Plasma amyloid β levels are driven by genetic variants near APOE, BACE1, APP, PSEN2: A genome-wide association study in over 12,000 non-demented participants

Vincent Damotte1 | Sven J. van der Lee2,3 | Vincent Chouraki1,4 | Benjamin Grenier-Boley1 | Jeannette Simino5 | Hieab Adams6 | Giuseppe Tosto7,8 | Charles White9,10 | Natalie Terzikhan3,11 | Carlos Cruchaga12 | Maria J. Knol3 | Shuo Li13,14 | Susanna Schraen15 | Megan L. Grove16 | Claudia Satizabal4,14 | Najaf Amin3 | Claudine Berr17 | Steven Younkin18 | Alzheimer's Disease Neuroimaging Initiative1 | Rebecca F. Gottesman19,20 | Luc Buée1,21 | Alexa Beiser4,13,14 | David S. Knopman22 | Andre Uitterlinden23 | Charles DeCarli24 | Jan Bressler16 | Anita DeStefano4,13,14 | Jean-François Dartigues25 | Qiong Yang13,14 | Eric Boerwinkle16,26 | Christophe Tzourio25 | Myriam Fornage16,27 | M. Arfan Ikram6 | Philippe Amouyel1 | Phil de Jager9,10,28 | Christiane Reitz7,8,29,30 | Thomas H. Mosley31 | Jean-Charles Lambert1 | Sudha Seshadri4,14,32 | Cornelia M. van Duijn3,33

1 Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, Lille, France
2 Alzheimer Center Amsterdam, Department of Neurology, Amsterdam Neuroscience, Vrije Universiteit Amsterdam, Amsterdam UMC, Amsterdam, the Netherlands
3 Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands
4 Department of Neurology, Boston University School of Medicine, Boston, Massachusetts, USA
5 Gertrude C. Ford MIND Center, Department of Data Science, John D. Bower School of Population Health, University of Mississippi Medical Center, Jackson, Mississippi, USA
6 Departments of Epidemiology, Neurology, and Radiology and Nuclear Medicine, Erasmus Medical Center, Rotterdam, the Netherlands
7 Taub Institute for Research on Alzheimer’s Disease and the Aging Brain, Columbia University, New York, New York, USA
8 Gertrude H. Sergievsky CenterColumbia University, New York, New York, USA
9 Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Departments of Neurology and Psychiatry, Brigham and Women’s Hospital, Boston, Massachusetts, USA
10 Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts, USA
11 Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium
12 Department of Psychiatry, Washington University in St. Louis, Saint Louis, Missouri, USA
13 Department of Biostatistics, Boston University School of Public Health, Boston, Massachusetts, USA
14 The Framingham Heart Study, Framingham, Massachusetts, USA
15 Université Lille, CHU-Lille, InsermUF de Neurobiologie, CBPG, Lille, France
16 Human Genetics Center, Department of Epidemiology, Human Genetics, and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, Texas, USA
17 INSERM U1061University of Montpellier, Montpellier, France
1 INTRODUCTION

Amyloid beta (Aβ) deposition is one of the hallmarks of Alzheimer’s disease (AD). Aβ peptides are the products of the catalytic processing of the Aβ precursor protein (APP) by the β-secretase, BACE1, and the γ-secretase complex.1 Aβ peptides are able to self-assemble in soluble Aβ oligomers but also in insoluble fibrils that can aggregate as plaques in the brain parenchyma or in the wall of blood vessels where they constitute defining hallmarks of AD and cerebral amyloid angiopathy (CAA), which is seen in many patients.3 Aβ peptides are mainly produced not only in the brain where APP and BACE1 are both highly expressed,1 but also in circulating blood platelets,4 in the pancreas,5 and the kidneys.6 There is strong evidence pointing toward a central role of Aβ peptides in the pathophysiology of AD.7 In the past decades, studies have shown that a large variety of rare mutations in genes involved in Aβ production, including APP, PSEN1, and PSEN2, lead to autosomal dominant early-onset forms of AD and to lobar hemorrhage from cerebral amyloid angiopathy.8 Moreover, apolipoprotein E (APOE) ε4, the major
genetic risk factor for AD in the general population,9 has been implicated in Aβ aggregation, deposition, and clearance, both in the brain and in blood vessels.7,10 Although for a long time the Aβ pathway did not emerge in our genome-wide association studies (GWAS) of AD,11 our most recent GWAS study highlighted the Aβ processing pathway and APP catabolic process pathway in late-onset Alzheimer’s disease (LOAD).12 We and others have also explored the genetics of Aβ through GWAS on quantitative measures of Aβ peptides, in the cerebrospinal fluid (CSF) or brain, through Pittsburgh Compound B (PiB) positron emission tomography (PET) scan or autopsy.13–17 Combining the effect of AD genetic loci in a genetic risk score shows that the combined AD genes are statistically significantly related to CSF Aβ42.17

Although Aβ can be assessed in CSF and brain (PiB PET), these tests are of limited use for clinical and epidemiological studies in the population, either because of lower compliance (CSF) or higher costs (PiB PET). The recent success of blood-based biomarkers (phosphorylated tau [p-tau] and neurofilament light [NfL]) fueled our interest in Aβ metabolism in blood. Unlike p-tau and NfL, Aβ peptides in the blood circulation are not brain specific. Aβ peptides produced in the brain can be degraded locally or transported into the CSF and the blood stream.18 However, Aβ peptides in the circulation can also be derived from blood platelets, kidney, or pancreas. Although the brain-derived Aβ peptides in the circulation cannot be distinguished from Aβ derived from blood platelets, the kidneys, or the pancreas, a recent study using immuno-precipitation coupled with mass spectrometry to measure plasma Aβ1-40/Aβ1-42 and APP/Aβ1-42 ratios was able to accurately predict individual brain Aβ-positive or -negative status.19 Also, studies assessing Aβ1-40 and Aβ1-42 using immunoassays show that these can predict Aβ status in the brain as assessed by PiB PET20 and that changes in the blood and plasma occur simultaneously.21

Earlier, we have also shown that plasma Aβ concentrations are prospectively associated with the risk of developing AD in the future.22–25 Despite the fact that we have used less sensitive techniques to measure plasma Aβ levels, we found modest but significant correlation with amyloid burden in the CSF and in the brain.26,27 Whereas Aβ levels may or may not prove to be an effective blood biomarker panel for predicting AD risk in patients with cognitive impairments, the association with future AD suggests Aβ could be an endophenotype, that is, a quantitative biological trait that is an intermediate between one or more disease genes (e.g., APOE, APP, PSEN, or other AD genes) and the disease of interest, AD. Endophenotypes can be associated to different diseases, for example, the endophenotype blood pressure is associated to brain, heart, kidney, and dementia and is relevant for early prevention. For instance, endophenotypes played a key role in developing prevention for cardiovascular disease, targeting intermediate endophenotypes such as cholesterol, glucose, and blood pressure. Building upon our findings that plasma Aβ concentrations are associated with developing AD in the future,22–25 a question that remains to be answered is whether plasma Aβ levels are driven by the genes implicated in AD.

To answer this question, we conducted a GWAS, hypothesizing that if we find that AD genes primarily determine plasma Aβ levels, it is likely that plasma Aβ is an endophenotype for AD. Alternatively, if we find that plasma Aβ is primarily associated to genes implicated in blood platelet function, or kidney or pancreas pathology, the findings argue against the hypothesis that Aβ in blood is an endophenotype for AD. We previously conducted a GWAS meta-analysis of plasma Aβ levels in 3528 non-demented participants, but failed to find genome-wide significant associations,28 indicating a lack of power related to the measurement or the sample size. At present, the more sensitive measures are not yet available in large samples with genome-wide genetic data. We therefore aimed to increase the studied sample size of our previous work. The present study is a GWAS meta-analysis of plasma Aβ levels in more than 12,000 individuals aiming to elucidate processes that determine plasma Aβ.

2 METHODS

2.1 Study populations

We included data from 12,369 European-descent participants from eight studies, the Framingham Heart Study (FHS; n = 6735), the Rotterdam study (RS; n = 1958), the Three City Study (3C; n = 1954), the Atherosclerosis Risk in Communities Study (ARIC; n = 830), the Washington Heights-Inwood Community Aging Project (WHICAP; n = 193), the Epidemiological Prevention Study of Zoetermeer (EPOZ; n = 397), the Alzheimer’s Disease Neuroimaging Initiative (ADNI; n = 173), and the Erasmus Rucphen Family Study (ERF; n = 129). In each study, we excluded participants with prevalent dementia at the time of blood sampling used for plasma Aβ assessment (see Materials and Methods 1 in supporting information for a detailed description of each study).

2.2 Plasma Aβ assessment

Each study used different protocols for blood sampling, plasma extraction, and storage and plasma Aβ assessment that have been detailed in previous publications.22,23,25,29–31 In the FHS, RS, and 3C studies, plasma Aβ levels were measured at different times because of cost considerations. Various assays were used to quantify plasma Aβ1-40 and Aβ1-42 levels (see Materials and Methods 2 in supporting information for a detailed description of the protocols used in each study and Table S1 in supporting information for baseline characteristics of the study populations).

2.3 Genotyping

Each study used different genotyping platforms as previously published.11 After applying pre-imputation variant and sample filters, genotypes were imputed using the 1000 Genomes phase 1 version 3 (all ethnicities) imputation panel and various imputation pipelines (see Methods 3 in supporting information). APOE genotyping was performed as part of protocols specific to each study (see Methods 4 in supporting information).
2.4 | Statistical analyses

2.4.1 | Plasma Aβ levels

Plasma Aβ levels were expressed as pg mL⁻¹. In each study and for each Aβ dosage, we excluded values that were over or below four standard deviations around the mean. To study the variations of plasma Aβ levels in a consistent way across studies, we performed a ranked-based inverse normal transformation of plasma Aβ levels in each study. If they were significantly associated with plasma Aβ levels, this transformation was performed after adjusting for batch effect and other technical artifacts.

2.4.2 | Genome-wide association studies

Each study performed GWAS of plasma Aβ1-40 and Aβ1-42 levels and Aβ1-42/Aβ1-40 ratio using 1000 Genomes imputed data. According to the imputation pipelines used, genetic information was available either as allele dosages or genotype probabilities. In each study, we excluded results from variants that had low imputation quality (r² or info score < 0.3), variants with low frequency (minor allele frequency < 0.005 or minor allele count < 7), and variants that were available in a small number of participants (n < 30). Association of genetic variations with plasma Aβ levels were assessed in linear regression models adjusted for sex and age at blood collection. If significantly associated with plasma Aβ levels, principal components were added in the models to account for population structure.

2.4.3 | Genome-wide meta-analysis

Before meta-analysis, we applied a series of filters and quality check that were previously published (see Figures S1 and S2 in supporting information). We performed an inverse variance weighted genome-wide meta-analysis, accounting for genomic inflation factors using the METAL software. Finally, we retained variants that had been meta-analyzed at least in the three largest available populations (FHS, RS, and 3C). Statistical significance was defined as a P-value below 5×10⁻⁸. Signals with P-values between 1×10⁻⁵ and 5×10⁻⁸ were considered suggestive. Additional graphs and analyses were done using R v3.6.1. To confirm the APOE signal we obtained in our genome-wide meta-analysis, we reran our analysis using genotyped APOE ε4 and APOE ε2 status, adjusting for age and sex.

2.4.4 | Gene-based and pathway analyses

We tested aggregated effects of single nucleotide polymorphisms (SNPs) located within genes using the multi-marker analysis of genomic annotation (MAGMA) v1.07 tool. For each dosage, a total of 18,089 genes were tested, resulting in a significance threshold of 2.76×10⁻⁶. Pathway analyses were also performed with MAGMA v1.07. The following gene sets were used: GO (biological process, cellular component and molecular function, KEGG, Biocarta, and Reactome). Pathway P-values were corrected for multiple testing using the false discovery rate (FDR) method.

2.4.5 | Expression quantitative trait loci (eQTL) analysis

We looked at effect on gene expression of (1) genome-wide significant variants and (2) variants with a P-value below 10⁻⁵ and belonging to the same loci as the genome-wide significant variants. We used the GTEx v8 dataset (https://gtexportal.org) and considered the following tissues: whole blood, kidney, pancreas, lymphoblastoid cell line, and brain (amygdala, anterior cingulate cortex, caudate basal ganglia, cerebellar hemisphere, cerebellum, cortex, frontal cortex, hippocampus, hypothalamus, nucleus accumbens [basal ganglia], putamen basal [basal ganglia], cerebral spinal cord, substantia nigra).

2.4.6 | Association analyses with Aβ brain deposition

We related allelic variation at the SNP of interest with a standard measure of amyloid burden in the brain on PET imaging in 193 middle-aged, dementia-free FHS participants (see Materials and Methods 5...
in supporting information for a detailed description of the protocols used). As a pre-specified hypothesis, we examined this association separately for persons with at least one APOE ε4 allele and those without. We report the odds ratio of having a positive amyloid scan associated with having a single copy of the allele of interest, using additive genetic models adjusted for age and sex.

2.4.7 Association with AD

For significant variants and genes, we checked for association with AD. Summary statistics from the most recent genetic meta-analyses of AD were used.12,37

3 RESULTS

3.1 Genome-wide significant variants associated with plasma Aβ levels

After meta-analysis, we identified 21 variants reaching genome-wide significance across two loci (Figures S3 to S8 in supporting information).

The first locus was located on chromosome 19, in the APOE gene, with significant associations with plasma Aβ1-42 levels and plasma Aβ1-42/Aβ1-40 ratio (Figures 1 and 2). For both associations, the most significant variant was rs429358 with $P$-values of $9.01 \times 10^{-13}$ and $6.46 \times 10^{-20}$ for Aβ1-42 levels and Aβ1-42/Aβ1-40 ratio, respectively (Table 1). The minor allele of this variant, which denotes APOE ε4, was associated with lower plasma Aβ1-42 levels (effect size = $-0.167$ standard deviations (SD); 95% confidence interval (CI) = $[-0.212; -0.121]$ and lower plasma Aβ1-42/Aβ1-40 ratio (effect size = $-0.212$ SD; 95% CI = $[-0.257; -0.121]$; Table 1 and Figure S9 in supporting information). We confirmed these associations using the directly genotyped APOE ε4 status (Figure S10 in supporting information).

The second genome-wide significant locus was an intronic variant in the RNF214 gene. The function on RNF214 is largely unknown. The gene is located on chromosome 11, near the BACE1 gene. BACE1 encodes β-secretase and is involved in the initial, Aβ-producing step of APP processing (Figure 3). For the most significant variant, rs650585, the minor allele was associated with lower plasma Aβ1-40 levels (effect size = $-0.073$ SD; 95% CI = $[-0.121; -0.0212]$ and lower plasma Aβ1-42/Aβ1-40 ratio (effect size = $-0.212$ SD; 95% CI = $[-0.257; -0.121]$; Table 1 and Figure S9 in supporting information). This variant is in linkage disequilibrium (LD; $R^2$ = 0.75, 1000 Genomes phase 3) with a BACE1 synonymous variant, rs638405, which was also associated with plasma Aβ1-40 levels (effect size = $-0.071$ SD, $P$-value = $1.21 \times 10^{-7}$). For
FIGURE 2  Association of frequent genetic variants with plasma amyloid beta (Aβ)1-42/Aβ1-40 ratio in the apolipoprotein E locus

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Association of top variants from genome-wide significant loci with plasma Aβ levels and amyloid-related traits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EAF</td>
</tr>
<tr>
<td>rs650585(chr11:117110740, T/C, intron; RNF214/BACE1)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>Aβ1-40</td>
</tr>
<tr>
<td></td>
<td>Aβ1-42</td>
</tr>
<tr>
<td></td>
<td>Aβ1-42/Aβ1-40 ratio</td>
</tr>
<tr>
<td></td>
<td>AD risk$^a$</td>
</tr>
<tr>
<td>rs429358(chr19:45411941, C/T, missense; APOE)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>Aβ1-40</td>
</tr>
<tr>
<td></td>
<td>Aβ1-42</td>
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<td></td>
<td>Aβ1-42/Aβ1-40 ratio</td>
</tr>
<tr>
<td></td>
<td>AD risk$^a$</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ, amyloid beta; AD: Alzheimer’s disease; EAF: effect allele frequency; SNP, single nucleotide polymorphism.

Notes: For plasma measures, “Effect” represents the mean variation of the standardized variable (i.e., transformed so that mean = 0 and standard deviation = 1). In each block, the rsID of the top SNP is followed by its GRCh37 position, effect/non-effect alleles, functional category and closest genes. $^a$results obtained from Kunkle et al.12
plasma Aβ1-40 levels, eQTL analysis showed an effect of variants belonging to loci significantly associated with plasma Aβ levels mainly on the expression of CEP164 and BACE1 in blood, and on the expression of CEP164 in several brain regions (Table S2 in supporting information). No effect on RNF214 expression was found. For plasma Aβ1-42 levels and Aβ1-42/Aβ1-40 ratio, we only observed an effect on the expression of NECTIN2.

3.2 Gene and pathway-based analyses of plasma Aβ levels

Next, we performed gene-based tests (Table 2, Figures S6-S8). We again observed the APOE, RNF214, and BACE1 genes ($P = 3.87 \times 10^{-13}$, $P = 2.33 \times 10^{-7}$, and $P = 3.2 \times 10^{-9}$, respectively), for which we had identified genome-wide significant single variant associations. In addition to these genes, four genes showed gene-wide significant signals ($P < 2.76 \times 10^{-6}$). We found that the APP and PSEN2 genes were associated with plasma Aβ1-40 levels ($P = 1.67 \times 10^{-7}$ and $P = 2.63 \times 10^{-6}$, respectively). Interestingly, at the SNP level, there were two peaks reaching suggestive evidence for association with Aβ1-40 levels in APP gene (Figure S11 in supporting information), probably explaining its strong association at the gene level. The two other genes were CCK, associated with plasma Aβ1-40 levels ($P = 2.63 \times 10^{-6}$), and ZNF397, associated with plasma Aβ1-42/1-40 ratio ($P = 2.27 \times 10^{-6}$). The formal pathway analyses did not yield any significant results (Tables S3-S5 in supporting information).

3.3 Association of the BACE1 locus with PET Aβ deposition

We tested the association of the top hit rs650585 from the BACE1 locus (see above) with Aβ deposition in the brain from subsets of the FHS population. We found an association of rs650585 with an increase of deposition of Aβ in FHS-Gen3 only among APOE ε4–positive individuals ($P = 0.02$; Table S6 in supporting information).

3.4 Variants associated with plasma amyloid associate with the risk of AD

The APOE ε4 allele is known to be associated with a higher risk of AD.38 We did not find significant evidence for association between the
TABLE 2  Associations of variants aggregated according to genes with plasma Aβ levels  

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Chromosome</th>
<th>Start position</th>
<th>Stop position</th>
<th>N. SNPs</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSEN2</td>
<td>1</td>
<td>227,057,885</td>
<td>227,083,806</td>
<td>84</td>
<td>2.63 × 10⁻⁶</td>
</tr>
<tr>
<td>CCK</td>
<td>3</td>
<td>42,299,317</td>
<td>42,307,699</td>
<td>20</td>
<td>2.63 × 10⁻⁶</td>
</tr>
<tr>
<td>RNF214</td>
<td>11</td>
<td>117,103,341</td>
<td>117,157,161</td>
<td>143</td>
<td>2.33 × 10⁻⁷</td>
</tr>
<tr>
<td>BACE1</td>
<td>11</td>
<td>117,156,402</td>
<td>117,186,975</td>
<td>70</td>
<td>3.20 × 10⁻⁹</td>
</tr>
<tr>
<td>APP</td>
<td>21</td>
<td>27,252,861</td>
<td>27,543,446</td>
<td>787</td>
<td>1.67 × 10⁻⁷</td>
</tr>
<tr>
<td>APOE</td>
<td>19</td>
<td>45,409,011</td>
<td>45,412,650</td>
<td>2</td>
<td>3.14 × 10⁻¹⁰</td>
</tr>
<tr>
<td>APOC1</td>
<td>19</td>
<td>45,417,504</td>
<td>45,422,606</td>
<td>3</td>
<td>2.52 × 10⁻⁹</td>
</tr>
<tr>
<td>ZNF397</td>
<td>18</td>
<td>32,820,994</td>
<td>32,847,097</td>
<td>48</td>
<td>2.27 × 10⁻⁶</td>
</tr>
<tr>
<td>APOE</td>
<td>19</td>
<td>45,409,011</td>
<td>45,412,650</td>
<td>2</td>
<td>3.87 × 10⁻¹³</td>
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<td>19</td>
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<td>3</td>
<td>6.79 × 10⁻¹³</td>
</tr>
</tbody>
</table>

Start and stop positions are given according to GRCh37. Gene-wide significance level is computed for 18,089 genes, i.e., 2.76 × 10⁻⁶.  
Abbreviations: Aβ, amyloid beta; SNP, single nucleotide polymorphism.

4 | DISCUSSION

To uncover the genes that determine plasma Aβ levels, we performed a GWAS of plasma Aβ in 12,369 non-demented subjects. Although we did not use recently developed high sensitivity methods, we found that plasma Aβ levels are determined by variants in and near the major AD genes: APOE, BACE1, PSEN2, and APP. The proteins these genes encode for are known to be involved in Aβ processing. A novel finding is that the variants near the BACE1 gene were found to be associated with Aβ in the brain as measured by PET imaging and these variants were also found to be associated with the risk of AD. We also identified additional signals for new genes implicated in Aβ levels in blood, CCK and ZNF397.

The BACE1 region encompasses several genes (PCSK7, RNF214, BACE1, CEP164) and a BACE1 anti-sense long non-coding RNA (BACE1-AS). Although the top variant in the GWAS is located in an intron of RNF214, the gene-based and eQTL analyses suggest BACE1 is likely the causal gene in the region. The fact that β-secretase activity of BACE1 is necessary for Aβ peptide production makes it highly likely that BACE1 or a local regulation of BACE1 explains most likely the association of the region to plasma Aβ levels. We also found gene-wide significant associations with plasma Aβ1-40 levels in APP and PSEN2, two major actors of Aβ metabolism. The APP gene is a key driver of its own metabolism in blood and PSEN2 is a key player of the γ-secretases, which process the APP C99 fragment into Aβ peptides.3 APOE ε4 allele had the strongest association with Aβ1-42 levels but was not even nominally associated with Aβ1-40. This suggests that the APOE ε4 isofrom is not involved early in the process of Aβ peptide production but rather in more downstream events, such as Aβ aggregation or clearance. These results might also illustrate the greater propensity of Aβ1-42 peptides to aggregate compared to Aβ1-40, and the influence of APOE isoforms in the regulation of this aggregation process.10

Interestingly, associations of APOE ε2 with plasma Aβ levels were not significant and effect sizes were very small. Contrary to APOE ε4, the effect of APOE ε2 on amyloid markers has been much less well studied and research has been focused on specific brain regions.39 Alternatively, other, Aβ-independent, mechanisms such as vascular pathology may explain the lower risk of AD observed in APOE ε2 carriers.40

As to the novel genes identified, CCK and ZNF397, to date these have not been associated with Aβ peptides in the circulation such as blood platelets, kidney, or pancreas pathology. The CCK gene is located in a region that was reported in a GWAS of neurofibrillary tangles but not Aβ.16 CCK or cholecystokinin is a neuropeptide that is widely distributed in the brain and highly expressed in brain regions like the hippocampus. Sulfated cholecystokinin-B may modulate neuronal activity in the brain but its function in the brain is far from clear. The protein is located in axons, dendrites, and the neuronal cell body and is involved in gastrin signaling and insulin secretion but also in neuron...
migration. CCK regulates pancreatic enzyme secretion and gastrointestinal motility, and acts as a satiety signal. CCK is released simultaneously from intestinal cells and neurons in response to a meal and thus may be implicated in the metabolic effects seen in and outside the brain including weight loss. The diseases associated with CCK include cholecystitis and biliary dyskinesia, the latter being of interest in light of the finding that bile acids have been found to be associated to the risk of AD and brain pathology. The other novel locus, the ZNF397 gene, encodes a protein with a N-terminal SCAN domain, and the longer isoform contains nine C2H2-type zinc finger repeats in the C-terminal domain. The protein localizes to centromeres during interphase and early prophase, and different isoforms can repress or activate transcription in transfection studies. Interestingly, the SNP rs509477, suggestively associated with CSF Aβ1-42 in a small association study, is located in an enhancer of ZNF397 (Genecards: GH18J034976), acting in the hippocampus middle, anterior caudate, and cingulate gyrus brain regions. Although this SNP was not associated with any Aβ levels or ratio in our study, our findings do support the hypothesis that ZNF397 plays a role in Aβ metabolism.

Our analysis shows associations of plasma Aβ levels mainly with genes that have been previously identified as involved in AD (APOE, APP, PSEN2), and other genes that are nominally associated to AD and are expressed in the brain. According to the hypothesis outlined in the introduction, it is likely that plasma Aβ is an endophenotype for AD. Although we cannot prove the origin, our findings suggest also that Aβ peptides measured in the blood circulation for a large part originate from the brain rather than from the pancreas or the kidney. This hypothesis is in line with recent observations showing correlation of Aβ levels in blood with its levels in CSF as well as with its deposition in brain as assessed by PET imaging.

Plasma Aβ has been long considered a poor predictive biomarker of AD risk, partially due to lack of precision and reproducibility in the assays that were available. A previous meta-analysis reported that plasma Aβ levels were not useful to make a clinical diagnosis of AD. However, as assays improved, several of the large cohorts participating in the present study have reported that low plasma Aβ42 and Aβ42/40 ratio levels were modestly associated with risk of development of AD after several years of follow-up, suggesting that they are valid endophenotypes of at least one biological process underlying AD risk. The results of the present study are consistent with the hypothesis that Aβ in blood reflects some aspects of brain AD pathophysiology and this view is strengthened by our present observation that APOE ε4 is both associated with low plasma Aβ42 and Aβ42/40 ratio and high AD risk. Some of studies have also reported that this association remained significant after adjusting for APOE ε4. Hence, plasma Aβ levels could prove useful as a biomarker of amyloid metabolism pathways in the brain and could be an accessible marker of target engagement for preventive interventions focused on this pathway. In this light there are intriguing reports that hemodialysis or peritoneal dialysis are able to lower Aβ not only in the blood, but also in the brain. Further, the association we observed between variants near BACE1 and plasma Aβ40 is also of interest in the light of the recent (disappointing) trials testing BACE inhibitors. Measuring Aβ40 in blood might help us understand the overall failure of these trials or identify responsive subgroups if we examined genetic variation among trial participants and the lack of association of these variants with AD risk could be further investigated.

Our study has several strengths. First, it, is, to date, the largest study of circulating amyloid peptides. This enabled us to identify biological factors underlying peripheral Aβ metabolism and the overlap of the genetic signals with those underlying brain pathology and AD risk, suggesting blood levels of Aβ may have clinical utility. Second, this study was conducted in non-demented participants and therefore is relevant for the study of early amyloid pathophysiological processes. Third, we carefully normalized the plasma Aβ data before running GWAS, thus taking into account some of the heterogeneity that has been described when using plasma Aβ levels.

Our study also has limitations. The state of current knowledge makes it difficult to ascertain if there is a causal role of plasma Aβ on the brain’s accrual of amyloid and further experimental research in this area is needed. Second, the assays used in this study non-selectively measured Aβ concentrations and could not distinguish monomers from oligomers of Aβ, whether free or protein-bound. Therefore, our interpretation of the present results might differ from other studies in which assays selectively measured monomers or oligomers of Aβ. Future studies with the novel assays that allow measurements of each form of Aβ will facilitate interpretation with regard to the balance between Aβ production, aggregation, and clearance. Thus, although our approach explores brain neurobiology through the study of plasma levels, the imperfect instrument used to determine this plasma endophenotype and the higher inter-assay variability requires further research of Aβ biomarkers in blood using state-of-the-art technology.

In summary, our results indicate that genetic determinants of plasma Aβ40 and Aβ42 levels are close to genes known to be central actors in APP metabolism in AD. Increasing the statistical power of plasma Aβ analyses may potentially lead to the identification of currently unknown players in Aβ metabolism; novel hypotheses; and, hopefully, new preventive or therapeutic targets against AD. In the future, the role of these genetic variants also needs to be explored further in AD animal models.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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