
Top Tertile, N (%)	62 (37.0)	31 (37.8)	117 (33.2)	52 (53.6)	34 (47.9)	66 (33.3)
Ptrend (tertiles)	*ns*	*ns*	*ref*	*<.001*	*0.02*	*ref*

* p<0.05,

** p<0.001 comparing cases to controls

Table 4

Association of hsCRP and IL6 in serum with Alzheimer's disease

	Nested case-control (incident) sample				Patient case-control (prevalent) sample			
	OR*	**95% CI**	**OR****	**95% CI**	**OR***	**95% CI**	**OR****	**95% CI**
hsCRP (mg/L)								
75th percentile Tertiles	0.93	0.51–1.68	1.38	0.69–2.79	0.88	0.43–1.78	0.64	0.20–2.06
top	1	ref	1	ref	1	ref	1	ref
middle	0.93	0.53–1.64	1.01	0.53–1.89	1.13	0.55–2.28	1.69	0.69–4.18
bottom	0.84	0.47–1.50	1.31	0.67–2.53	0.84	0.40–1.74	1.16	0.40–3.36
P_{trend}	*0.55*		*0.46*		*0.64*		*0.65*	
IL6 (ng/L)								
75th percentile Tertiles	1.10	0.62–1.95	1.49	0.75–2.95	2.24	1.27–3.95	2.13	0.94–4.82
top	1	ref	1	ref	1	ref	1	ref
middle	1.04	0.53–2.05	0.77	0.34–1.76	1.14	0.53–2.48	3.29	0.70–15.34
bottom	1.34	0.69–2.62	1.32	0.59–2.94	1.87	0.94–3.74	1.81	0.51–6.37
P_{trend}	*0.36*		*0.38*		*0.05*		*0.11*	

Odds Ratios (OR) with 95% Confidence Intervals (CI) from conditional logistic regression.

* Matched on age at blood draw and sex.

** Matched on age at blood draw and sex and adjusted for APOE4, BMI, ever smoking, high blood pressure, education, diabetes, CHD and stroke.