

RESEARCH ARTICLE

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 Lee^{2,3} | Vincent Chouraki^{1,4} | Benjamin Grenier-Boley¹ | Jeannette Simino⁵ | Hieab Adams⁶ | Giuseppe Tosto^{7,8} | Charles White^{9,10} | Natalie Terzikhan^{3,11} | Carlos Cruchaga¹² | Maria J. Knol³ | Shuo Li^{13,14} | Susanna Schraen¹⁵ | Megan L. Grove¹⁶ | Claudia Satizabal^{4,14} | Najaf Amin³ | Claudine Berr¹⁷ | Steven Younkin¹⁸ | Alzheimer's Disease Neuroimaging Initiative¹ | Rebecca F. Gottesman^{19,20} | Luc Buée^{1,21} | Alexa Beiser^{4,13,14} | David S. Knopman²² | Andre Uitterlinden²³ | Charles DeCarli²⁴ | Jan Bressler¹⁶ | Anita DeStefano^{4,13,14} | Jean-François Dartigues²⁵ | Qiong Yang^{13,14} | Eric Boerwinkle^{16,26} | Christophe Tzourio²⁵ | Myriam Fornage^{16,27} | M. Arfan Ikram⁶ | Philippe Amouyel¹ | Phil de Jager^{9,10,28} | Christiane Reitz^{7,8,29,30} | Thomas H. Mosley³¹ | Jean-Charles Lambert¹ | Sudha Seshadri^{4,14,32} | Cornelia M. van Duijn^{3,33}

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 Center Columbia 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 U1061 University of Montpellier, Montpellier, France

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¹⁸ Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA

¹⁹ Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

²⁰ Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

²¹ Institut National de la Santé et de la Recherche Médicale (INSERM, Université de Lille, Lille, France

²² Department of Neurology, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

²³ Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

²⁴ Department of Neurology, University of California at Davis, Davis, California, USA

²⁵ Bordeaux Population Health Research Center, INSERM, UMR1219Bordeaux University, Bordeaux, France

²⁶ Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, USA

²⁷ Brown Foundation Institute of Molecular Medicine, McGovern Medical SchoolThe University of Texas Health Science Center at Houston, Houston, Texas, USA

²⁸ Center for Translational & Systems Neuroimmunology, Department of Neurology, Columbia University Medical Center, New York, New York, New York, USA

²⁹ Department of Neurology, Columbia University, New York, New York, USA

³⁰ Department of Epidemiology, Columbia University, New York, New York, USA

³¹ Department of Medicine, Gertrude C. Ford MIND CenterUniversity of Mississippi Medical Center, Jackson, Mississippi, USA

³² Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, UT Health San Antonio, San Antonio, Texas, USA

³³ Nuffield Department of Population Health, University of Oxford, Oxford, UK

Correspondence

Cornelia M van Duijn, Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, Old Road Campus, Oxford OX3 7LF, UK.

E-mail: Cornelia.vanDuijn@ndph.ox.ac.uk

Vincent Damotte, Sven J. van der Lee, Jean-Charles Lambert, Sudha Seshadri, Cornelia M. van Duijn, and Vincent Chouraki contributed equally to this work.

Abstract

Introduction: There is increasing interest in plasma amyloid beta ($A\beta$) as an endophenotype of Alzheimer's disease (AD). Identifying the genetic determinants of plasma $A\beta$ levels may elucidate important biological processes that determine plasma $A\beta$ measures.

Methods: We included 12,369 non-demented participants from eight population-based studies. Imputed genetic data and measured plasma $A\beta$ 1-40, $A\beta$ 1-42 levels and $A\beta$ 1-42/ $A\beta$ 1-40 ratio were used to perform genome-wide association studies, and gene-based and pathway analyses. Significant variants and genes were followed up for their association with brain positron emission tomography $A\beta$ deposition and AD risk.

Results: Single-variant analysis identified associations with apolipoprotein E (*APOE*) for $A\beta$ 1-42 and $A\beta$ 1-42/ $A\beta$ 1-40 ratio, and *BACE1* for $A\beta$ 1-40. Gene-based analysis of $A\beta$ 1-40 additionally identified associations for *APP*, *PSEN2*, *CCK*, and *ZNF397*. There was suggestive evidence for interaction between a *BACE1* variant and *APOE* ϵ 4 on brain $A\beta$ deposition.

Discussion: Identification of variants near/in known major $A\beta$ -processing genes strengthens the relevance of plasma- $A\beta$ levels as an endophenotype of AD.

KEYWORDS

Alzheimer's disease, *APOE*, *APP*, *BACE1*, endophenotype, genetic epidemiology, genome-wide association study, plasma amyloid beta levels, plasma biomarkers, preclinical biomarkers, *PSEN2*

1 | INTRODUCTION

Amyloid beta ($A\beta$) deposition is one of the hallmarks of Alzheimer's disease (AD). $A\beta$ peptides are the products of the catalytic processing of the $A\beta$ precursor protein (APP) by the β -secretase, *BACE1*, and the γ -secretase complex.¹ $A\beta$ peptides are able to self-assemble in soluble $A\beta$ 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 AD² and cerebral amyloid angiopathy (CAA),

which is seen in many patients.³ $A\beta$ 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 $A\beta$ 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 $A\beta$ 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*) ϵ 4, the major

genetic risk factor for AD in the general population,⁹ has been implicated in A β aggregation, deposition, and clearance, both in the brain and in blood vessels.^{7,10} Although for a long time the A β pathway did not emerge in our genome-wide association studies (GWAS) of AD,¹¹ our most recent GWAS study highlighted the A β processing pathway and APP catabolic process pathway in late-onset Alzheimer's disease (LOAD).¹² We and others have also explored the genetics of A β through GWAS on quantitative measures of A β 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 A β 42.¹⁷

Although A β 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 A β metabolism in blood. Unlike p-tau and NFL, A β peptides in the blood circulation are not brain specific. A β peptides produced in the brain can be degraded locally or transported into the CSF and the blood stream.¹⁸ However, A β peptides in the circulation can also be derived from blood platelets, kidney, or pancreas. Although the brain-derived A β peptides in the circulation cannot be distinguished from A β derived from blood platelets, the kidneys, or the pancreas, a recent study using immunoprecipitation coupled with mass spectrometry to measure plasma A β 1-40/A β 1-42 and APP/A β 1-42 ratios was able to accurately predict individual brain A β -positive or -negative status.¹⁹ Also, studies assessing A β 1-40 and A β 1-42 using immunoassays show that these can predict A β 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 A β 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 A β levels, we found modest but significant correlation with amyloid burden in the CSF and in the brain.^{26,27} Whereas A β 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 A β 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 A β concentrations are associated with developing AD in the future,²²⁻²⁵ a question that remains to be answered is whether plasma A β 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 A β levels, it is likely that plasma A β is an endophenotype for AD. Alternatively, if we

find that plasma A β is primarily associated to genes implicated in blood platelet function, or kidney or pancreas pathology, the findings argue against the hypothesis that A β in blood is an endophenotype for AD. We previously conducted a GWAS meta-analysis of plasma A β 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 A β levels in more than 12,000 individuals aiming to elucidate processes that determine plasma A β .

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 A β assessment (see Materials and Methods 1 in supporting information for a detailed description of each study).

2.2 | Plasma A β assessment

Each study used different protocols for blood sampling, plasma extraction, and storage and plasma A β assessment that have been detailed in previous publications.^{22,23,25,29-31} In the FHS, RS, and 3C studies, plasma A β levels were measured at different times because of cost considerations. Various assays were used to quantify plasma A β 1-40 and A β 1-42 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).

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional (e.g., PubMed) sources and meeting abstracts and presentations. Genome-wide association studies have not yet identified variants associated with plasma amyloid beta ($A\beta$)1-40, $A\beta$ 1-42 levels and $A\beta$ 1-42/ $A\beta$ 1-40 ratio, probably due to limited sample sizes.
2. Interpretation: Our findings identified two genome-wide significant loci in apolipoprotein E (APOE) and BACE1 regions associated with plasma $A\beta$ levels in 12,369 non-demented subjects. A gene-based approach confirmed the association with APOE and BACE1 genes and identified additional signals in APP, PSEN2, CCK, and ZNF397 genes. We also showed a suggestive interaction between the most significant BACE1 variant and APOE ϵ 4 with $A\beta$ deposition in the brain using positron emission tomography imaging.
3. Future directions: We propose to further explore the biology underlying both circulating and brain amyloid levels using larger, multiomic samples, new plasma beta-amyloid assays, and induced pluripotent stem cells-based methods.

2.4 | Statistical analyses**2.4.1 | Plasma $A\beta$ levels**

Plasma $A\beta$ levels were expressed as pg mL^{-1} . In each study and for each $A\beta$ dosage, we excluded values that were over or below four standard deviations around the mean. To study the variations of plasma $A\beta$ levels in a consistent way across studies, we performed a ranked-based inverse normal transformation of plasma $A\beta$ levels in each study. If they were significantly associated with plasma $A\beta$ 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 $A\beta$ 1-40 and $A\beta$ 1-42 levels and $A\beta$ 1-42/ $A\beta$ 1-40 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 (r^2 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 $A\beta$ levels were assessed in linear regression models adjusted for sex and age at blood collection. If significantly

associated with plasma $A\beta$ 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).³² We performed an inverse variance weighted genome-wide meta-analysis, accounting for genomic inflation factors using the METAL software.³³ 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 ϵ 4 and APOE ϵ 2 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.³⁴ 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 v1.07.³⁴ 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], cervical spinal cord, substantia nigra).

2.4.6 | Association analyses with $A\beta$ brain deposition

We related allelic variation at the SNP of interest with a standard measure of amyloid burden in the brain on PET imaging³⁵ in 193 middle-aged, dementia-free FHS participants³⁶ (see Materials and Methods 5

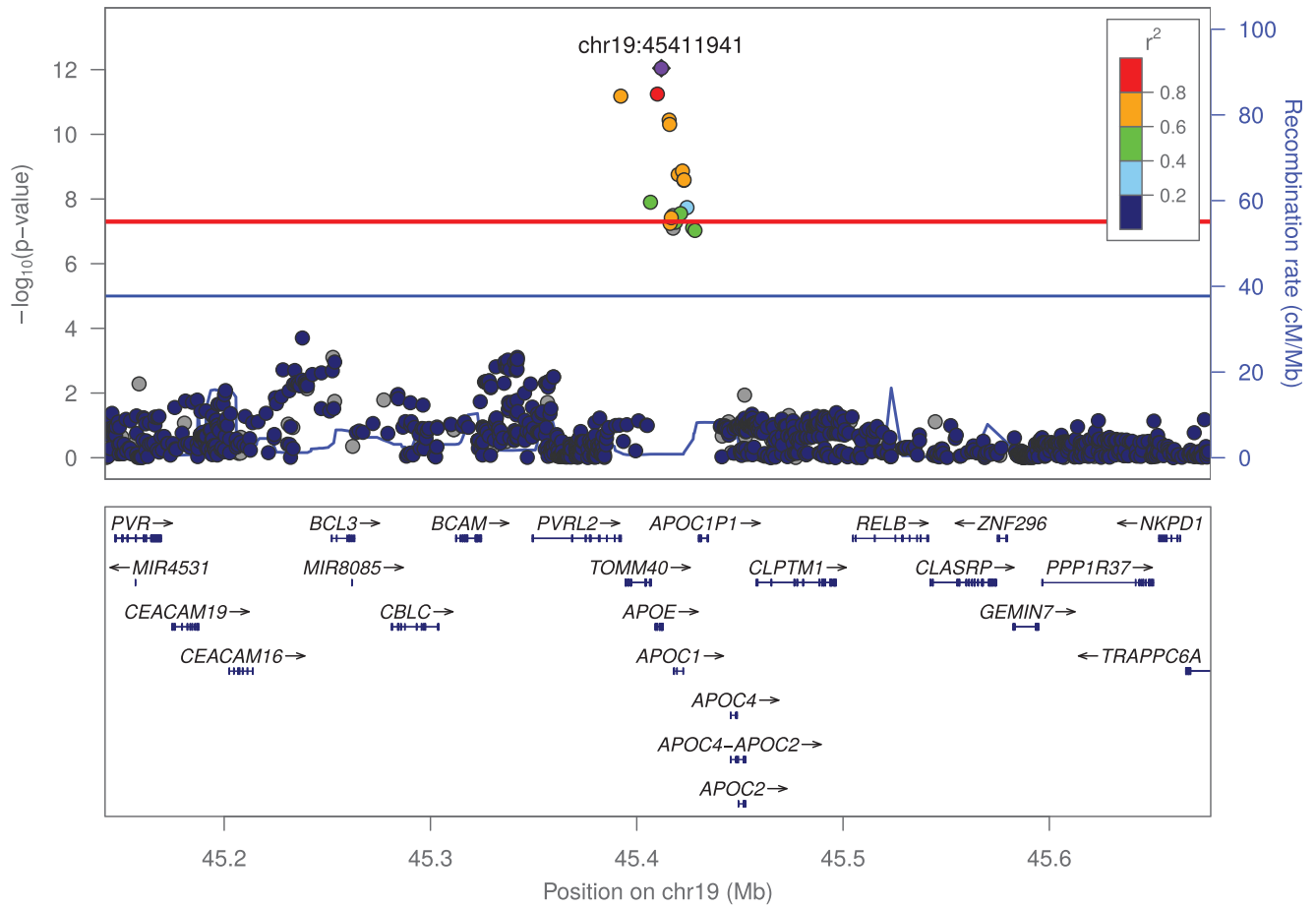


FIGURE 1 Association of frequent genetic variants with plasma amyloid beta ($A\beta$)1-42 in the apolipoprotein E locus

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* ϵ 4 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 $A\beta$ 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 $A\beta$ 1-42 levels and plasma $A\beta$ 1-42/ $A\beta$ 1-40 ratio (Figures 1 and 2). For both associations, the most significant variant was rs429358 with *P*-values of 9.01×10^{-13} and 6.46×10^{-20} for $A\beta$ 1-42 levels and $A\beta$ 1-42/ $A\beta$ 1-40 ratio, respectively (Table 1). The minor allele of this variant, which denotes *APOE* ϵ 4, was associated with lower plasma $A\beta$ 1-42 levels (effect size = -0.167 standard deviations (SD); 95% confidence interval (CI) = $[-0.212; -0.121]$) and lower plasma $A\beta$ 1-42/ $A\beta$ 1-40 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* ϵ 4 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, $A\beta$ -producing step of APP processing (Figure 3). For the most significant variant, rs650585, the minor allele was associated with lower plasma $A\beta$ 1-40 levels (effect size = -0.073 SD; 95% CI = $[-0.099; -0.047]$; *P*-value = 2.56×10^{-8} ; Table 1 and Figure S9). 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 $A\beta$ 1-40 levels (effect size = -0.071 SD, *P*-value = 1.21×10^{-7}). For

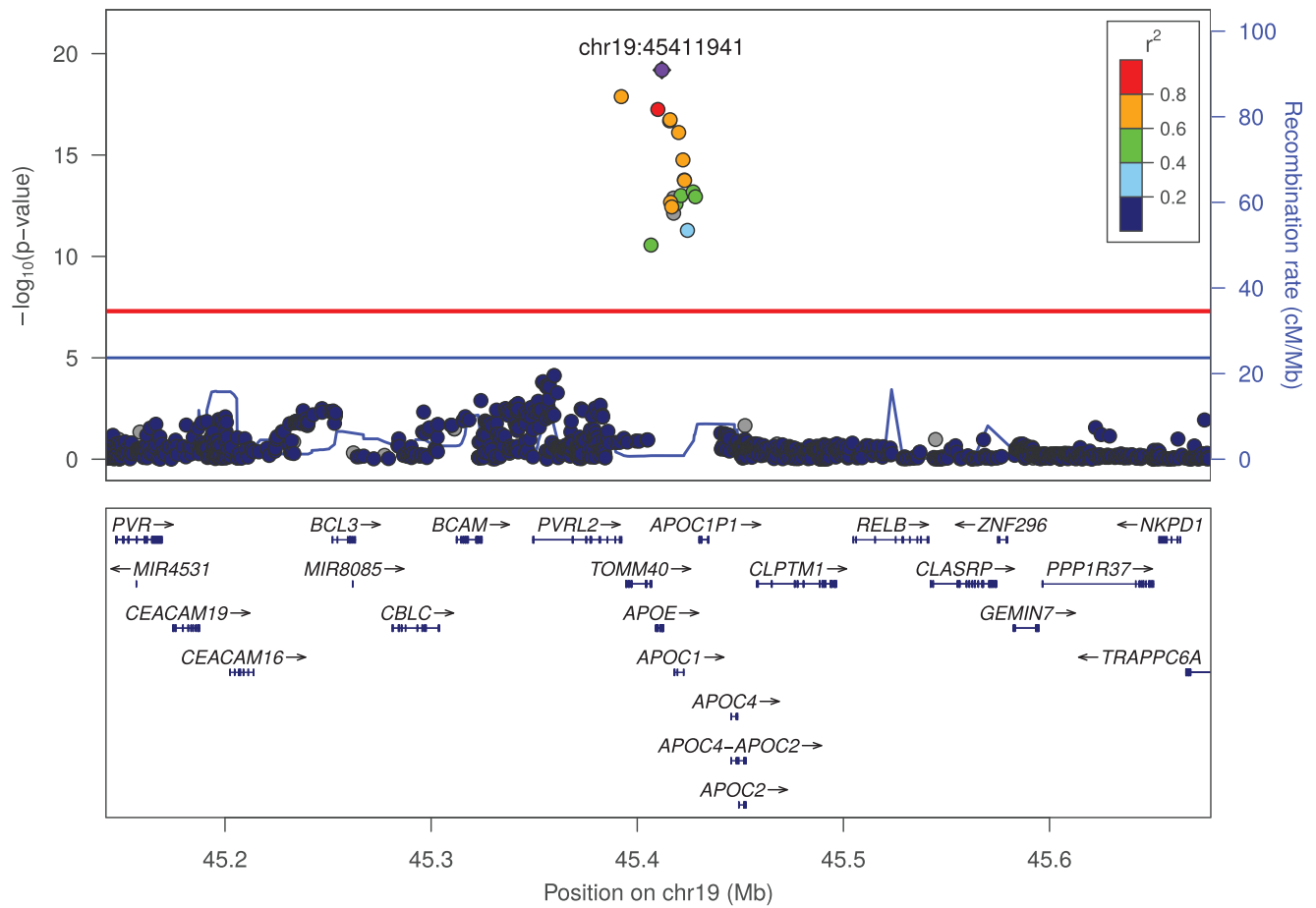


FIGURE 2 Association of frequent genetic variants with plasma amyloid beta ($A\beta$)1-42/ $A\beta$ 1-40 ratio in the apolipoprotein E locus

TABLE 1 Association of top variants from genome-wide significant loci with plasma $A\beta$ levels and amyloid-related traits

	EAF	Effect	Standard Error	P-value	r^2
rs650585 (chr11:117110740, T/C, intron; RNF214/BACE1)					
<i>Plasma</i>					
$A\beta$ 1-40	41.3%	-0.073	0.013	2.56×10^{-8}	3.1%
$A\beta$ 1-42	41.3%	-0.035	0.013	9.57×10^{-3}	27.8%
$A\beta$ 1-42/ $A\beta$ 1-40 ratio	41.4%	0.033	0.013	1.39×10^{-2}	0.0%
<i>AD risk</i> ^a	40.8%	0.033	0.015	2.30×10^{-2}	8.3%
rs429358 (chr19:45411941, C/T, missense; APOE)					
<i>Plasma</i>					
$A\beta$ 1-40	13.4%	0.023	0.023	3.11×10^{-1}	23.7%
$A\beta$ 1-42	13.4%	-0.167	0.023	9.01×10^{-13}	32.3%
$A\beta$ 1-42/ $A\beta$ 1-40 ratio	13.4%	-0.212	0.023	6.46×10^{-20}	52.6%
<i>AD risk</i> ^a	21.6%	1.20	0.019	$.00 \times 10^{+00}$	72.1%

Abbreviations: $A\beta$, 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. ^aresults obtained from Kunkle et al.¹²

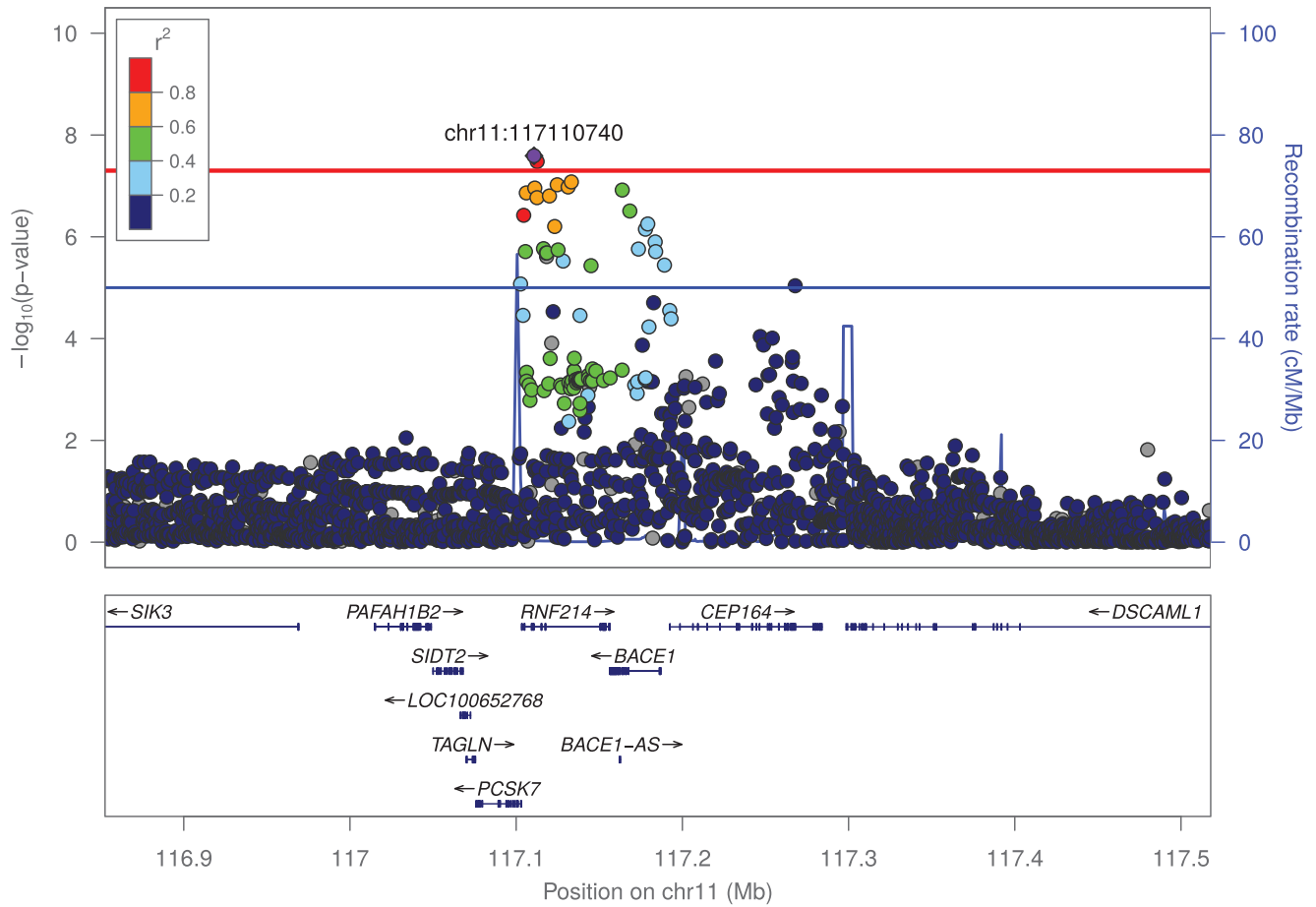


FIGURE 3 Association of frequent genetic variants with plasma amyloid beta ($A\beta$)1-40 in the *BACE1* locus

plasma $A\beta$ 1-40 levels, eQTL analysis showed an effect of variants belonging to loci significantly associated with plasma $A\beta$ 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 $A\beta$ 1-42 levels and $A\beta$ 1-42/ $A\beta$ 1-40 ratio, we only observed an effect on the expression of *NECTIN2*.

3.2 | Gene and pathway-based analyses of plasma $A\beta$ 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 $A\beta$ 1-40 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 $A\beta$ 1-40 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 $A\beta$ 1-40 levels ($P = 2.63 \times 10^{-6}$), and *ZNF397*, associated with plasma $A\beta$ 1-42/1-40 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 $A\beta$ deposition

We tested the association of the top hit rs650585 from the *BACE1* locus (see above) with $A\beta$ deposition in the brain from subsets of the FHS population. We found an association of rs650585 with an increase of deposition of $A\beta$ in FHS-Gen3 only among *APOE* ϵ 4-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* ϵ 4 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 A β levels

Gene symbol	Chromosome	Start position	Stop position	N. SNPs	P-value
<i>Plasma Aβ1-40</i>					
PSEN2	1	227,057,885	227,083,806	84	2.63×10^{-6}
CCK	3	42,299,317	42,307,699	20	2.63×10^{-6}
RNF214	11	117,103,341	117,157,161	143	2.33×10^{-7}
BACE1	11	117,156,402	117,186,975	70	3.20×10^{-9}
APP	21	27,252,861	27,543,446	787	1.67×10^{-7}
<i>Plasma Aβ1-42</i>					
APOE	19	45,409,011	45,412,650	2	3.14×10^{-10}
APOC1	19	45,417,504	45,422,606	3	2.52×10^{-9}
<i>Plasma Aβ1-42/Aβ1-40 ratio</i>					
ZNF397	18	32,820,994	32,847,097	48	2.27×10^{-6}
APOE	19	45,409,011	45,412,650	2	3.87×10^{-13}
APOC1	19	45,417,504	45,422,606	3	6.79×10^{-13}

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: A β , amyloid beta; SNP, single nucleotide polymorphism.

protect variant for AD, APOE ϵ 2, and circulating A β peptides levels (Figure S10). A significant association of APP gene with AD ($P = 8.42 \times 10^{-7}$) was reported.³⁷ Interestingly, one of the two peaks in APP suggestively associated with A β 1-40 levels (Figure S11) was also associated with AD, whereas the second peak was not (Figure S12 in supporting information).³⁷ Nominal significant associations of RNF214 ($P = 4.8 \times 10^{-5}$) and BACE1 ($P = 1.1 \times 10^{-3}$) with AD were reported while PSEN2 was close to nominal association ($P = 5.1 \times 10^{-2}$).³⁷

4 | DISCUSSION

To uncover the genes that determine plasma A β levels, we performed a GWAS of plasma A β in 12,369 non-demented subjects. Although we did not use recently developed high sensitivity assays, we found that plasma A β 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 A β processing. A novel finding is that the variants near the BACE1 gene were found to be associated with A β 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 A β 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 A β peptide production makes it highly likely that BACE1 or a local regulation of BACE1 explains most likely the association of the region to plasma A β levels. We also found gene-wide

significant associations with plasma A β 1-40 levels in APP and PSEN2, two major actors of A β 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 A β peptides.¹ The top variants at the PSEN2 and BACE1 loci were also nominally significantly associated with A β 1-42 levels in the same direction as A β 1-40 levels, which is in agreement with the finding that PSEN2 and BACE1 activities indifferently produce A β 40 and A β 42 peptides. Conversely, the APOE ϵ 4 allele had the strongest association with A β 1-42 levels but was not even nominally associated with A β 1-40. This suggests that the APOE ϵ 4 isoform is not involved early in the process of A β peptide production but rather in more downstream events, such as A β aggregation or clearance. These results might also illustrate the greater propensity of A β 1-42 peptides to aggregate compared to A β 1-40, and the influence of APOE isoforms in the regulation of this aggregation process.¹⁰ Interestingly, associations of APOE ϵ 2 with plasma A β levels were not significant and effect sizes were very small. Contrary to APOE ϵ 4, the effect of APOE ϵ 2 on amyloid markers has been much less well studied and research has been focused on specific brain regions.³⁹ Alternatively, other, A β -independent, mechanisms such as vascular pathology may explain the lower risk of AD observed in APOE ϵ 2 carriers.⁴⁰

As to the novel genes identified, CCK and ZNF397, to date these have not been associated with A β 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 A β .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-8 may modulate neuronal activity in the brain⁴¹ 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.⁴² 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 A β 1-42 in a small association study,⁴³ is located in an enhancer of *ZNF397* (Genecards: GH18J034976), acting in the hippocampus middle, anterior caudate, and cingulate gyrus brain regions.⁴⁴ Although this SNP was not associated with any A β levels or ratio in our study, our findings do support the hypothesis that *ZNF397* plays a role in A β metabolism.

Our analysis shows associations of plasma A β 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 A β is an endophenotype for AD. Although we cannot prove the origin, our findings suggest also that A β 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 A β levels in blood with its levels in CSF as well as with its deposition in brain as assessed by PET imaging.^{19,45}

Plasma A β 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 A β levels were not useful to make a clinical diagnosis of AD.⁴⁶ However, as assays improved, several of the large cohorts participating in the present study have reported that low plasma A β 42 and A β 42/40 ratio levels were modestly associated with risk of development of AD after several years of follow-up,²²⁻²⁵ 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 A β in blood reflects some aspects of brain AD pathophysiology and this view is strengthened by our present observation that *APOE* ϵ 4 is both associated with low plasma A β 42 and A β 42/40 ratio and high AD risk. Some of studies have also reported that this association remained significant after adjusting for *APOE* ϵ 4.²⁵ Hence, plasma A β 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 A β not only in the blood, but also in the brain.^{47,48} Further, the association we observed between variants near *BACE1* and plasma A β 40 is also of interest in the light of the recent (disappointing) trials testing *BACE* inhibitors. Measuring A β 40 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.⁴⁹

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 A β metabolism and the overlap of the genetic signals with those underlying brain pathology and AD risk, suggesting blood levels of A β 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 A β data before running GWAS, thus taking into account some of the heterogeneity that has been described when using plasma A β levels.

Our study also has limitations. The state of current knowledge makes it difficult to ascertain if there is a causal role of plasma A β 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 A β concentrations and could not distinguish monomers from oligomers of A β , 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 A β .⁵⁰ Future studies with the novel assays that allow measurements of each form of A β will facilitate interpretation with regard to the balance between A β 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 A β biomarkers in blood using state-of-the-art technology.

In summary, our results indicate that genetic determinants of plasma A β 40 and A β 42 levels are close to genes known to be central actors in APP metabolism in AD. Increasing the statistical power of plasma A β analyses may potentially lead to the identification of currently unknown players in A β 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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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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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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