

Genome-wide Association Study Links *APOE* ϵ 4 and *BACE1* Variants with Plasma Amyloid β Levels

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Running Title

A 1000 Genomes GWAS of plasma A β levels

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Abstract

Background: Amyloid β ($A\beta$) 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. Impairment of the $A\beta$ production/clearance balance is the major pathophysiological hypothesis in Alzheimer's disease (AD). Plasma $A\beta$ levels are easy to measure in large numbers and therefore can be used as an endophenotype to study the genetics of $A\beta$ and its relevance to AD.

Methods: We performed genome-wide association studies (GWAS) of plasma $A\beta$ 1-40, $A\beta$ 1-42 and $A\beta$ 1-42/ $A\beta$ 1-40 ratio in 12,366 non-demented participants across 8 studies, using genetic data imputed on the 1000 Genomes phase 1 version 3 reference panel. To gain further insight, we performed LD-score regression analysis of plasma $A\beta$ -42 and $A\beta$ -40 levels using previously published GWAS of AD and other related traits, and pathway analyses.

Results: We identified 21 variants reaching genome-wide significance across two loci. The most significant locus spanned the APOE gene, with significant associations with plasma $A\beta$ 42 levels ($p = 9.01 \times 10^{-13}$) and plasma $A\beta$ 42/ $A\beta$ 40 ratio ($p = 6.46 \times 10^{-20}$). The second locus was located on chromosome 11, near the BACE1 gene ($p = 2.56 \times 10^{-8}$). We also observed suggestive evidence of association ($p < 1 \times 10^{-5}$) around genes involved in $A\beta$ metabolism including *APP* and *PSEN2*.

Conclusion: Using plasma $A\beta$ 40 and $A\beta$ 42 levels, this GWAS was able to identify relevant and central actors of the APP metabolism in AD. Overall, this study strengthens the utility of plasma $A\beta$ levels both as an endophenotype and a biomarker.

Introduction

Amyloid β (A β) peptides are the products of the catalytic processing of the A β precursor protein (APP) by the β -secretase, BACE1 and the γ -secretase complex.¹ A β peptides are mainly produced in the brain where APP and BACE1 are both highly expressed,¹ but also in circulating blood platelets² and in the pancreas.¹ A β peptides are able to self-assemble in soluble A β oligomers but also in insoluble fibrils that can aggregate as plaques in the brain parenchyma or in the wall of pial blood vessels where they constitute defining hallmarks of Alzheimer's disease (AD)³ and cerebral amyloid angiopathy (CAA),⁴ respectively.

There is strong evidence pointing toward a central role of A β peptides in the pathophysiology of AD, although the exact role remains controversial.⁵ Studies have shown that a large variety of individually rare mutations in genes involved in A β production, including *APP*, *PSEN1* and *PSEN2*, lead to autosomal dominant early-onset forms of AD (EOAD) and to lobar hemorrhage from cerebral amyloid angiopathy.⁶ Moreover, Apolipoprotein E (*APOE*) ϵ 4, the major genetic risk factor for late-onset AD (LOAD) in the general population,⁷ has been implicated in A β aggregation, deposition and clearance, both in brain and in blood vessels.⁸

These findings are the basis of drug discovery efforts targeting A β production and clearance that are currently under consideration in clinical trials, although unfortunately with negative results up to now.⁹ Although these results appear to contradict the amyloid hypothesis, several explanations have been advanced.¹⁰ One major concern is the ineffectiveness of intervention after the onset of clinical symptoms which likely result from irreversible neurodegeneration. Since the amyloid accumulation process precedes the clinical onset by decades,^{11,12} early intervention in high risk groups such as Down syndrome patients and *APOE* ϵ 4/ ϵ 4 carriers remains to be evaluated. Another concern is the lack of knowledge concerning the precise mechanisms involving A β in the pathophysiology of LOAD. Indeed, except for *APOE* and *SORL1*, only a small number of genes identified by genome-wide association studies (GWAS) of LOAD¹³ have been linked to APP metabolism^{14,15} and A β -related pathways have not yet emerged in formal enrichment analyses.¹⁶ Moreover, the genes involved in autosomal dominant forms of EOAD have not been detected in GWAS of LOAD.

To address these questions regarding the amyloid hypothesis, we and others have directly explored the genetics of A β through GWAS on quantitative measures of A β peptides, either in the cerebrospinal fluid (CSF) or in the brain, through Pittsburgh Compound B (PiB) PET scan or autopsy.¹⁷⁻²¹ Combining the effect of AD genetic loci resulted in statistically significant effects on CSF A β 42, suggesting that amyloid metabolism is also involved in LOAD.²¹ Nevertheless, these studies are limited in their sample size due to low acceptability of lumbar puncture and brain donation and high cost of PiB PET, and therefore may lack statistical power necessary in genetic association research. A β peptides produced in the brain can be degraded locally or transported into the CSF and the blood stream where they can be easily detected.²² Although the brain-derived A β peptides in the circulation cannot be distinguished from A β derived from blood platelets or pancreas, plasma A β levels are modestly but significantly correlated with amyloid burden in the CSF and in the brain.^{23,24} Our groups

have also independently shown that plasma A β concentrations are prospectively associated with the future risk of developing AD,²⁵⁻²⁸ suggesting that there is indeed a link between mechanisms controlling A β concentrations in plasma and AD pathophysiological processes in the brain and that circulating A β peptides can be used as a marker for brain amyloid metabolism. Alternatively, it has also been postulated that the relation of plasma A β with subsequent AD is reflecting general physiological processes in the brain, platelets and kidney, thus giving information on A β peptides production/clearance in each of these tissues.²⁹

In this context, we set out to discover genetic determinants of circulating A β . We previously conducted a GWAS of plasma A β levels in 3,528 non-demented participants, but failed to find genome-wide significant associations.²⁹ The present study extends our previous work by performing a GWAS using a sample size (n=12,366) that is more than three times larger.

Methods

Study population

We included data from 12,366 European-descent participants from eight studies, the Framingham Heart Study (FHS; n=6,735), the Rotterdam study (n=1,958), the Three City Study (3C; n=1,954), the Atherosclerosis Risk in Communities Study (ARIC; n=830), the Washington Heights-Inwood Community Aging Project (WHICAP; n=190), the Epidemiological Prevention study 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 Supplementary Methods 1 for a detailed description of each study).

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.^{25,26,28,30-32} In the FHS, Rotterdam and 3C study, 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 Supplementary Methods 2 for a detailed description of the protocols used in each study and Supplementary Table 1 for baseline characteristics of the study populations).

Genotyping

Each study used different genotyping platforms as previously published.¹³ 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 Supplementary Methods 3). *APOE* ϵ genotyping was performed as part of protocols specific to each study (see Supplementary Methods 4).

Statistical analyses

Plasma A β levels

Plasma A β levels were expressed as pg/mL. In each study and for each A β dosage, we excluded values that were over or below 4 standard deviations around the mean. To study the variations of plasma A β levels in a consistent way across studies, we performed a ranked-based inverse normal transformation of plasma A β levels in each study. If they were significantly associated with plasma A β levels, this transformation was performed after adjusting for batch effect and other technical artifacts.

Genome-wide association studies

Each study performed genome-wide association studies of plasma A β 1-40 and A β 1-42 levels and A β 1-42/A β 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 small number of participant ($n < 30$). Association of genetic variations with plasma A β levels were assessed in linear regression models adjusted for sex and age at blood collection. If significantly associated with plasma A β levels, principal components were added in the models to account for population structure.

Genome-wide meta-analysis

Before meta-analysis, we applied a series of filters and quality check that were previously published (see Supplementary Figures 1 and 2).³³ We performed an inverse variance weighted genome-wide meta-analysis, accounting for genomic inflation factors using the METAL software.³⁴ Finally, we retained variants that had been meta-analyzed at least in the 3 largest available populations (FHS ($n=6,735$), Rotterdam Study (RS; $n=1,958$) and Three City Study (3C; $n=1,954$)). 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 v3.4.1 (Vienna, Austria).

Confirmation of the APOE ϵ 4 signal

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.

Annotation

Variant information were retrieved using the Feb 2009 (grch37) assembly of the human genome and dbSNP v147 in the UCSC Table Browser web tool (<https://genome.ucsc.edu/cgi-bin/hgTables> accessed on 2017-07-25) and the CADD database version 1.3 (<http://cadd.gs.washington.edu/download> accessed on 2017-07-26).³⁵ We considered that variants that were less than 250kb apart from one another belonged to the same locus. We then used the Ensembl Variant Effect Predictor (VEP, <http://grch37.ensembl.org/info/docs/tools/vep/index.html> accessed on 2017-07-25)³⁶ to relate those variants to nearby genes of potential interest. We also searched if those variants were also eQTL for nearby genes using data from the Genotype-Tissue Expression (GTEx) project (<https://gtexportal.org/home/>)³⁷ using the Ensembl REST API (http://rest.ensembl.org/documentation/info/species_variant, data retrieved on 2017-07-31). We corrected for multiple testing by computing False Discovery Rate (FDR), using an FDR threshold of 0.05. Finally, we cross-checked our results with previously published GWAS of AD¹³ amyloid-related brain pathology,¹⁹ and CSF A β 42 levels.²¹

Genetic correlations

To gain further insight, we used the LD Score software v1.0.0^{38,39} and previously published GWAS to compute genetic correlations between plasma A β levels and ratio and AD,¹³ Parkinson's disease,⁴⁰ cognition,⁴¹ hippocampal volume,⁴² intracranial volume,⁴³ white matter lesions,⁴⁴ brain lobes volumes (unpublished data) and subcortical brain structures volumes.⁴⁵

Pathway over-representation analysis

We used the ConsensusPathDB-human website (<http://cpdb.molgen.mpg.de/> accessed on 2017-08-01)⁴⁶ and a curated list of genes related to A β ⁴⁷ to check whether the genes we annotated using GTEx were over-represented in biochemical pathways (see Supplementary Table 2 for the complete list of genes). In order to detect new pathways, we performed a second pathway analysis after excluding genes from loci known for their involvement in A β metabolism, namely *APOE* (including *APOE*, *PVRL2*, *NKPD1*, *CTB-129P6.4*, *APOC1* and *VASP*), *APP* (including *APP*, *AP000230.1* and *AP001596.6*), *PSEN2* (including *PSEN2* and *ADCK3*) and *BACE1* (including *BACE1*, *SIDT2*, *TAGLN*, *RP11-109L13.1*, *RNF214* and *CEP164*). Statistical significance was assessed using hypergeometric tests and corrected for multiple testing using Q-value.

Results

Genome-wide significant variants associated with plasma A β levels

After meta-analysis, we identified 21 variants reaching genome-wide significance across two loci (Supplementary Figures 3 to 8).

The first locus was located on chromosome 19, in the *APOE* gene, with significant associations with plasma A β 1-42 levels and plasma A β 1-42/A β 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 β 1-42 levels and A β 1-42/A β 1-40 ratio, respectively (Table 1). The minor allele of this variant, which denotes the *APOE* ϵ 4, was associated with lower plasma A β 1-42 levels (effect size=-0.167 standard deviations (SD); 95% confidence interval (CI)=[-0.212 ; -0.121]) and lower plasma A β 1-42/A β 1-40 ratio (effect size=-0.212 SD; 95% CI=[-0.257 ; -0.121]; Table 1 and Supplementary Figure 9). We confirmed these associations using the directly genotyped *APOE* ϵ 4 status (Supplementary Figure 10). We did not find significant associations between genotyped *APOE* ϵ 2 and circulating A β peptides levels, despite the protective effect of this variant on the risk of AD (Supplementary Figure 10).

The second genome-wide significant locus was located on chromosome 11, near the *BACE1* gene that encodes the β -secretase and is involved in the initial, A β -producing step of APP processing (Figure 3). For the most significant variant, rs650585 (CADD score=6), the minor allele was associated with lower plasma A β 1-40 levels (effect size=-0.073 SD; 95%CI=[-0.099 ; -0.047]; p-value= 2.56×10^{-8} ; Table 1 and Supplementary Figure 9). This variant was in moderate LD ($R^2=0.58$) with a *BACE1* synonymous variant, rs638405 (CADD score=11), which was also associated with plasma A β 1-40 levels (effect size=-0.071 SD, p-value= 1.21×10^{-7}). Both variants were associated with *BACE1* expression in testis (FDR= 4.52×10^{-11} and FDR= 2.62×10^{-38} , respectively). rs638405 was also associated with CEP164 expression in the cerebellum (FDR= 2.05×10^{-6} ; Supplementary Table 2). Of note, this locus also contained a missense variant in the CEP164 gene that was associated with plasma A β 1-40 levels with suggestive levels of significance (rs573455, effect size=-0.057 SD; p-value= 9.16×10^{-6}).

GWAS suggestive hits

Besides genome-wide significant signals at the *APOE* and *BACE1* loci, signals reaching suggestive levels of association ($p < 1 \times 10^{-5}$) for at least one of the three plasma A β measures were identified for 240 variants across 73 loci.

Interestingly, we found suggestive levels of association spread across three peaks within and nearby *APP*, one of the core genes of amyloid metabolism (Figure 4). Two independent variants located within *APP* were suggestively associated with plasma A β 1-40 levels: rs150707803 (effect size=-0.184 SD; p-value= 2.10×10^{-6}) and rs436011

(effect size=0.061 SD; p-value= 3.92×10^{-6}) (Figure 4A and Table 2). SNPs within this second locus, including rs436011, were associated with APP expression in the “Esophagus – Muscularis” (FDR= 6.29×10^{-7} for rs436011; Supplementary Table 2). The top variant of the third locus, rs199744263, was located upstream of *APP* (Figure 4B). This variant was associated with lower A β 1-42/A β 1-40 ratio (effect size=-0.075 SD; p-value= 2.43×10^{-6} ; Table 2). SNPs from this locus were associated with brain expression of *AP000230.1*, a lincRNA located directly upstream of *APP* (e.g. for rs199744263, FDR= 8.33×10^{-5} for cerebellar hemisphere expression; Supplementary Table 2).

In addition to *BACE1* and *APP*, we explored genetic associations around other genes closely involved with A β production, namely *PSEN1* and *PSEN2* which are part of the γ -secretase complex and *ADAM10*, which encode for the α -secretase, and is involved in a competing, non-A β producing, processing of APP. There was a suggestive association with plasma A β 1-40 at the *PSEN2* locus (Table 3, Supplementary Figure 11). In this locus, the top variant, rs2246221 (effect size= 0.057 SD; p-value= 8.09×10^{-6}) was also associated with *PSEN2* expression in spleen (FDR= 2.35×10^{-6}), thyroid (FDR= 1.77×10^{-5}), lung (FDR= 8.08×10^{-5}), skin (FDR= 1.76×10^{-2}) and transformed fibroblasts (FDR= 6.25×10^{-23} ; Supplementary Table 2). Finally, we found a suggestive association with plasma A β 1-40 within *RGS6* which is located approximately 600kb upstream of *PSEN1* but did not find any evidence of eQTL linking the two loci (Table 3, Supplementary Figure 12).

To minimize risk of false positive signal among the remaining suggestive loci, we prioritized 12 loci containing exonic variants or variants with a CADD score higher than 10 (Supplementary Table 3). Among these variants, rs11123523 (CADD=12.2), located near the *TMEM37* gene, showed the lowest p-value. This variant was associated with plasma A β 1-40 levels (effect size=-0.074; p-value= 2.80×10^{-7}) and was associated with expression of a nearby gene, *SCTR*, in thyroid (FDR= 3.34×10^{-10}), testis (FDR= 9.45×10^{-3}) and tibial nerve (FDR= 1.63×10^{-2}). The only exonic variant was rs704, a missense variant of the *VTN* gene associated with plasma A β 1-40 levels (effect size=-0.060; p-value= 5.81×10^{-6}).

Genetic overlap with other A β -related traits and diseases

In order to put those genome-wide significant variants in the context of amyloid-related pathophysiology of AD, we compared them with results obtained from CSF A β 42, AD brain pathology and AD risk in GWAS (Table 1). The *APOE* ϵ 4 allele was also associated with lower CSF A β 42 levels, higher brain levels of neuritic plaques, diffuse plaques and neurofibrillary tangles and higher risk of AD. For the *BACE1* locus, we did not find genome-wide significant or suggestive associations of rs650585 with any of the aforementioned traits. Among suggestive variants, no genome-wide significant or suggestive association was found for any of the other amyloid-related traits or for AD risk.

To improve statistical power, we performed a genetic correlation analysis of GWAS results of multiple traits using LD regression. On a genome-wide scale (Figure 5), we observed a nominally significant negative genetic correlation between variants modulating plasma A β 1-40 levels and hippocampal volume (r_g =-0.64, p =0.03), and a

positive correlation between variants modulating plasma A β 1-42 levels and white matter lesions ($r_g=0.42$, $p=0.05$).

Pathway over-representation analysis

After annotation using GTEx, we obtained a list of 41 genes located nearby our suggestive and significant signals that we used to perform a pathway over-representation analysis. Using ConsensusPathDB-human and a curated list of genes related to A β as references, we found significant over-representation in several biochemical pathways, related to Alzheimer's disease (ConsensusPathDB Q-value= 5.73×10^{-4}) and generation of A β (curated list p-value= 4.18×10^{-3} ; ConsensusPathDB Q-value= 4.44×10^{-4} ; Supplementary Table 4). These results were driven by genes previously known for their involvement in A β metabolism (*APOE*, *BACE1*, *APP*, *PSEN2*) as the AD and A β pathways were no longer significant after removing these genes from the analysis (Supplementary Table 5).

Discussion

Previous genome-wide association studies of plasma A β 40 and A β 42 levels have failed to uncover genome-wide significant findings. In this study, we identified two genome-wide significant loci associated with plasma A β levels in up to 12,366 non-demented subjects of European ancestry. The top variant in the first locus, rs429358, a well-known non-synonymous variant that encodes for the APOE4 isoform, was associated with lower circulating A β 42 levels and A β 42/40 ratio. In the second, located near *BACE1*, rs650585 was associated with lower plasma A β 40 levels.

The *BACE1* region encompasses several genes (*PCSK7*, *RNF214*, *BACE1*, *CEP164*) and a *BACE1* anti-sense long non-coding RNA (*BACE1-AS*). Since the β -secretase activity of *BACE1* is necessary for A β peptide production, it is likely that *BACE1* or a local regulation of *BACE1* expression are responsible for this signal. We also found suggestive associations with plasma A β 40 levels near *APP* and *PSEN2*, two major actors of the A β metabolism. *APP* is obviously a central element of its own metabolism and *PSEN2* is a key component of the γ -secretase which processes the APP C99 fragment into A β peptides.¹ We speculate that the effect of the variants on the expression/biological activation of these key elements of β -amyloid processing is strong enough to allow their detection at the plasma level, despite the influence of many other simultaneous biological processes, e.g. secretion, interaction with other proteins, degradation and/or clearance. Moreover, the top variants at the *PSEN2* and *BACE1* locus were also nominally associated with A β 42 levels in the same direction as A β 40 levels, which is in agreement with knowledge 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 42 levels but was not even nominally associated with A β 40. In line with our previous comment, this suggests that the APOE4 isoform is not involved in the early process of A β peptide production but in more downstream events, such as A β aggregation or clearance. These results might also illustrate the greater ability to aggregate of A β 42 peptides compared to A β 40, and the influence of APOE isoforms in the regulation of this process.⁸ 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 studied and seems to be focused on specific brain regions, which could explain why we could not detect any association.⁴⁸ This could also suggest that other, A β -independent, mechanisms are involved in the lower risk of AD observed in APOE ϵ 2 carriers.⁴⁹

Given the presence of suggestive associations around known APP/A β genes, it is likely that novel and relevant signals exist within this range of statistical significance, awaiting definite validation. In order to minimize the risk of false positive result, we used exonic location and CADD score as additional filters to screen for SNPs of interest. Among those, we observed an association between a missense mutation of the *VTN* gene and lower plasma A β 40 levels. *VTN* encodes for vitronectin, a glycoprotein present in abundance in the plasma and the extracellular matrix and involved in early regulation of thrombogenesis and tissue repair.⁵⁰ Vitronectin has been associated with amyloid deposits,⁵¹ including A β , both at the level of amyloid plaques in the brain⁵² and near

the retinal pigment epithelium in the aging eye.⁵³ Modest correlation between plasma vitronectin and brain amyloid burden measured by PiB PET has been reported.⁵⁴ Vitronectin is involved in microglial activation,⁵⁵ which is relevant to AD pathophysiology.⁵⁶ Vitronectin might also be involved in small vessel disease. In a mouse model of CADASIL, an autosomal dominant disease responsible for stroke and cognitive impairment, reduction of vitronectin expression resulted in less white matter lesions.⁵⁷ Vitronectin could therefore represent a promising candidate to study mechanisms linking A β peptides with A β -related pathologies, such as AD, cerebral amyloid angiopathy and small vessel disease. Other potential genes of interest close to suggestive signals warrant further investigation. For example, *SCTR*, encoding for secretin receptor, is involved in a wide range of physiological functions, beyond the scope of this study. Interestingly though, a study reported that mice deficient for that gene display impaired hippocampal synaptic plasticity.⁵⁸

Although research on variants associated with levels of circulating amyloid peptides is of general interest for A β physiology, we are interpreting the findings primarily from the perspective of brain disorders, especially AD. When cross-checking our results with other published GWAS of CSF and brain A β , and AD, we observed consistent results only with the *APOE* ϵ 4 variant. As expected, this variant was associated with low plasma and CSF A β 42, high brain A β and AD risk, consistent with a differential effect of *APOE* isoforms on A β aggregation and clearance from the brain. When interpreting the absence of significant association between variants in the other loci and those same traits, one should keep in mind that their effect on plasma A β levels was generally smaller compared to that of *APOE* ϵ 4 so we might be underpowered to detect a significant association. It is also of interest that the LD-score regression analysis suggested a positive correlation of variants modulating plasma A β 1-42 levels and white matter lesions and a negative correlation between plasma A β 40 and hippocampal volume. Nevertheless, these genetic correlations were only nominally significant and await replication.

Plasma A β is usually considered as a poor biomarker of AD in the literature. A previous meta-analysis reported that plasma A β levels were not useful to make a clinical diagnosis of AD.⁵⁹ Nevertheless many of the cohorts participating in the present study have previously reported that low plasma A β 42 and A β 42/40 ratio levels were associated with development of AD after several years of follow-up.²⁵⁻²⁸ These results are consistent with an early, preclinical, involvement of A β in AD pathophysiology and are 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 those studies have also reported that this association remained significant after adjusting for *APOE* ϵ 4,²⁸ and we might hypothesize that variations of plasma A β levels are not only a side-effect of *APOE* ϵ 4, but are also involved in AD pathophysiology. As such, plasma A β levels would not be only useful as a biomarker of an active amyloid process in the brain but could also be considered as a therapeutic target. In favor of this hypothesis are reports that hemodialysis or peritoneal dialysis are able to lower A β in the brain.^{60,61} The association we observed between variants near *BACE1* and plasma A β 40 is also of interest in the light of the ongoing trials testing *BACE* inhibitors, even though the lack of association of these variants with AD risk should be further investigated.⁶²

Our study has several strengths. First, it is, to date, the largest study of circulating amyloid peptides. This enabled us to identify known actors of A β metabolism and, thus,

to be optimistic about the relevance of some of our suggestive signals. 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 has also limitations worth mentioning. As stated before, the state of current knowledge makes it hard for us to extrapolate the role of these actors from the plasma compartments to the brain and further research in this area is needed. Second, most studies used assays that only measured free monomeric forms of A β . Therefore, our interpretation of the present results might differ from other studies in which assays used non-selectively measured both monomers and oligomers of A β .⁶³ Future studies should carefully choose assays that allow measurements of each form of A β as this will facilitate interpretation with regard to the balance between A β production, aggregation and clearance. Finally we tried to prioritize signals of interest using strict criteria, thus omitting to mention other interesting signals that might be real. Therefore we hope that the unfiltered results from this study, will be helpful as a resource to the scientific community to further decipher the physiology of A β peptides and its links to pathophysiology of AD and other A β -related diseases.

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. Further increasing the statistical power of plasma A β analyses may potentially lead us to the identification of currently unknown players in A β metabolism, novel hypotheses and hopefully, new preventive or therapeutic targets against Alzheimer's disease.

Figures Legends

Figure 1. Association of frequent genetic variants with plasma A β 1-42 in the *APOE* locus

Figure 2. Association of frequent genetic variants with plasma A β 1-42/A β 1-40 ratio in the *APOE* locus

Figure 3. Association of frequent genetic variants with plasma A β 1-40 in the *BACE1* locus

Figure 4. Association of frequent genetic variants with plasma A β 1-40 (A) and A β 1-42/A β 1-40 ratio (B) in the *APP* locus

Figure 5. LD-score regression assessing genetic correlation between plasma A β levels, Alzheimer's and Parkinson's diseases, cognition, hippocampal and intracranial volumes, white matter lesions, brain lobes volumes and subcortical brain structures volumes
Notes: * denotes nominal significance ($p < 0.05$), "/" denotes missing values

Tables Legends

Table 1. Associations of top variants from genome-wide significant loci with plasma A β levels and amyloid-related traits

Table 2. Associations of top variants from the *APP* locus with plasma A β levels and amyloid-related traits

Table 3. Associations of top SNPs from the *PSEN1* and *PSEN2* locus with plasma A β levels and amyloid-related traits

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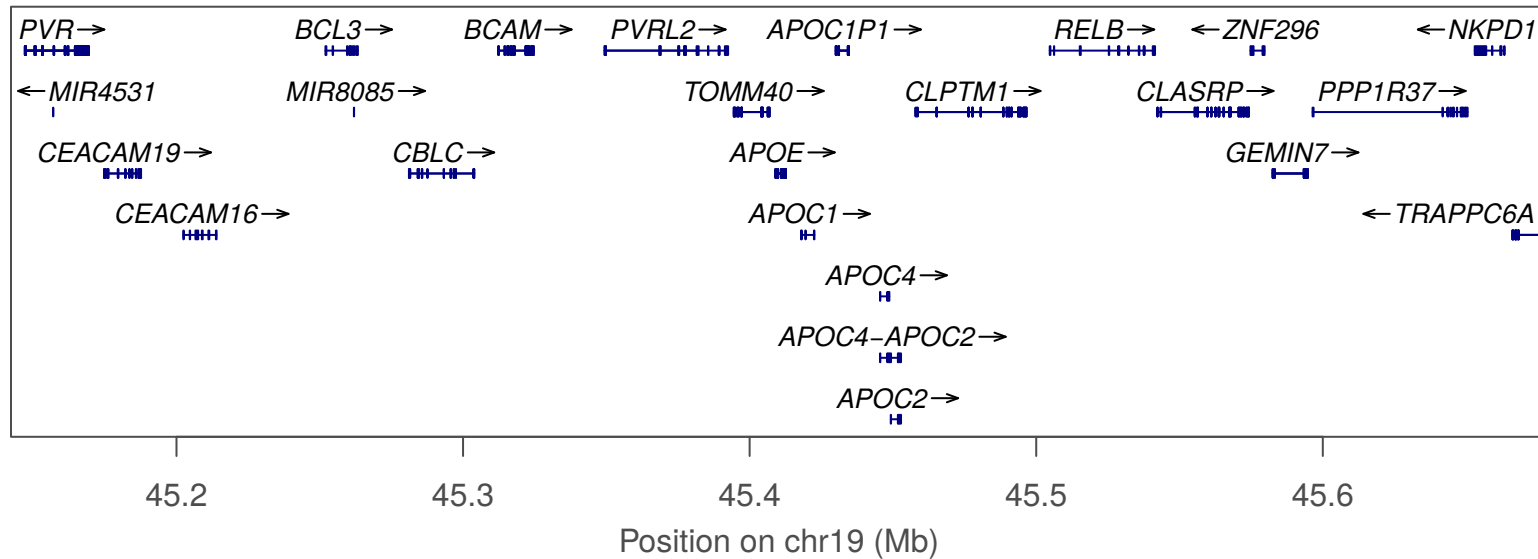
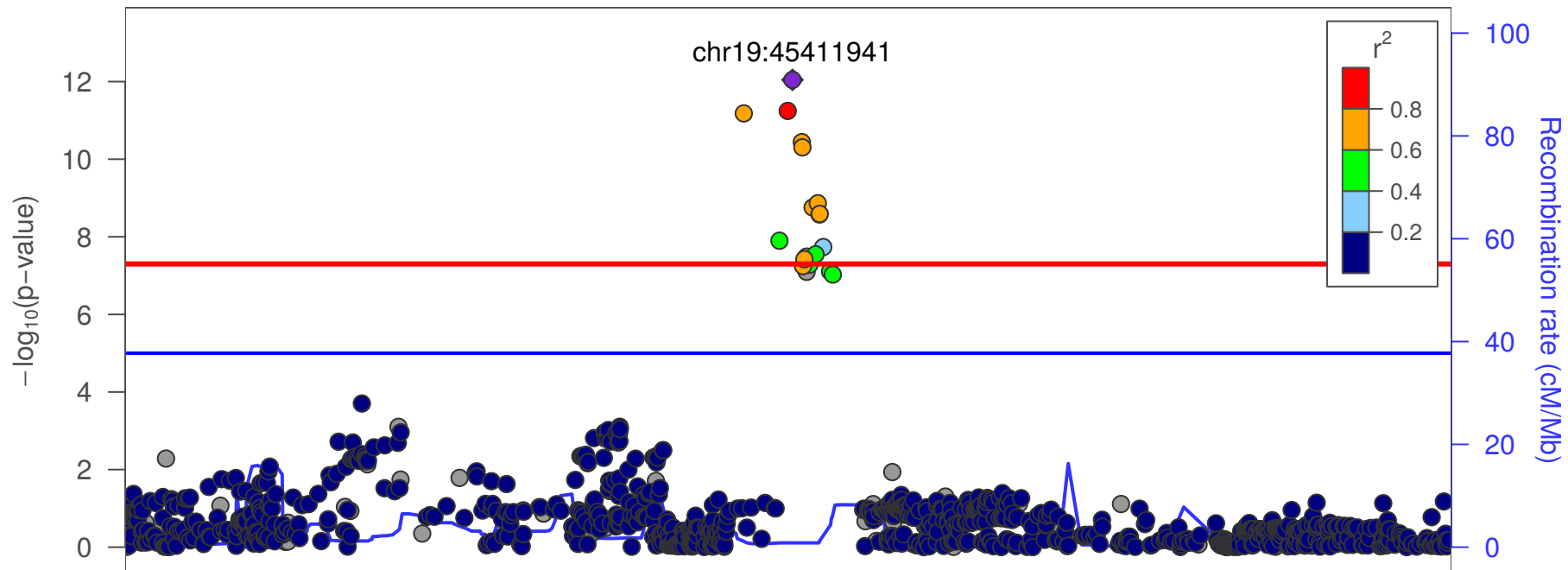
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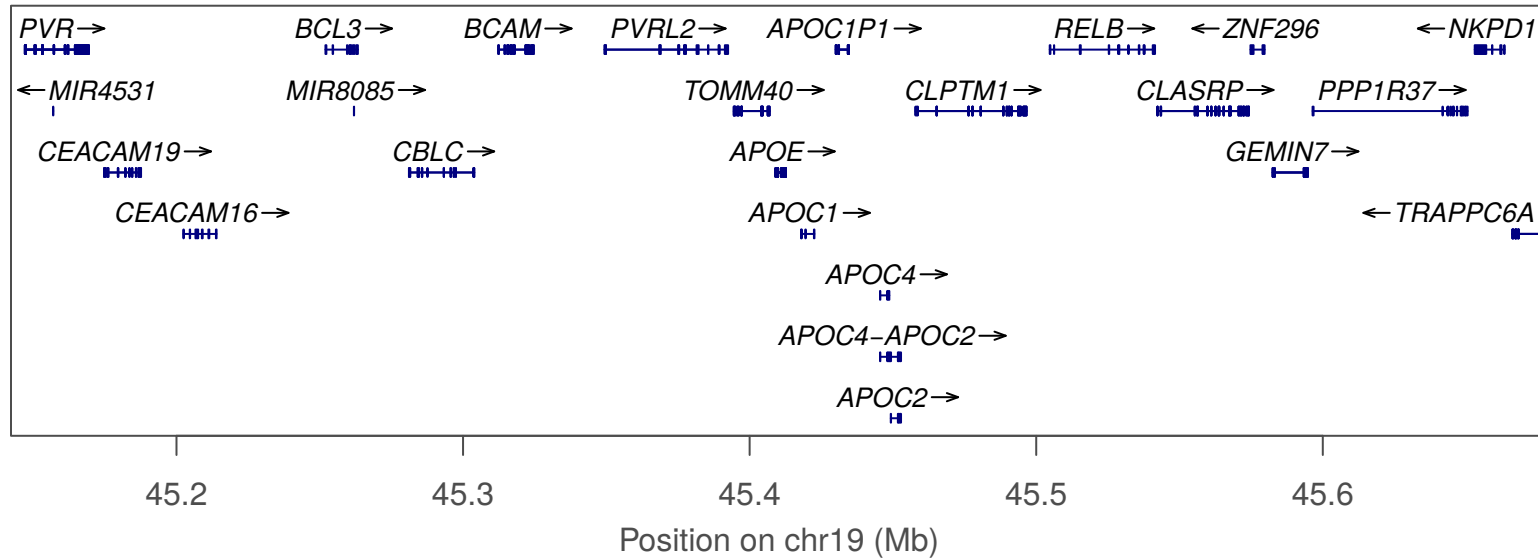
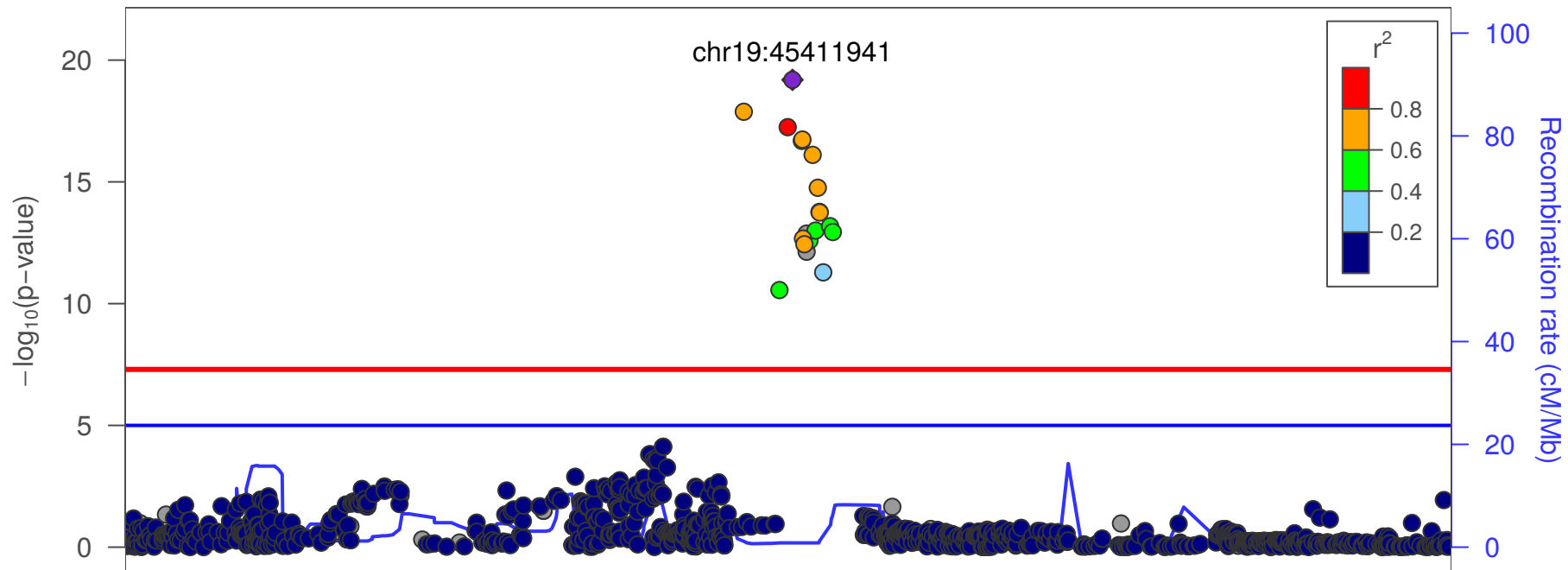
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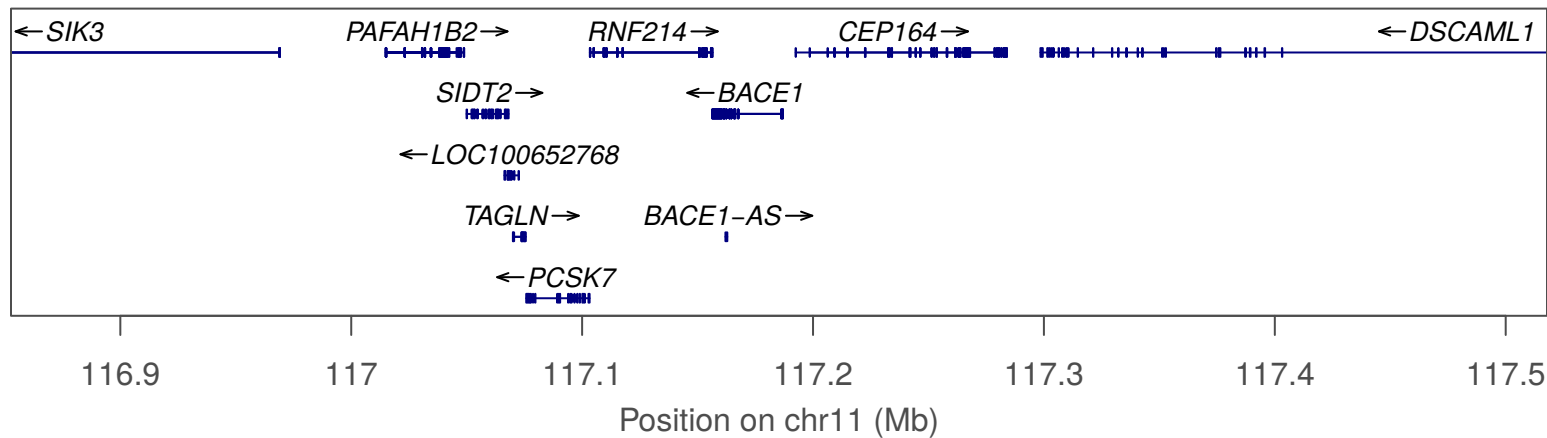
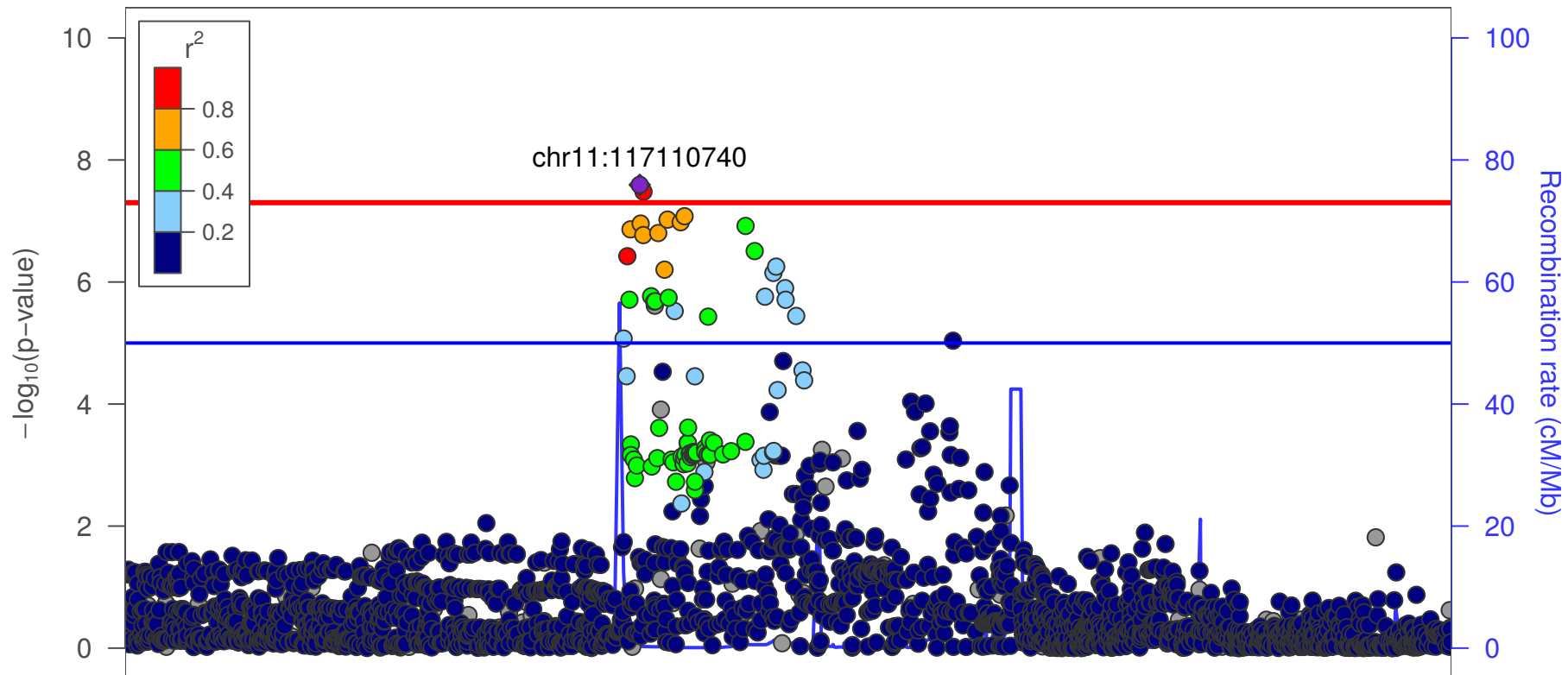
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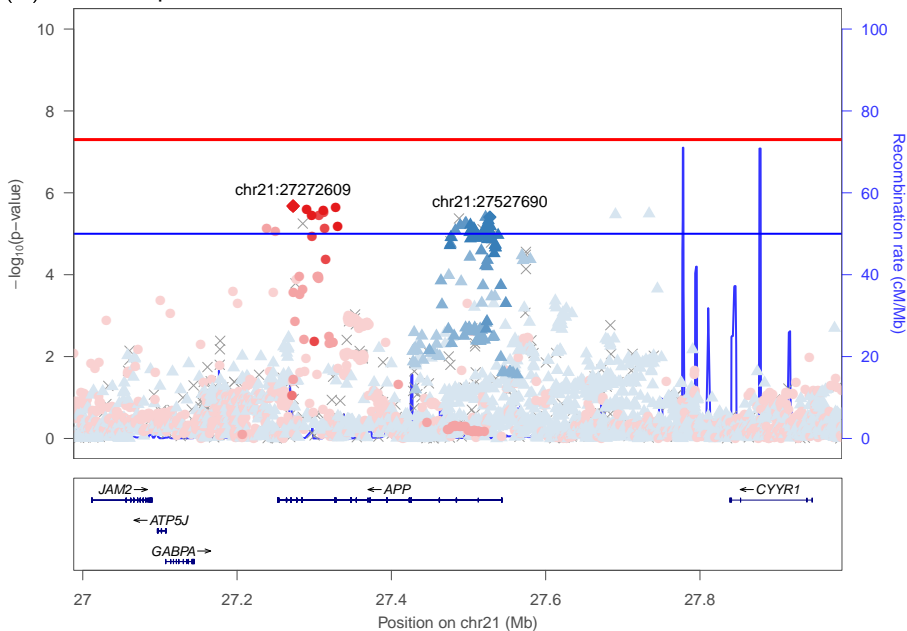
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(A) Plasma A β 1-40



(B) Plasma A β 1-42/A β 1-40 Ratio

