

## CSF Biomarkers

# Apolipoproteins and their subspecies in human cerebrospinal fluid and plasma

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### Abstract

**Introduction:** Subspecies of apolipoproteins can be defined by fractionating apolipoproteins based on the presence and absence of coexisting apolipoproteins.

**Methods:** We determined age- and sex-adjusted correlations of enzyme-linked immunosorbent assay-measured plasma and cerebrospinal fluid (CSF) apolipoproteins (apoA-I, apoC-III, apoE, and apoJ) or apolipoprotein subspecies (apoA-I with and without apoC-III, ApoE, or apoJ; apoE with and without apoC-III or apoJ) in 22 dementia-free participants.

**Results:** CSF apoE did not correlate with plasma apolipoproteins or their subspecies. CSF apoJ correlated most strongly with plasma apoA-I without apoJ ( $r = 0.7$ ). CSF apoA-I correlated similarly strong with plasma total apoA-I and all apoA-I subspecies ( $r \geq 0.4$ ) except for apoA-I with apoE ( $r = 0.3$ ) or apoA-I with apoJ ( $r = 0.3$ ). CSF apoC-III was most strongly correlated with plasma apoA-I with apoC-III ( $r = 0.7$ ).

**Discussion:** CSF levels of some apolipoproteins implicated in the pathophysiology of dementia might be better approximated by specific plasma apolipoprotein subspecies than total plasma concentrations.

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### Keywords:

Apolipoproteins; Brain; Lipoproteins; Apolipoprotein subspecies; Cerebrospinal fluid

## 1. Introduction

Evidence is accumulating that apolipoproteins, found in both plasma and cerebrospinal fluid (CSF), are involved in the pathophysiology of Alzheimer's disease [1]. Genetic variants of the apolipoprotein E (*APOE*) and J (clusterin, *CLU*) genes are known risk factors for Alzheimer's disease [2]. Apolipoprotein (apo) E and J have been suggested to play

an important role in the deposition and clearance of neurotoxic  $\beta$ -amyloid [3–5]. ApoE also protects the microtubule-associated protein tau from hyperphosphorylation [6]. ApoC-III was discovered to be a  $\beta$ -amyloid binding protein [7] and lower plasma apoC-III levels have been related to higher Alzheimer's disease prevalence in the Alzheimer's Disease Neuroimaging Initiative [8].

CSF biomarkers are sometimes used in clinical practice to support the diagnosis of AD [9,10]. However, the identification of markers that may reflect increased risk of future disease is a major mission in the field of Alzheimer's research [11]. For purposes of such large-scale studies and use in future clinical screenings, minimally invasive measures such as those obtained from plasma

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samples are highly desirable. Plasma apolipoproteins are primarily synthesized by the liver and intestine [12]. Because of differences in local synthesis and transport across the blood-brain barrier, it is important to understand the relationships of plasma and CSF levels of specific apolipoprotein subspecies to gain insight into which plasma apolipoproteins reflect CSF levels and which apolipoproteins are best measured in CSF.

## 2. Methods

This cross-sectional analysis was performed in nine men and 13 women (age 20–76 years, mean age 40 years), treated at the emergency department at the University Hospital Schleswig-Holstein, Campus Kiel, Germany between 2009 and 2015 for acute headache. Patients with a history of dementia, systemic or CSF inflammatory signs, or blood-brain barrier dysfunction (CSF-to-serum albumin ratios  $\geq 9 \times 10^3$ ) were excluded. Diagnoses were migraine and headache ( $n = 8$ ), common cold or sinusitis ( $n = 7$ ), skin sensation disturbance ( $n = 4$ ), syringomyelia ( $n = 1$ ), mild cognitive impairment ( $n = 1$ ), and suspected pseudotumor cerebri ( $n = 1$ ).

Informed consent for scientific analysis of diagnostic remnant samples collected for clinical care was obtained at the time of specimen collection. Information on age, sex, and diagnosis was collected from anonymized medical reports.

Plasma samples and CSF samples were obtained by venipuncture and lumbar puncture, respectively. In paired CSF and serum samples, concentrations of albumin were measured immediately by particle-enhanced immunologic turbidimetry (Roche Cobas, Switzerland). Remnant samples were stored at 4°C for up to 2 weeks after collection and stored at –80°C afterward. Samples were shipped on dry ice to the lipid laboratory of the Harvard Chan School of Public Health for apolipoprotein measurements.

Concentrations of apoC-III and apoJ were measured by sandwich enzyme-linked immunosorbent assay (ELISA). For apoA-I and apoE with and without specific apolipoproteins, a patented modified sandwich ELISA approach was used. The 96-well plates were coated with antibody to the apolipoprotein by which we desired to fractionate (apoC-III, apoE, or apoJ; Academy Biomedical Company Inc, Houston TX and R&D Systems, Minneapolis, MN). Diluted samples were incubated on these plates to bind lipoproteins containing that apolipoprotein. The unbound fraction was removed and apoA-I or apoE in this fraction was measured by sandwich ELISA on a second plate coated with anti-apoA-I or anti-apoE antibody. The fraction bound to the first plate was released by dissociation of the lipoprotein complex and transferred to a third plate coated with anti-apoA-I or anti-apoE antibody to quantify the concentration of apoA-I or apoE with the apoC-III, apoE, or apoJ. Both CSF and plasma samples from an individual were assayed together on the same 96-well plate for each measurement and the

coefficients of variation (CVs) were quite comparable. For instance, the average (standard deviation, SD) CV% for apoE in plasma was 4% (3%), whereas that for CSF was 5% (3%). Overall our CVs were less than 20% except for apoA-I without apoC-III in plasma (average CV 26%, SD 19%) and apoA-I with apoJ in plasma (average CV 20%, SD 14%) and in CSF (average CV 27%, SD 22%).

We examined the effect of length of time between thawing and start of assay protocol, comparing no delay, 2, 6, and 24 hours in the context of room temperature delay storage, and refrigerated (4°C) delay storage. In the room temperature storage samples, we observed a trend toward increasing concentrations of apoA-I with apoC-III with increasing delay time, along with increasing CV% for replicate measures. These effects are not found with storage at 4°C. We also examined the effect of freeze/thaw cycles comparing freshly drawn samples to aliquots frozen and assayed after one, three, and five freeze/thaw cycles. There was no effect of up to five freeze/thaw cycles on the measured concentration or CV% of the samples assayed. Samples are not affected by the act of freezing and thawing up to five times provided that they are kept chilled when in a thawed state. Partial correlation analysis was performed controlling for age and sex.

In sensitivity analysis, we determined unadjusted Pearson correlation coefficients of logarithmically transformed apolipoproteins and their subspecies. The CSF-to-serum albumin ratio correlates with the CSF-to-serum ratio of other blood-derived proteins, which also enter the brain by diffusion [13]. The CSF-to-serum albumin ratio does not correlate with the CSF-to-serum ratio of proteins with intrathecal synthesis or proteins actively transferred across the blood-CSF barrier [13,14]. Analyses were performed using SAS software, Version 9.4 (SAS Institute Inc, Cary, NC).

## 3. Results

ApoE and apoA-I were the quantitatively major apolipoproteins in both CSF and plasma (Table 1). Most of the apoE did not carry apoC-III or apoJ, and most of the apoA-I did not carry apoC-III, apoE, or apoJ.

CSF concentrations were approximately 10 times smaller than the plasma concentrations. Median concentrations in CSF relative to plasma concentrations were between 0.01% for apoC-III and 11.57% for apoE without apoC-III.

CSF and plasma apolipoprotein concentrations in paired samples correlated moderately with each other (all  $r \geq 0.4$ ) except for apoE ( $r = -0.01$ ,  $P = .95$ ).

CSF-to-serum albumin ratios (median 4.5; interquartile range: 3.7,  $5.5 \times 10^3$ ), a marker of blood-brain barrier integrity, correlated with the CSF-to-plasma ratio of apolipoproteins and their subspecies ( $r > 0.4$ ) except for apoE ( $r = -0.03$ ,  $P = .89$ ; Table 1). Correlations were higher for apoA-I with the apoC-III ( $r = 0.63$ ,  $P \leq .01$ ) than total apoC-III ( $r = 0.42$ ,  $P = .06$ ).

Table 1

Median concentrations of apolipoproteins and their subspecies in human CSF and plasma, and Spearman correlations of paired CSF and plasma concentrations and CSF-to-serum albumin ratio ( $n = 22$ )

Apolipoproteins and their subspecies	Median <sup>†</sup> concentration (IQR) in CSF, mg/dL	Median <sup>†</sup> concentration (IQR) in plasma, mg/dL	Proportion of plasma concentration, median (IQR) in CSF, %	Correlation paired CSF and plasma concentrations, Spearman $r$ ( $P$ value)*	Correlation CSF-to-serum albumin ratio with CSF-to-plasma apolipoprotein ratio, Spearman $r$ ( $P$ value)*
ApoE	0.727 (0.592, 0.966)	12.73 (8.27, 15.72)	5.98 (3.09, 11.18)	-0.01 (.95)	-0.03 (.89)
ApoE with apoC-III	0.076 (0.056, 0.106)	6.37 (4.15, 9.04)	1.29 (0.57, 1.93)	-0.12 (.61)	-0.18 (.44)
ApoE without apoC-III	0.654 (0.523, 0.859)	5.35 (4.23, 7.69)	11.57 (7.60, 18.33)	0.16 (.51)	0.03 (.90)
Proportion of apoE with apoC-III, %	11.4 (8.7, 12.8)	50.4 (45.1, 61.5)			
ApoE with apoJ	0.002 (0.001, 0.002)	0.23 (0.18, 0.33)	0.82 (0.46, 1.40)	-0.35 (.13)	-0.28 (.22)
ApoE without apoJ	0.725 (0.591, 0.964)	12.44 (8.13, 15.45)	6.08 (3.17, 11.38)	<0.01 (1.00)	-0.03 (.89)
Proportion of apoE with apoJ, %	0.2 (0.2, 0.3)	1.9 (1.7, 2.3)			
ApoJ	0.005 (0.003, 0.007)	4.75 (3.15, 6.48)	0.11 (0.08, 0.14)	0.58 (.01)	0.57 (.01)
ApoA-I	0.223 (0.175, 0.320)	155.3 (130.4, 176.6)	0.16 (0.12, 0.22)	0.53 (.02)	0.84 (<.01)
ApoA-I with apoC-III	0.006 (0.004, 0.010)	10.8 (8.2, 16.7)	0.05 (0.03, 0.08)	0.65 (<.01)	0.63 (<.01)
ApoA-I without apoC-III	0.217 (0.172, 0.310)	146.1 (120.8, 154.9)	0.16 (0.13, 0.24)	0.41 (.07)	0.86 (<.01)
Proportion of apoA-I with apoC-III, %	2.5 (2.0, 2.8)	7.5 (5.6, 9.7)			
ApoA-I with apoE	0.066 (0.057, 0.103)	6.05 (3.08, 9.64)	1.23 (0.93, 2.03)	0.75 (<.01)	-0.23 (.34)
ApoA-I without apoE	0.152 (0.120, 0.234)	149.1 (118.1, 172.3)	0.11 (0.08, 0.16)	0.60 (.01)	0.88 (<.01)
Proportion of apoA-I with apoE, %	33.0 (25.1, 39.2)	3.8 (2.0, 6.6)			
ApoA-I with apoJ	0.013 (0.005, 0.021)	9.05 (6.32, 11.14)	0.13 (0.05, 0.21)	0.45 (.04)	0.72 (<.01)
ApoA-I without apoJ	0.205 (0.171, 0.310)	141.0 (122.4, 166.6)	0.15 (0.12, 0.22)	0.55 (.01)	0.83 (<.01)
Proportion of apoA-I with apoJ, %	5.9 (3.0, 9.3)	5.5 (4.9, 7.6)			
ApoC-III	0.003 (0.002, 0.006)	23.6 (17.0, 29.8)	0.01 (0.01, 0.02)	0.62 (<.01)	0.42 (.06)

Abbreviations: CSF, cerebrospinal fluid; IQR, interquartile range.

\*Partial correlation controlling for age and sex.

<sup>†</sup>The median of the apolipoprotein subspecies do not sum up to the total apolipoprotein concentration because the ranking of the study participants by the two subspecies is not identical (the midpoint of the frequency distribution of the subspecies originates from different individuals). The median total apolipoprotein concentration is based on the sum of the subspecies from the same individual.

Next, we determined which plasma measures correlated most strongly with CSF total apolipoproteins. Consistent with its production in the central nervous system (CNS), total CSF apoE did not correlate with any plasma apolipoprotein or any of their subspecies (Fig. 1). For total CSF apoJ, the highest correlation was surprisingly observed for apoA-I without apoJ in plasma ( $r = 0.67$ ,  $P \leq .01$ ; statistically significant after correction for multiple testing). For comparison, the correlation of CSF apoJ with plasma apoA-I was only slightly higher ( $r = 0.70$ ,  $P \leq .01$ ; data not shown). CSF apoA-I correlated similarly high with plasma apoA-I and all plasma apoA-I subspecies ( $r \geq 0.42$ ) except for plasma apoA-I with apoE ( $r = 0.28$ ,  $P = .24$ ) and plasma apoA-I with apoJ ( $r = 0.32$ ,  $P = .17$ ). Total CSF apoC-III correlated somewhat more strongly with plasma subspecies apoA-I with apoC-III ( $r = 0.66$ ,  $P \leq .01$ ; statistically significant after correction for multiple testing) than with total plasma apoC-III ( $r = 0.62$ ,  $P \leq .01$ ) or plasma apoE with apoC-III ( $r = 0.56$ ,  $P = .01$ ). The sensitivity analysis of logarithmically transformed apolipoproteins and their subspecies

revealed similar results to the overall analysis (see [Supplementary Figs. 1-4](#)).

#### 4. Discussion

In this analysis, concentrations of apolipoproteins in CSF were 10 times smaller than plasma concentrations. CSF and plasma levels generally correlated moderately except for apoE, which is produced in the CNS itself. CSF levels of apolipoproteins were generally more strongly correlated with their specific corresponding apolipoprotein subtypes than with total plasma concentrations.

Few studies have examined the concordance of plasma and CSF lipoproteins, and to our knowledge, none have specified them with the granularity recorded here. Two studies investigating apolipoproteins in paired plasma and CSF samples [15,16] found that apolipoproteins were present in CSF at proportions of plasma concentrations similar to our results.

Concordant with previous observations [17,18], the minimal correlation of plasma and CSF apoE supports its

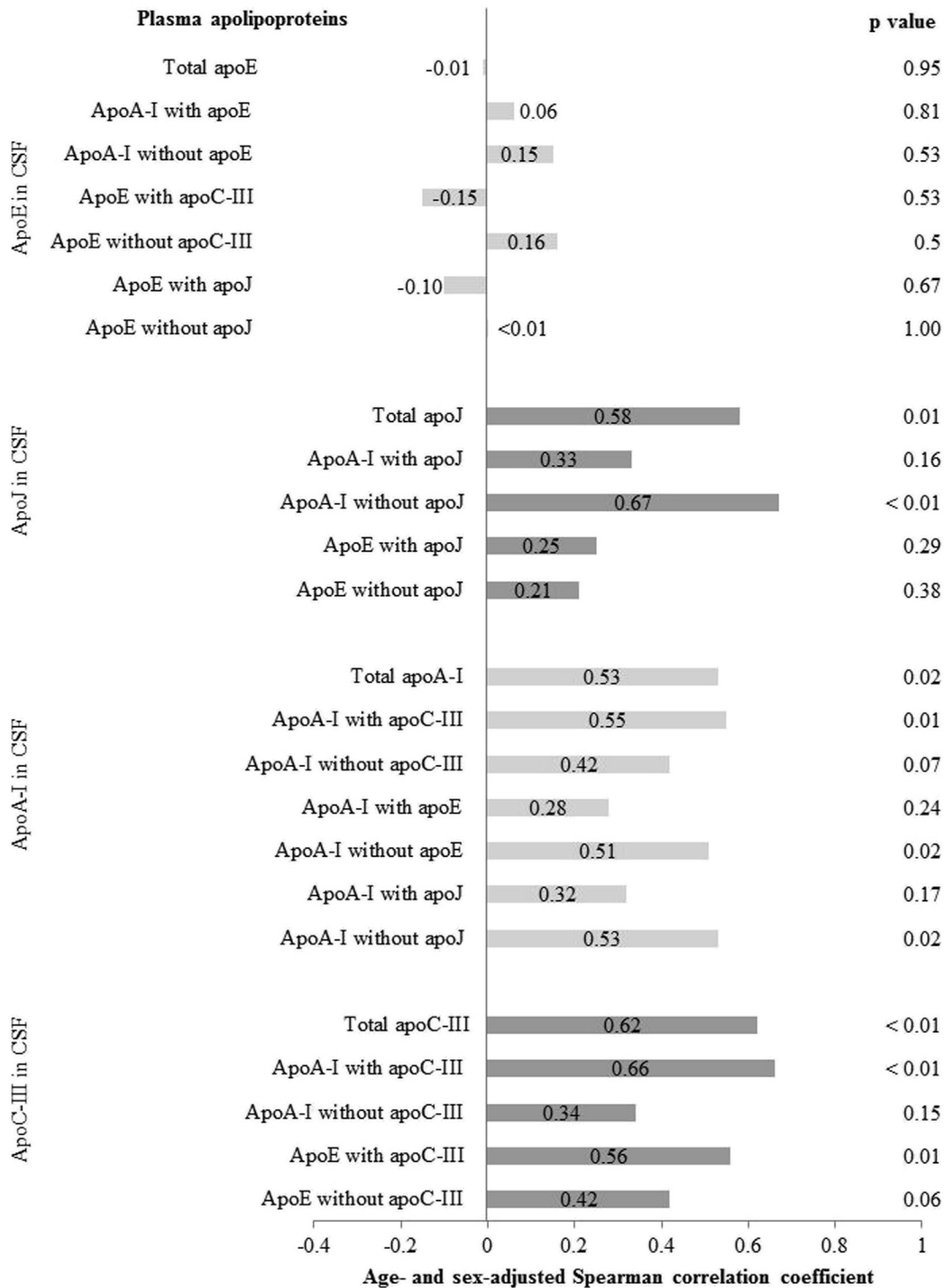


Fig. 1. Age- and sex-adjusted Spearman correlations of total CSF apolipoproteins with various plasma apolipoproteins and their subspecies (n = 22). Abbreviation: CSF, cerebrospinal fluid.

intrathecal synthesis and lack of transfer of apoE across the brain barrier interfaces [12,19]. Polymorphisms in the *APOE* gene result in three apoE isoforms, defining apoE phenotypes. A prior study has shown that in patients receiving liver transplantations the serum apoE phenotype converted to the donor apoE phenotype, whereas the CSF

apoE phenotype remained the same, supporting the hypothesis that peripheral and CNS apoE represent distinct pools [12].

In our study, CSF apoJ correlated even more strongly with plasma apoA-I without apoJ than it did with total plasma apoJ. The latter finding might be explained by several

observations. First, the transport of apoJ over the brain barrier has been shown to be saturated in animal experimental studies [20], suggesting that brain apoJ and plasma apoJ are distinct. Second, in CSF, most of apoJ has been found as a complex with apoA-I [21]. However, as brain apoA-I is plasma derived only [22], it might render plasma apoA-I and its major fraction that lacks apoJ more closely related to CSF apoJ than plasma apoJ.

The presence of apoA-I in the absence of apoA-I messenger RNA in the brain supports the hypothesis that peripheral apoA-I enters the brain [22]. A recent animal experimental study suggests that most of the brain apoA-I enters the CNS via the blood-CSF barrier formed by the choroid plexus epithelium [22]. The role of apoA-I in the brain is not well known. A recent cross-sectional study in cognitively healthy participants and participants with mild cognitive impairment or Alzheimer's disease found no correlation between CSF cholesterol efflux capacity and CSF apoA-I concentrations, suggesting a role of apoA-I beyond cholesterol efflux [23].

Plasma apoA-I containing apoC-III and not total plasma apoC-III correlated most strongly with CSF apoC-III, suggesting preferential crossing of apoC-III over barrier interfaces in the presence of coexisting proteins like apoA-I. This is further supported by our finding of higher correlation of CSF-to-serum ratio of albumin with apoA-I with apoC-III than total apoC-III. The transport mechanisms by which peripheral apoC-III enters the brain are unknown [24,25].

Strength of this study is the assessment of an unprecedented variety of apolipoproteins and their subspecies in paired samples of dementia-free individuals with a wide age range. A main limitation of the study is the small sample size and the limited generalizability of study findings to diseased populations. With progressing dementia, the blood-brain barrier might become dysfunctional, and associations between CSF and plasma apolipoproteins might change, although our results presumably apply to the long preclinical period for chronic diseases like Alzheimer's dementia. Indeed, the identification of minimally invasive plasma biomarkers that prospectively predict the risk of AD before the appearance of clinical symptoms would be instrumental for improved risk-stratification, drug development, early intervention, and prevention of Alzheimer's disease.

#### 4.1. Conclusion

Location and kinetics of synthesis and clearance and the ability of apolipoproteins and their subspecies to cross the brain barriers determine plasma and CSF levels. CSF and plasma levels generally correlated moderately with each other except for apoE, indicating that plasma apolipoprotein levels reflect CSF levels but apoE is best measured in CSF. However, the CSF concentration of apoC-III might be better approximated by the plasma concentration of apoA-I with apoC-III than the total plasma apoA-I

concentration. The findings support the necessity of further investigation of apolipoprotein subspecies in relation to Alzheimer's disease risk.

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Conflicts of Interest: Harvard University holds a patent for the measurement of apoA-I subspecies by apoC-III where Drs Jensen and Furtado are named coinventors.

#### Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.dadm.2017.01.007>.

#### RESEARCH IN CONTEXT

1. Systematic review: PubMed search identified few studies examining the concordance of plasma and cerebrospinal fluid lipoproteins, and to our knowledge, none have specified them with the granularity recorded here.
2. Interpretation: Cerebrospinal fluid levels of some apolipoproteins might be better reflected by specific apolipoprotein subspecies than total plasma concentrations.
3. Future directions: Prospective studies on apolipoprotein subspecies in relation to Alzheimer's disease risk are warranted.

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