# Genome-wide meta-analysis for Alzheimer's disease cerebrospinal fluid biomarkers

**Supplementary Information**

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## Supplementary results

*Genetic architecture*

To test which of the two proteins has a more comparable genetic background to AD, genetic correlations were calculated with previously published AD GWAS summary statistics, both for Aβ42 and pTau (Supplementary Table 4). Unfortunately, these results were inconclusive, presumably due to the low SNP-heritability for AD diagnosis.

*GWAS rsults for stratified subgroups*

Explorative meta-analyses were repeated stratified for *APOE* (*APOE* ε4 carriers (n=3,240) vs. *APOE* ε4 non-carriers (n=3,201)) and amyloid status (Amyloid normal levels (n=3,182) vs. amyloid abnormal levels (n=3,775)) for stage 1 (QQ plots and lambda shown in Supplementary Figure 9 and 10), of which the results are visualized in Supplementary Figures 11 and 12, and detailed in Supplementary Table 5. *APOE* ε4 carriers still harbored a strong *APOE* signal (Z=-17.92; $p$=8.59x10$^{-72}$), representing the dosage effect of the *APOE* ε4 allele, where rs429358-C decreased Aβ42 protein levels. The 3q28 (*GMNC*) locus was observed for pTau in both the *APOE* ε4 carriers (Z=5.63; $p$=1.85x10$^{-8}$) and non-carriers (Z=7.05; $p$=2.29x10$^{-12}$). We furthermore report a novel locus mapping to chromosome region 7q11.22 for Aβ42 (Z=-5.49; $p$=4.12x10$^{-8}$) in the *APOE* ε4 non-carriers.

Stratified for amyloid status, *APOE* remained strongly associated with decreased CSF Aβ42 levels, both within the abnormal-amyloid-level (Z=-10.03; $p$=1.14x10$^{-23}$) and normal-amyloid-level subgroups (Z=-14.49; $p$=1.40x10$^{-47}$). By contrast, *APOE* increased pTau levels in individuals with normal amyloid levels (Z=-8.53; $p$=1.50x10$^{-17}$), but not in those with abnormal amyloid levels (Z=-2.81; $p$=0.034). The 3q28 (*GMNC*) locus was significantly associated to pTau levels in individuals with both normal and abnormal amyloid levels. A novel locus (12q13.3) was observed for individuals with abnormal amyloid levels, decreasing Aβ42 protein levels (Z=-5.47; $p$=4.50x10$^{-8}$).

*Gene prioritization*

Besides the well-established causal *APOE* gene for the *APOE* locus, the genes *CR1* and *BIN1* have also been indicated as most likely causal genes in previous studies for the AD loci on genomic locations 1q32.2 and 2q14.3, respectively. As this previous work is based on thorough functional experiments, we therefore here only focused with the computational-based tool FUMA [1], on the replicated loci for which the causal gene has not been determined. These loci are the 3q28 and 16q24.2 associations for pTau. We report the most promising causal genes by interpreting positional mapping information, gene-based association tests and eQTL annotations in brain and immune tissue and cell types based on publicly available data (see Methods).

The lead SNP for the 3q28 locus is an intergenic variant 59 kb upstream of *GMNC,* the gene physically closest to the significant association signal. *GMNC* is the only gene within this locus showing an association to pTau based on the aggregated effect of 139 variants ($p$=5.00x$10^{-10}$; Supplementary Table 6). No significant eQTLs were annotated for significant variants within this locus. The lead SNP rs4843559 of 16q24.2 is an intronic variant in *C16orf95*, for which also a significant variant-aggregation association ($p$=4.03x$10^{-9}$) is observed (Supplementary Table 6). Rs4843559 is furthermore a significant eQTL in blood, increasing *ZCCHC14* gene expression.

The variant aggregation analysis showed no newly associated genes, besides the expected associations for genes in the *APOE* locus, *GMNC* and *C16orf95* (Supplementary Table 6).

**Supplementary reference**

1. Watanabe, K., et al., *Functional mapping and annotation of genetic associations with FUMA.* Nature Communications, 2017. **8**(1): p. 1826.

## Supplementary figures

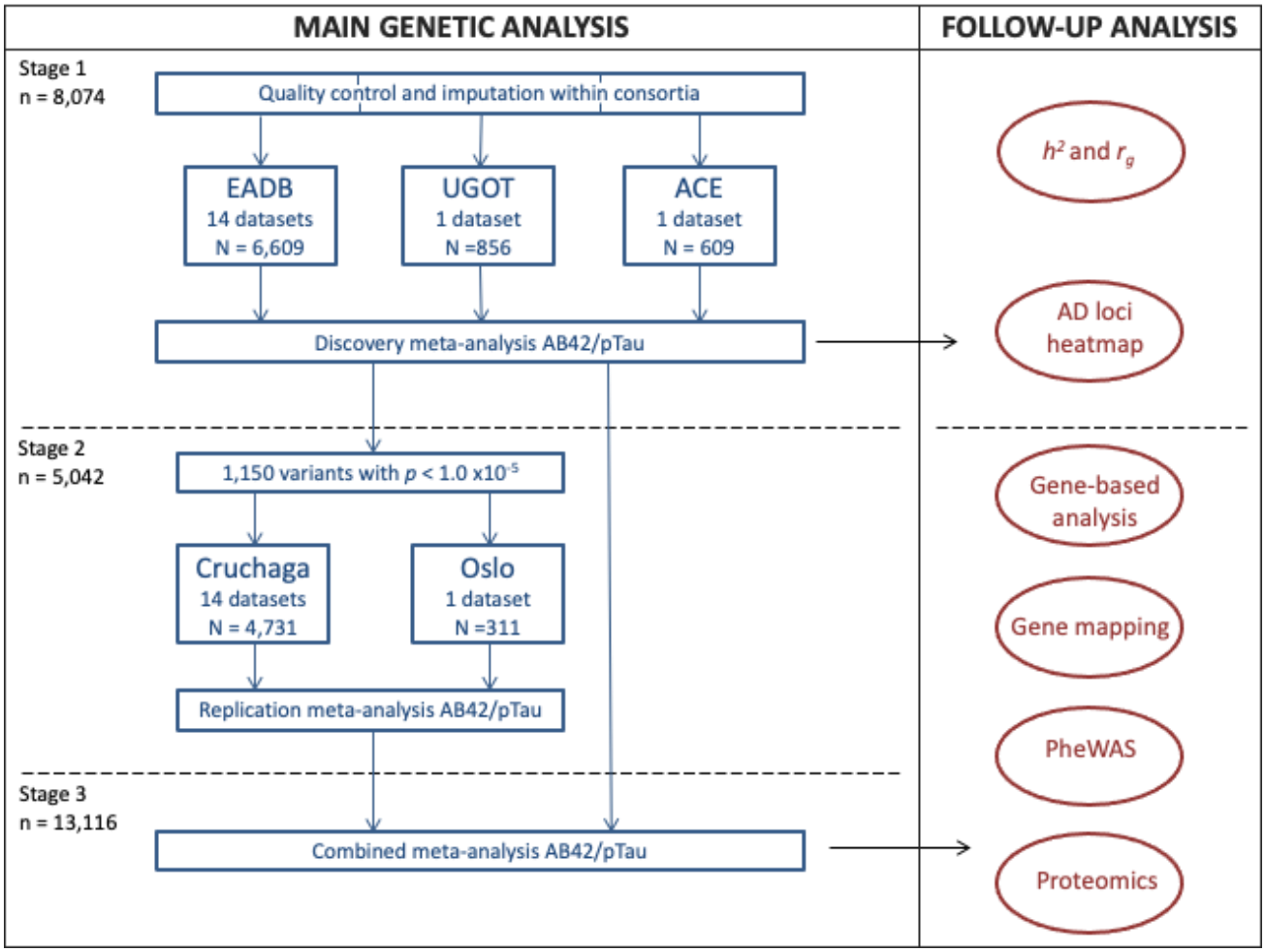


**Figure 1.** Study design of GWAS meta-analysis, and follow up analyses to explore genetic architecture and biological implications. For the follow-up analyses strategies reported in the first two red ovals, it was preferred to maintain n per variant similar in size. For the remaining follow-up analyses the most powerful results were preferred as input.

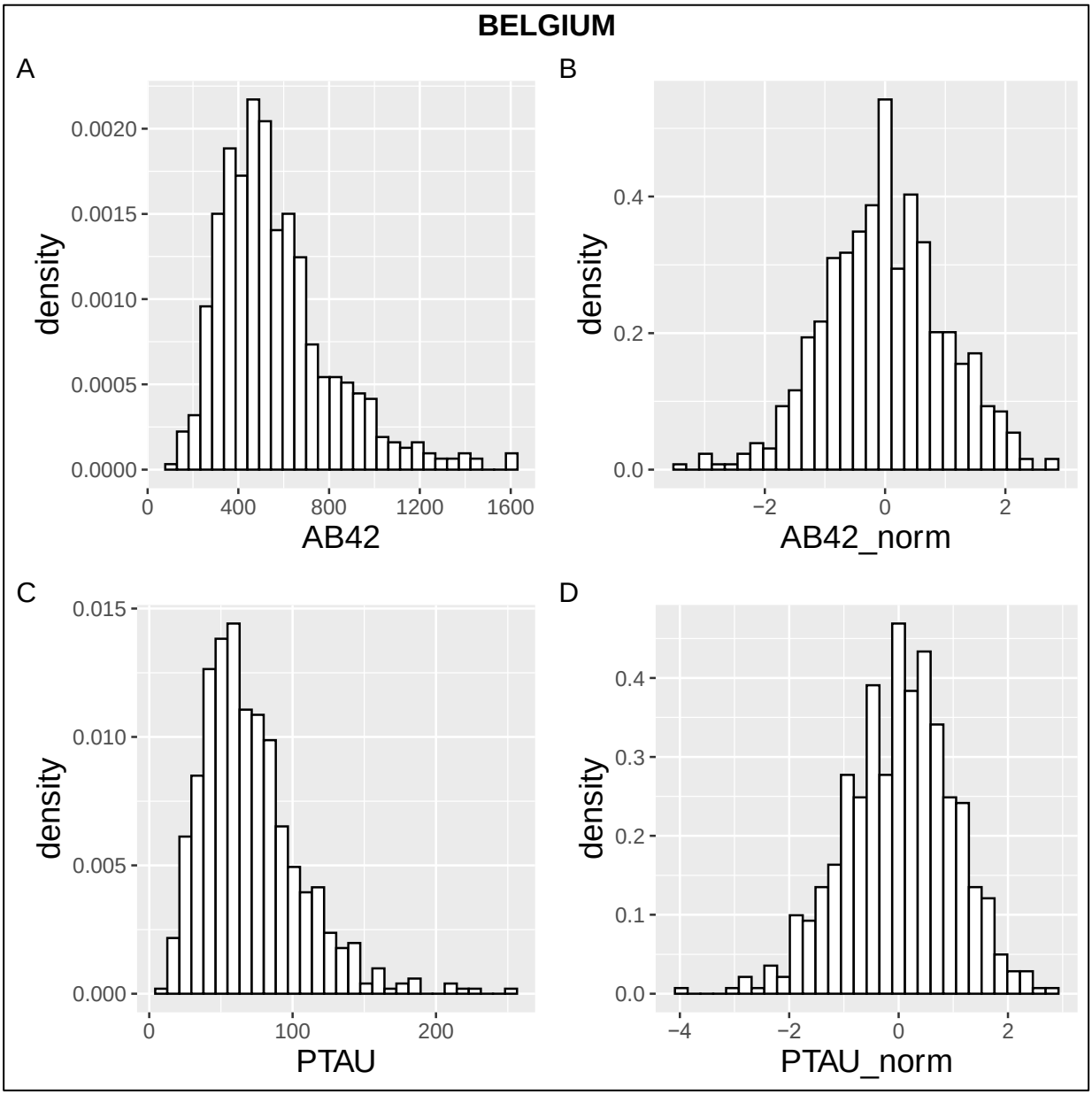


**Figure 2.** Raw and normalized CSF protein level distributions for the Belgium cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

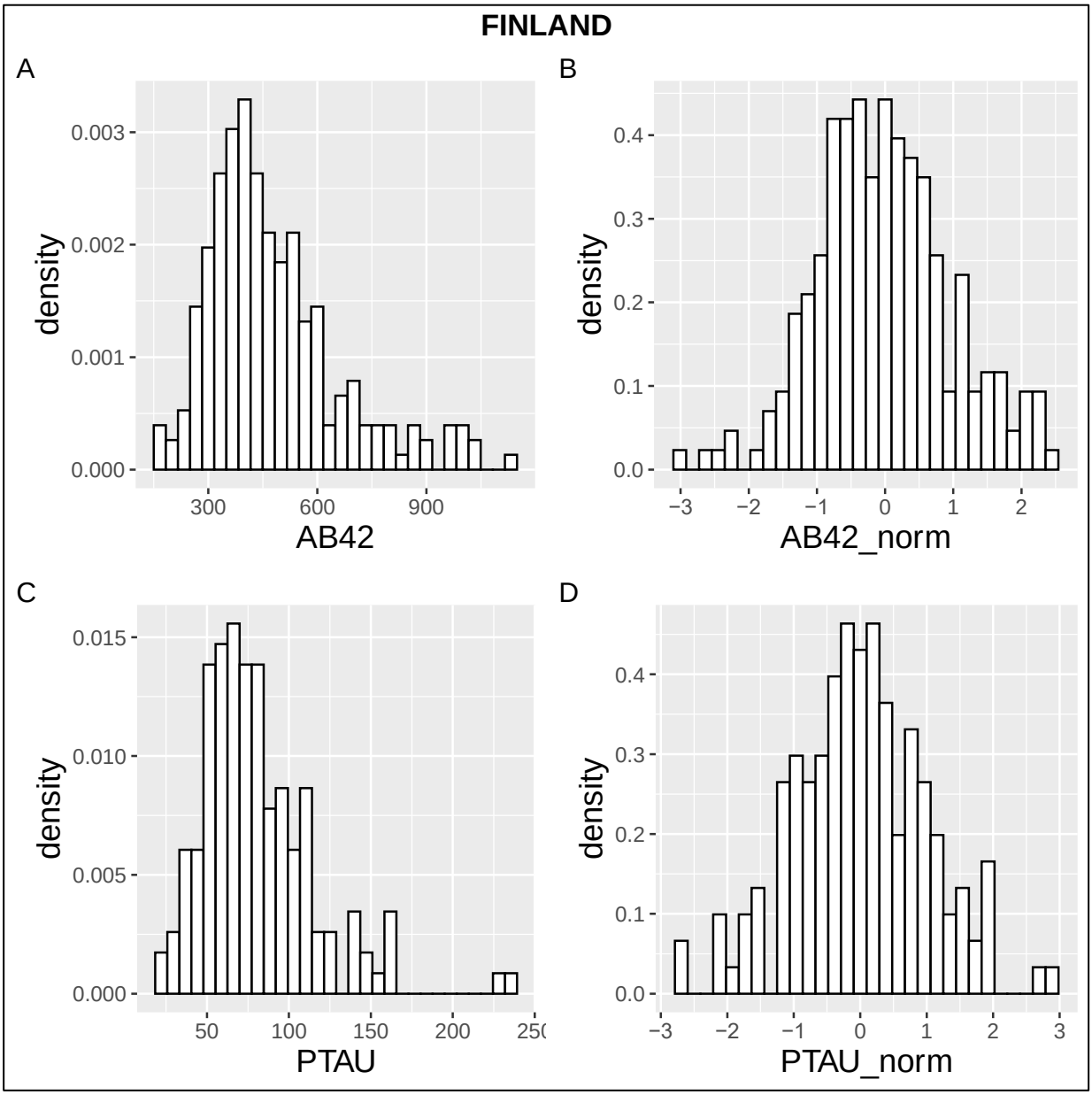


**Figure 3.** Raw and normalized CSF protein level distributions for the Finish cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

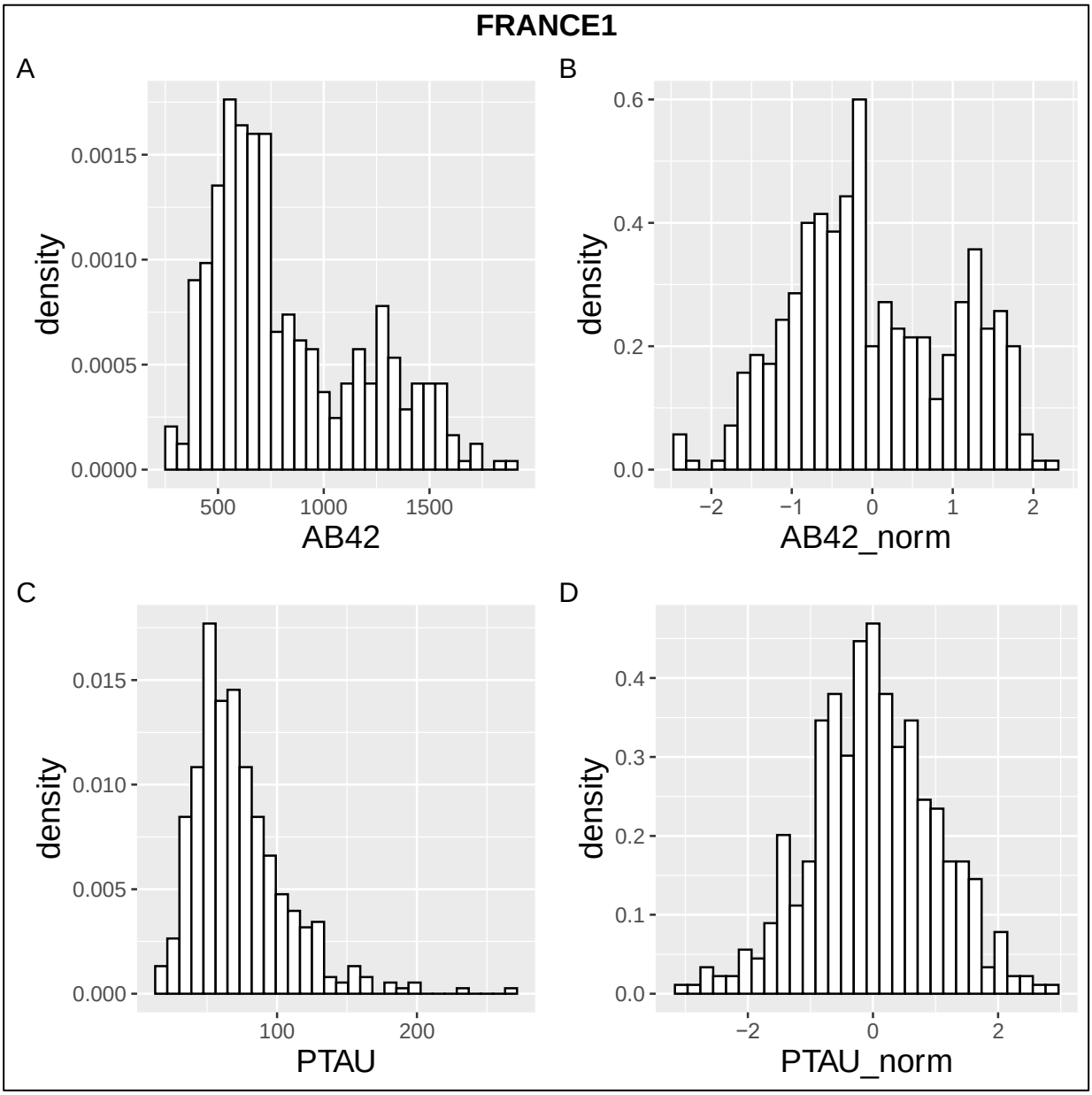


**Figure 4.** Raw and normalized CSF protein level distributions for the France1 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

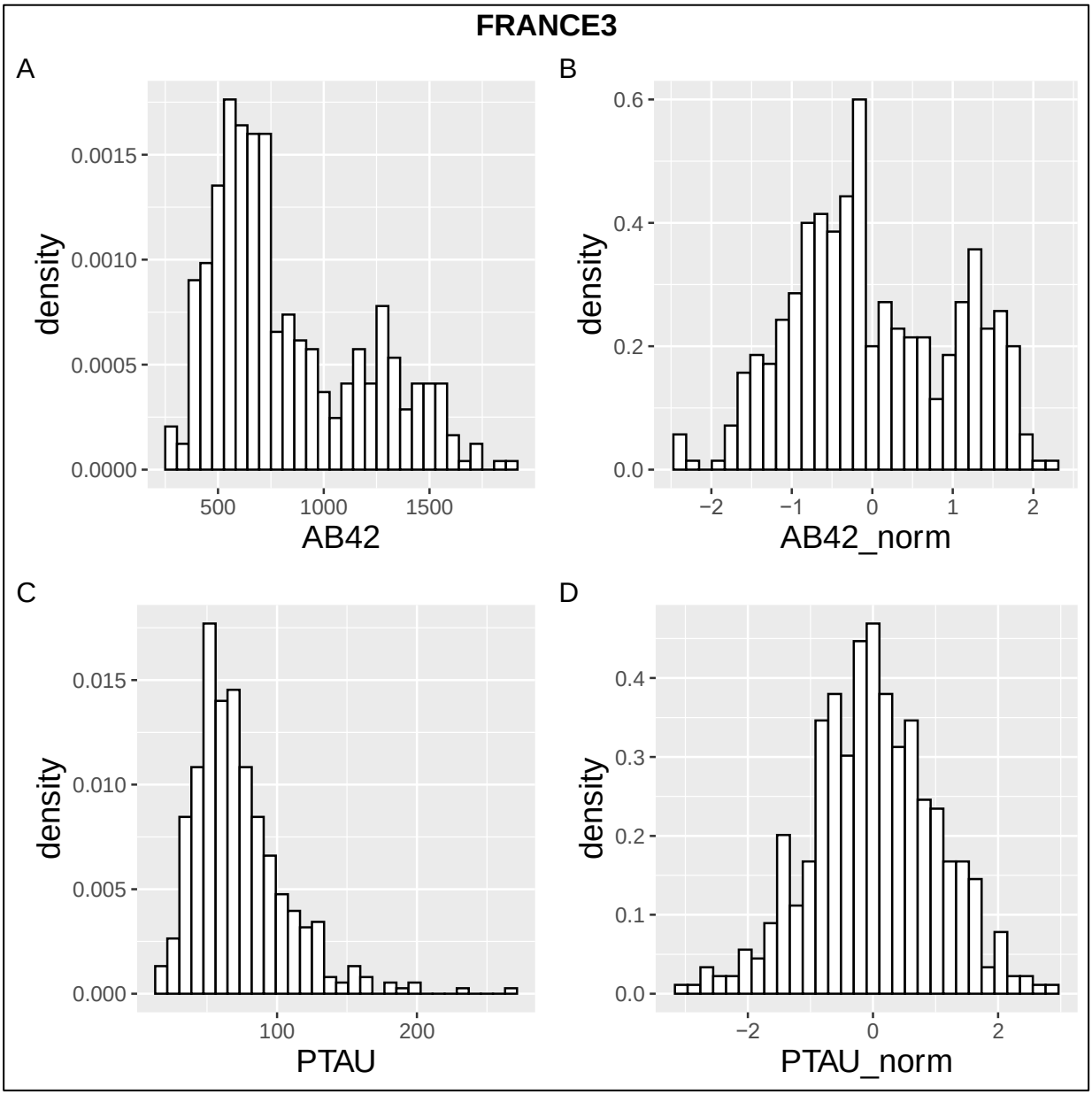


**Figure 5.** Raw and normalized CSF protein level distributions for the France3 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

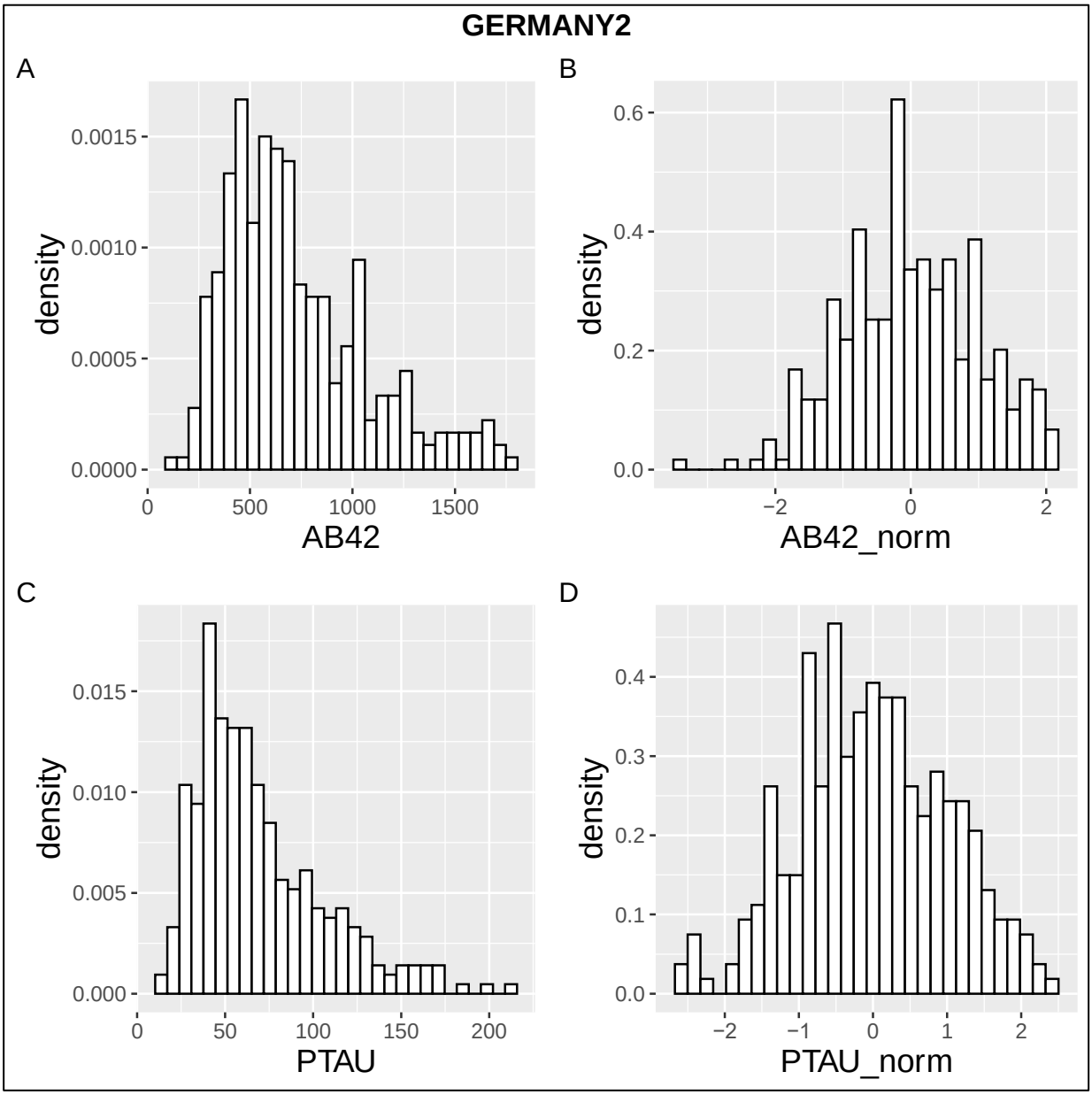


**Figure 6.** Raw and normalized CSF protein level distributions for the Germany2 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

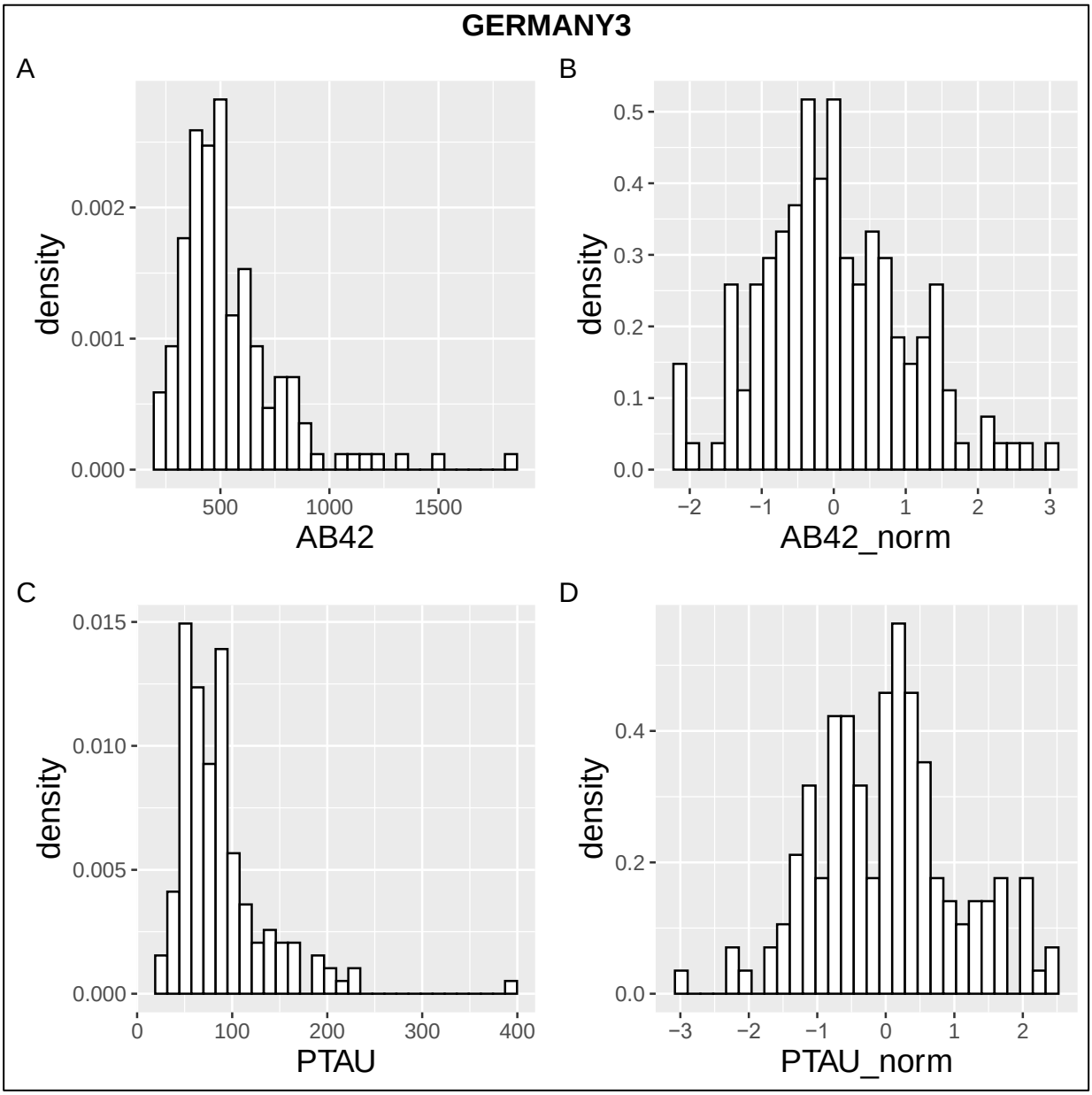


**Figure 7.** Raw and normalized CSF protein level distributions for the Germany3 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

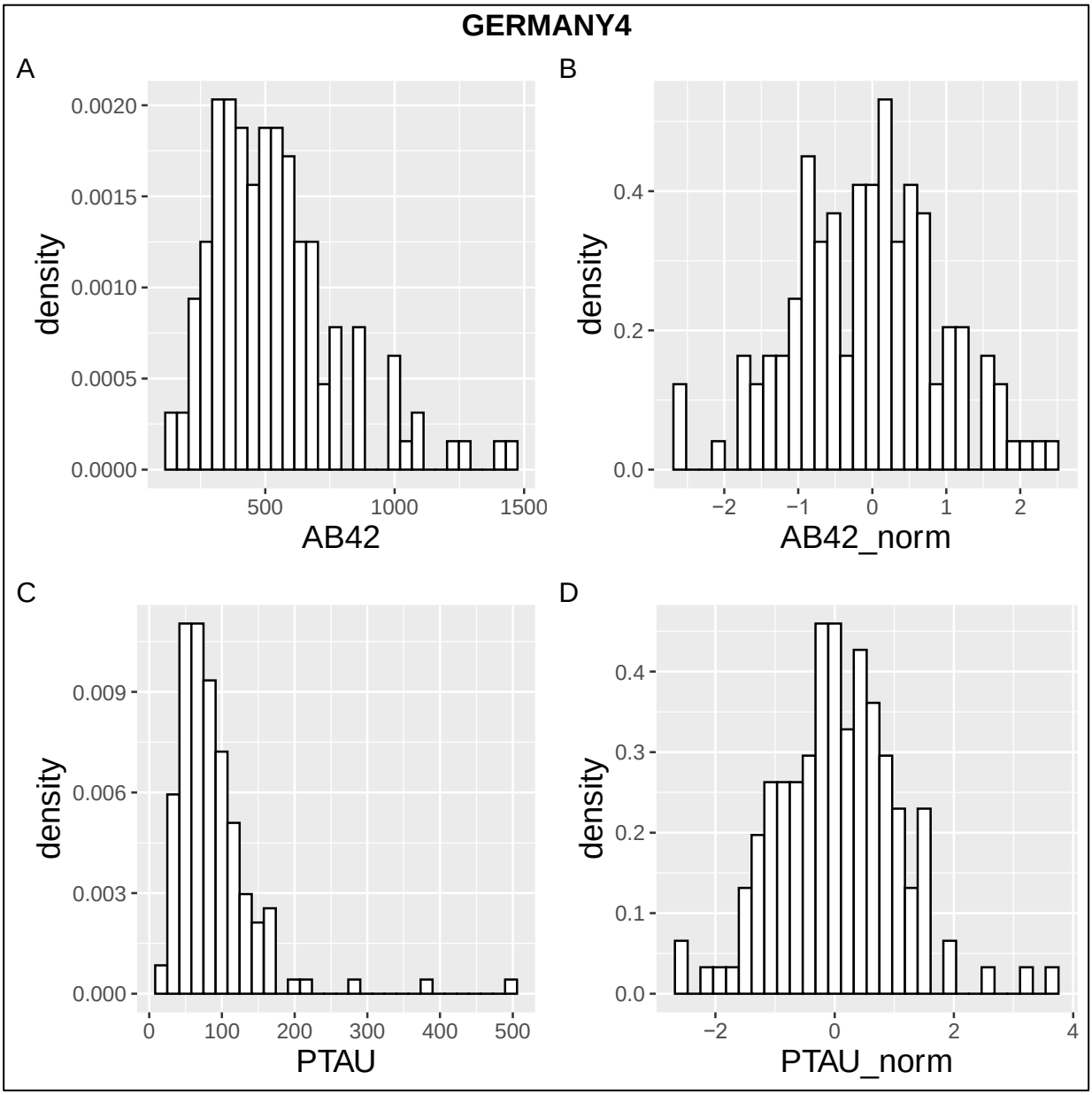


**Figure 8.** Raw and normalized CSF protein level distributions for the Germany4 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

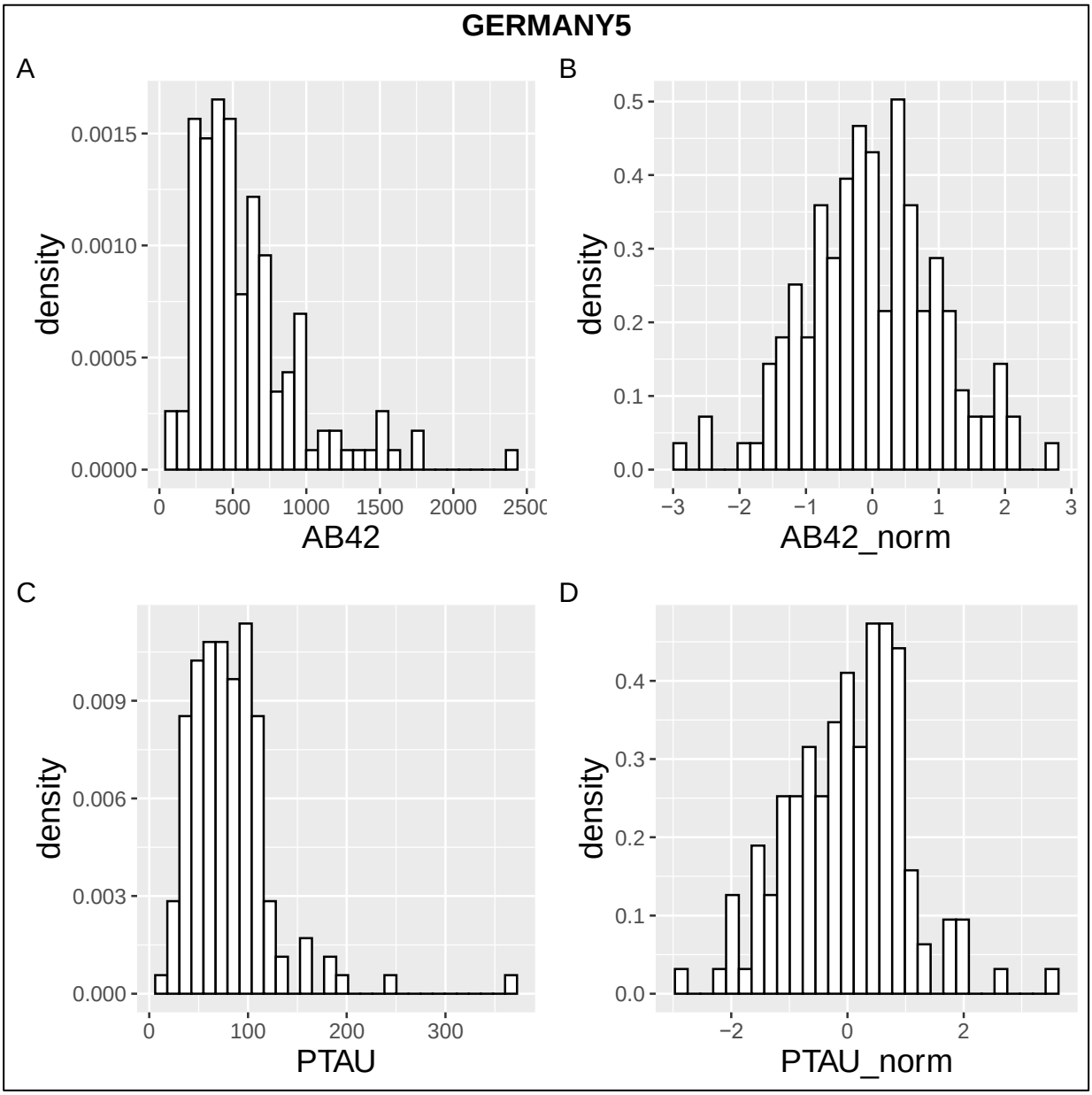


**Figure 9.** Raw and normalized CSF protein level distributions for the Germany5 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

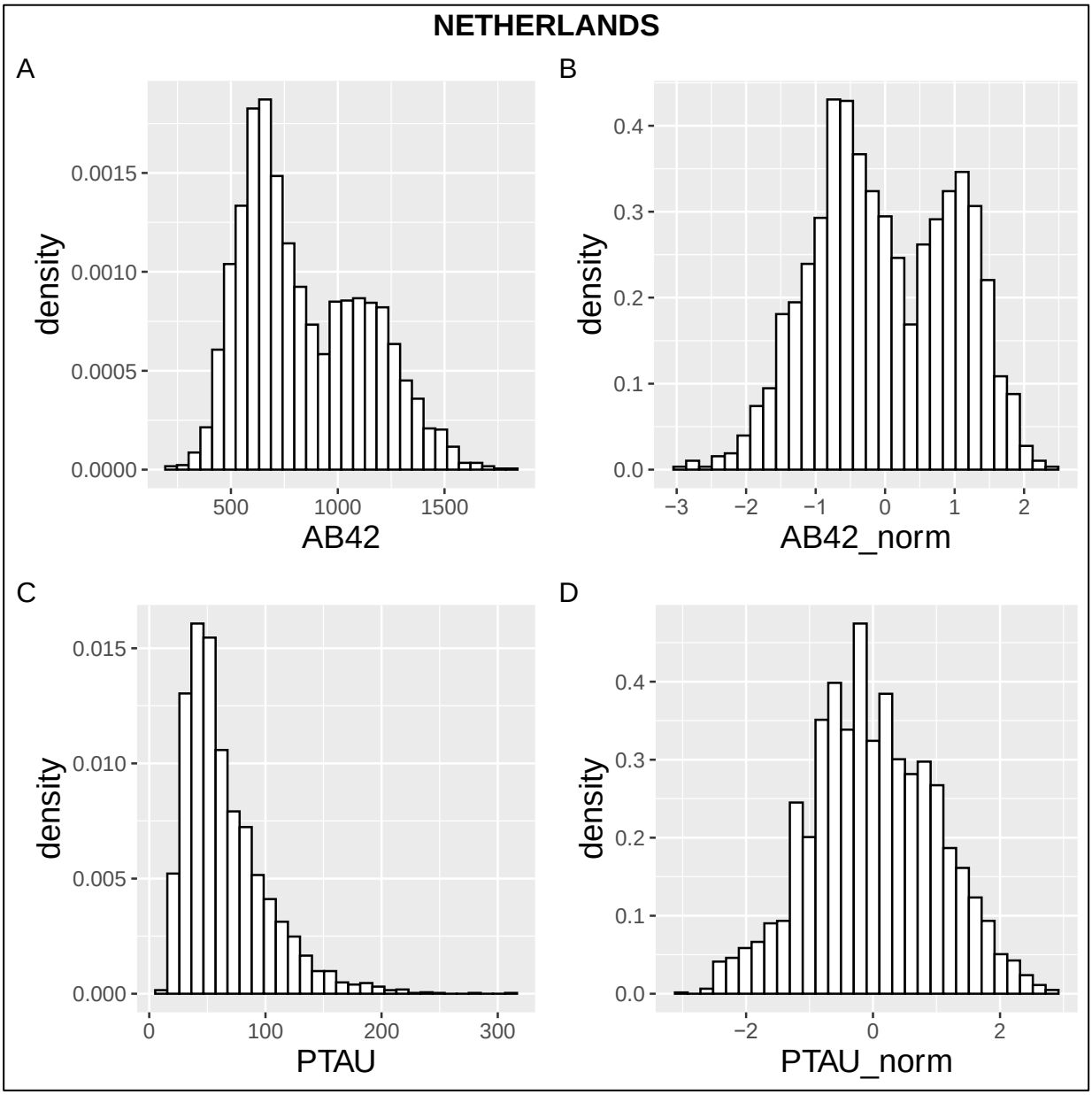


**Figure 10.** Raw and normalized CSF protein level distributions for the Netherlands cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

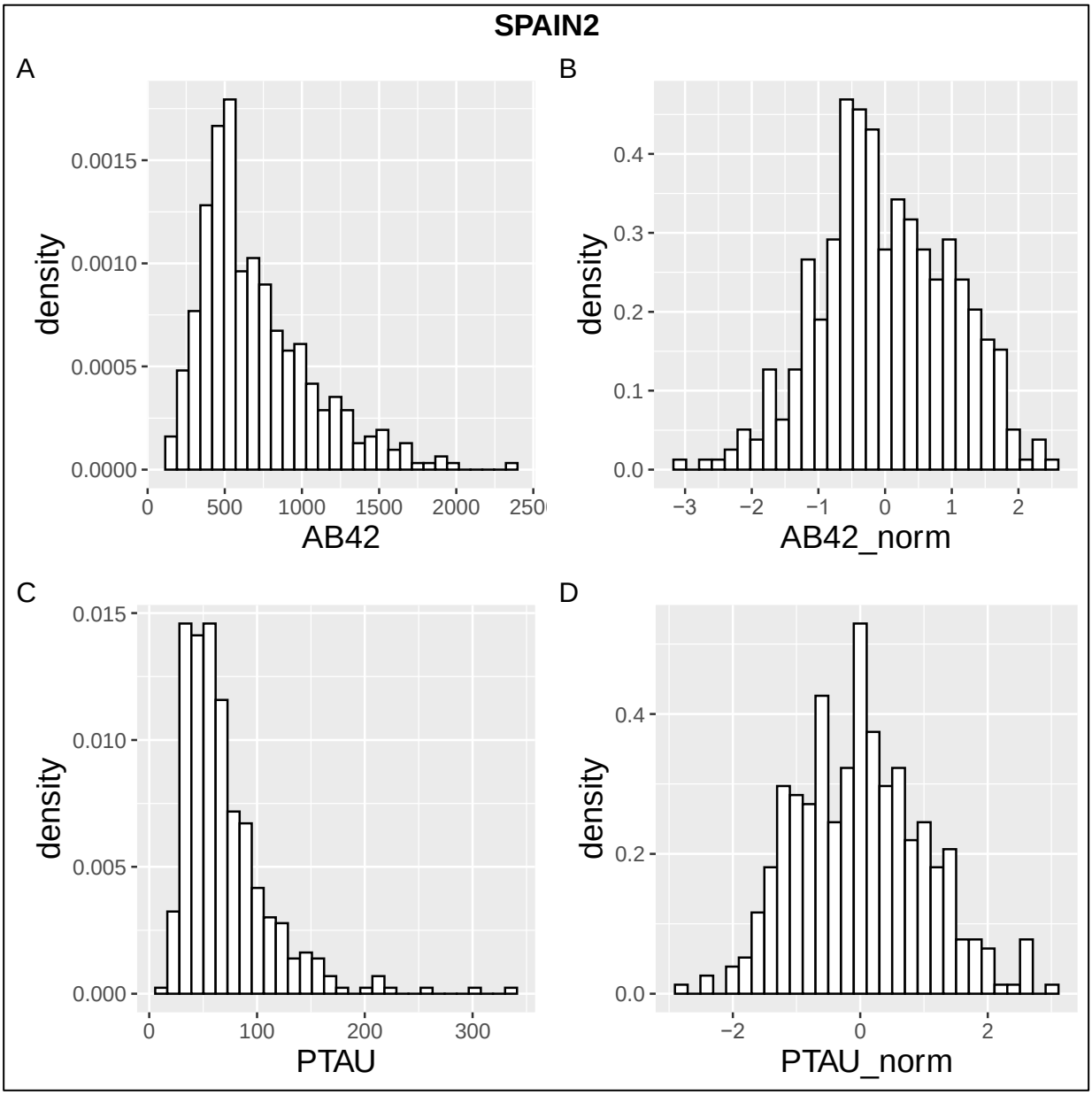


**Figure 11.** Raw and normalized CSF protein level distributions for the Spain2 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

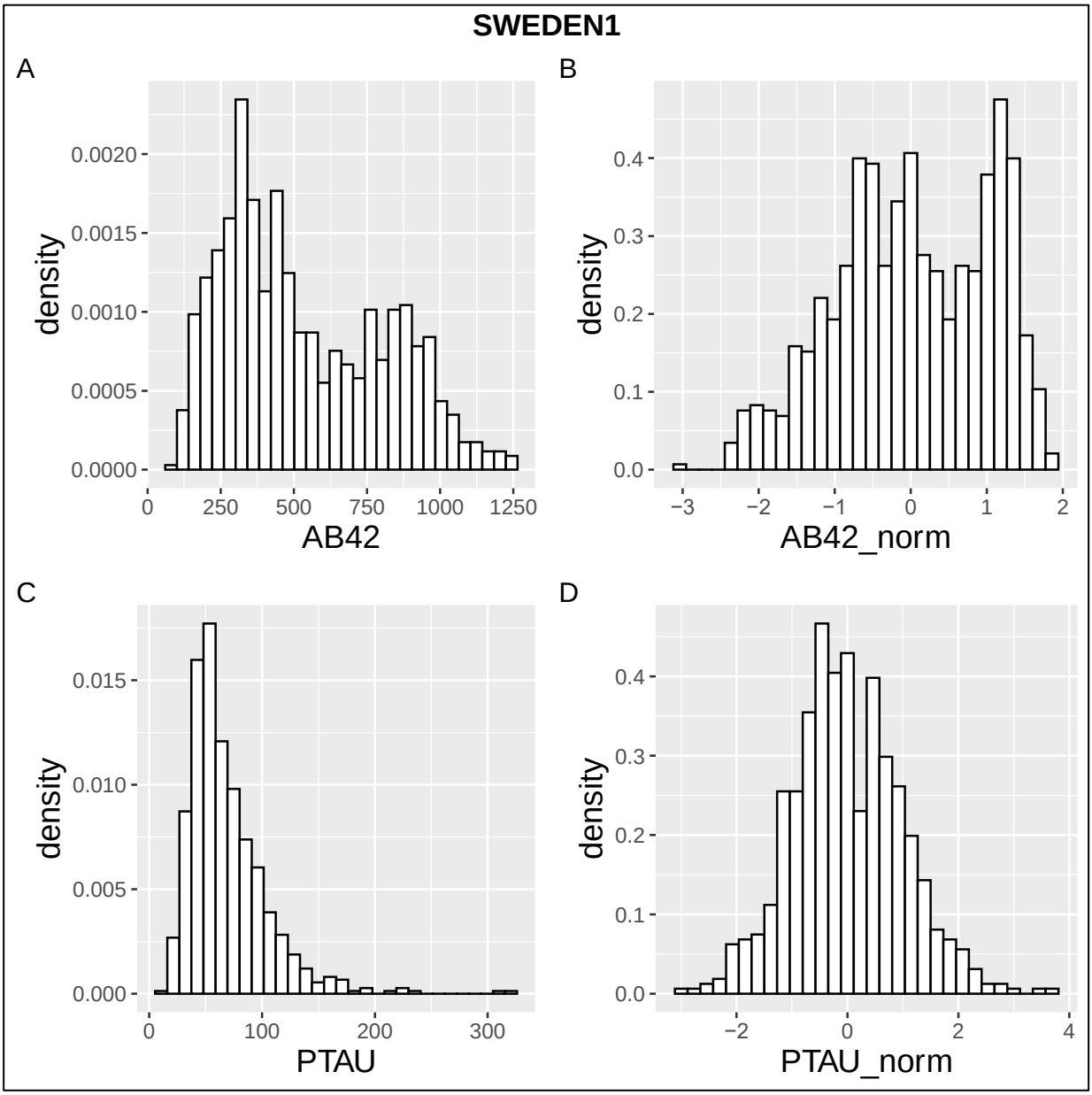


**Figure 12.** Raw and normalized CSF protein level distributions for the Sweden1 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

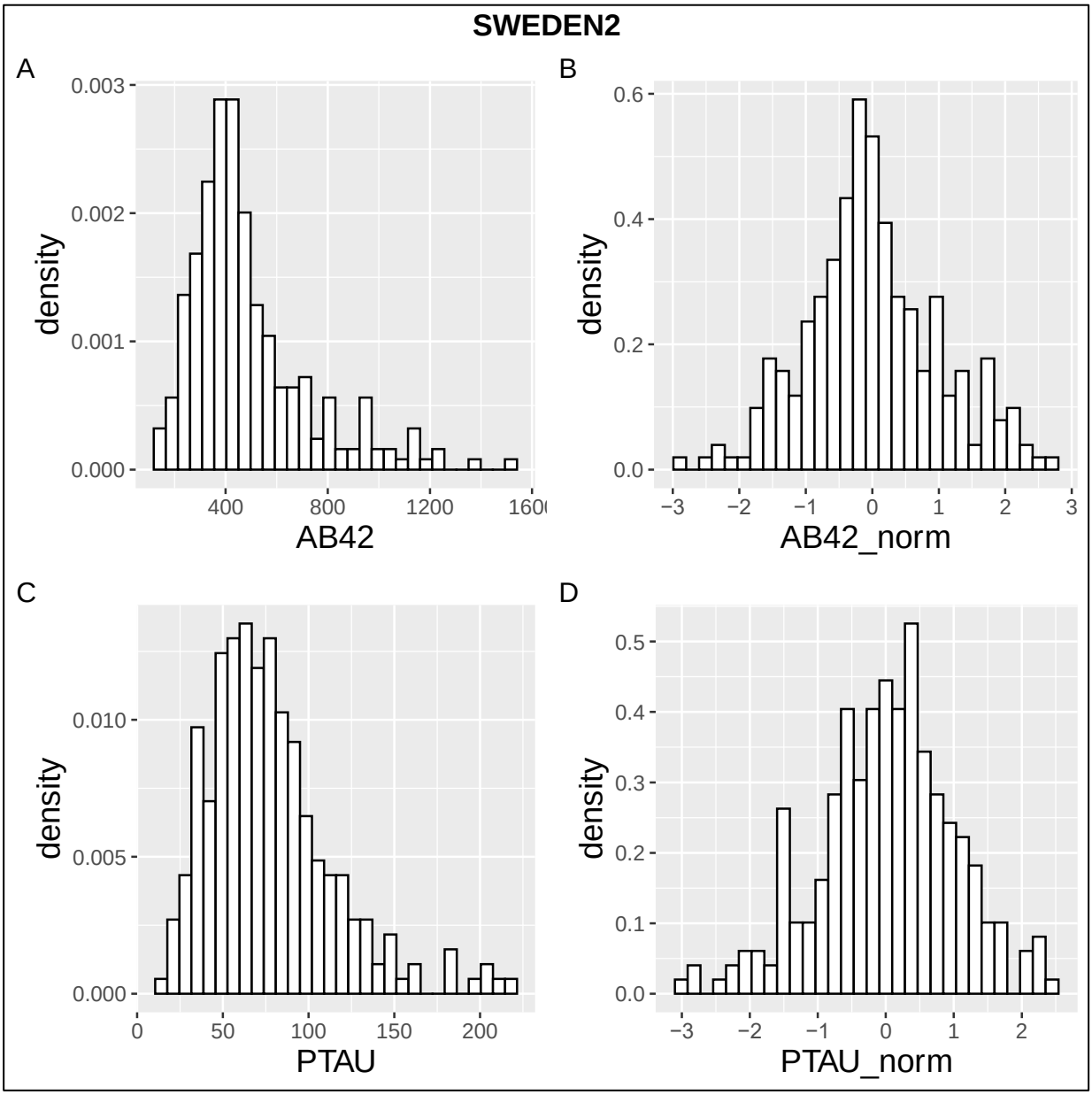


**Figure 13.** Raw and normalized CSF protein level distributions for the Sweden2 cohort. AB42 = amyloid-beta 42 protein levels; AB42_norm = normalized amyloid-beta 42 protein levels; PTAU = phosphorylated tau protein levels; PTAU_norm; normalized phosphorylated tau protein levels.

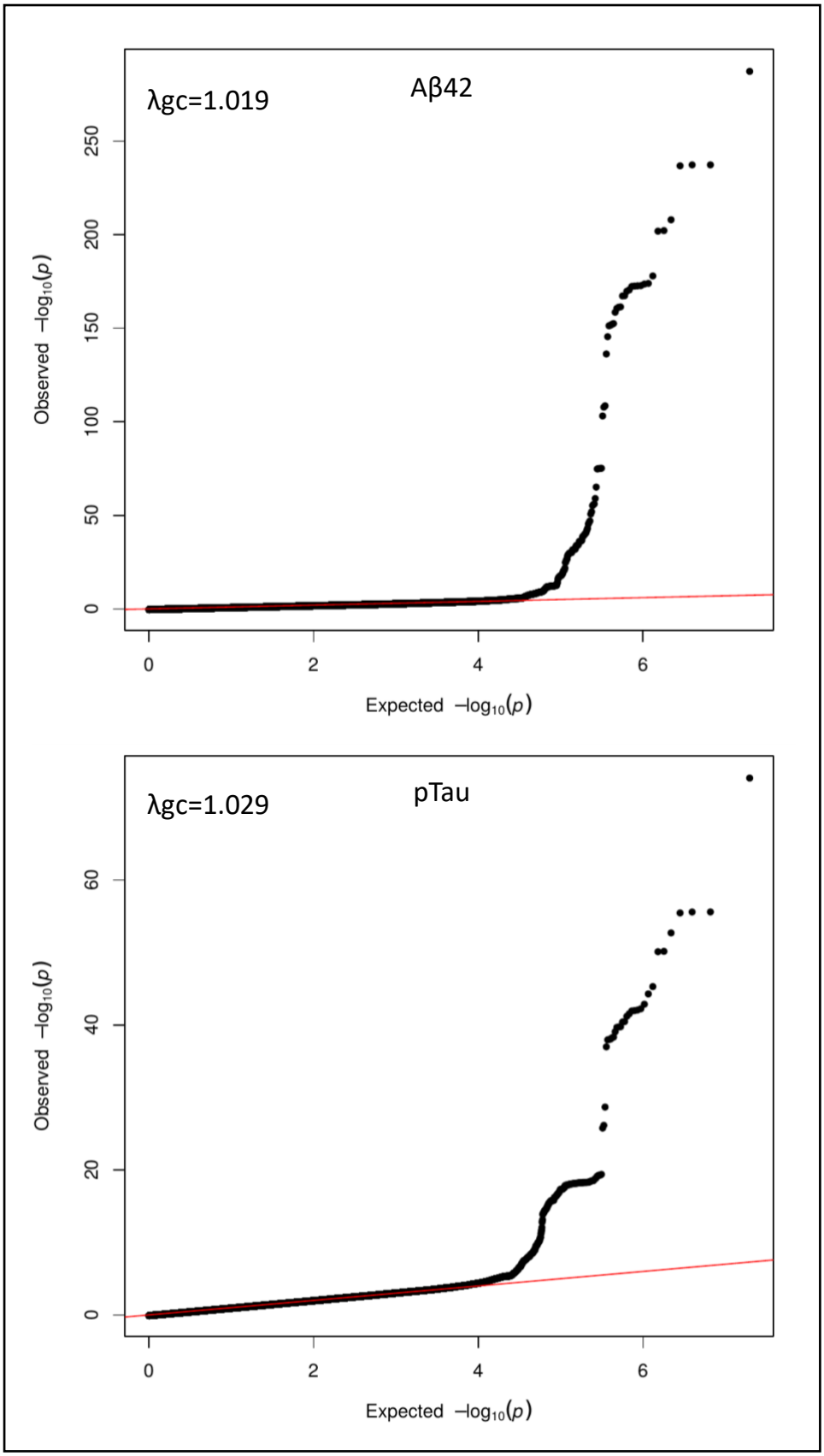


**Figure 14.** QQ plots of Aβ42 and pTau in stage 1. A graphical representation of the deviation of the observed P values from the null hypothesis.

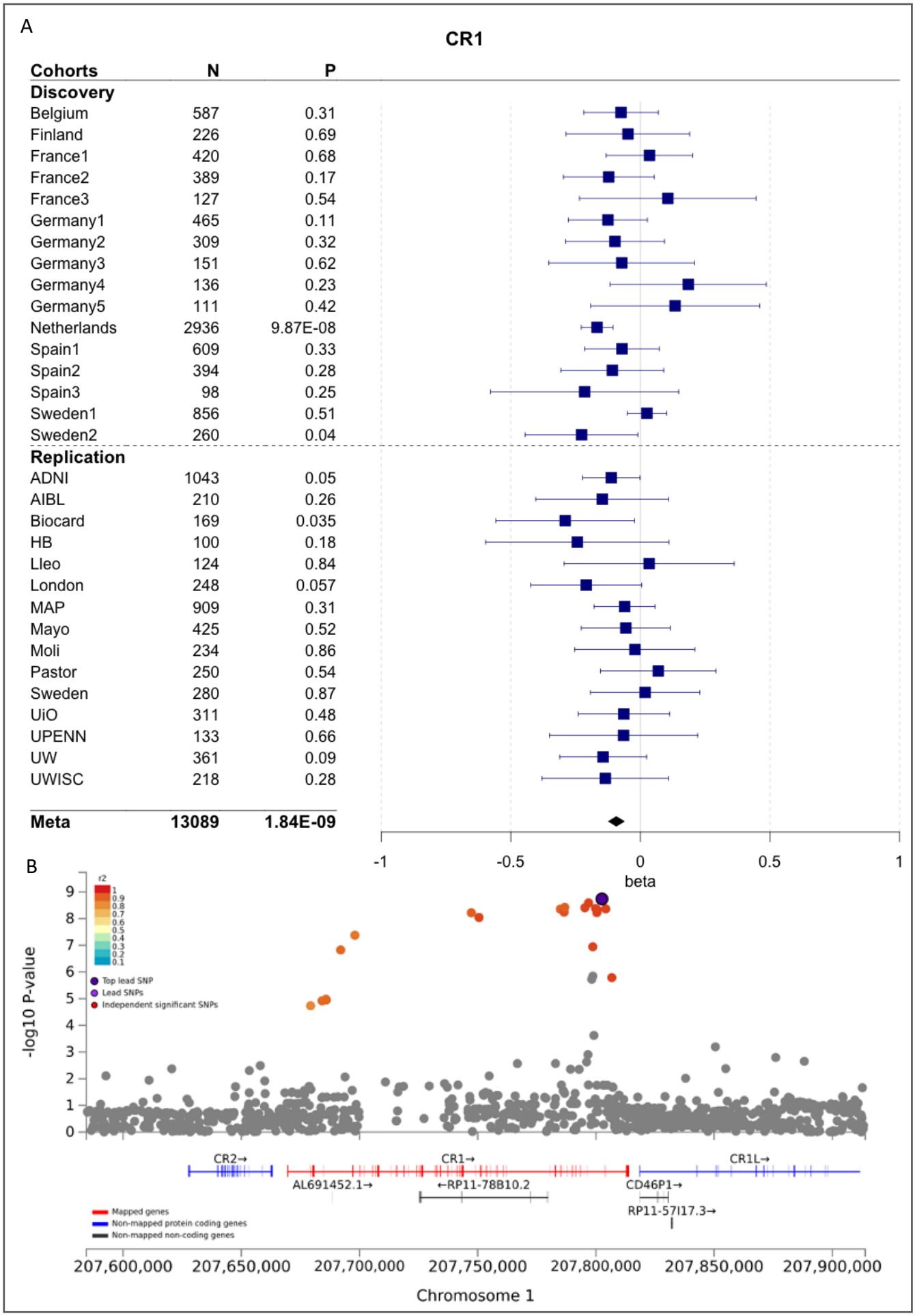


**Figure 15.** Forest (A) and LocusZoom (B) plots of *CR1* locus associating for AB42. Only the variants with an r2 > 0.6 with the lead SNP are color-coded.

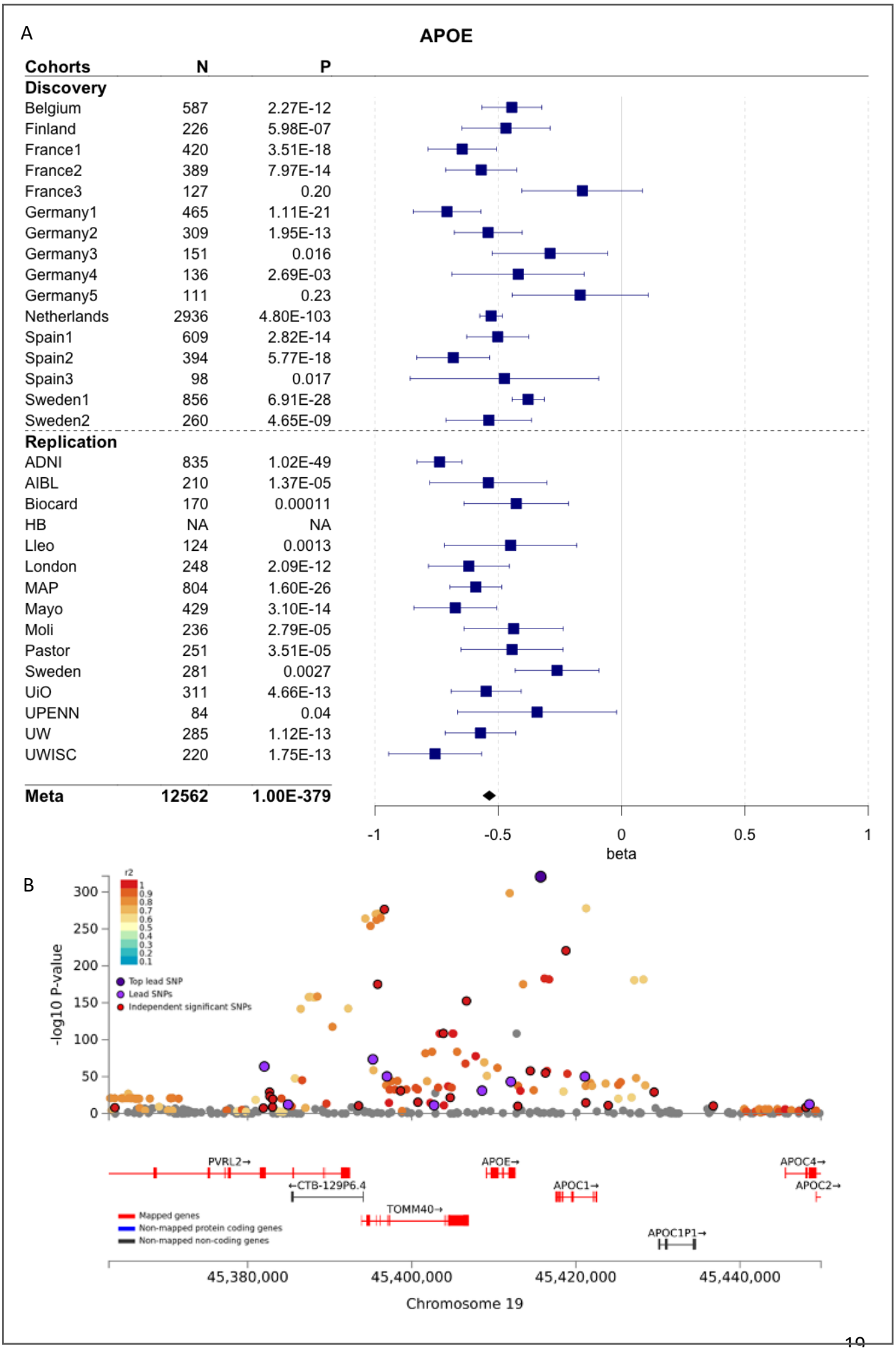


**Figure 16.** Forest (A) and LocusZoom (B) plots of *APOE* locus associating for AB42. Only the variants with an r2 > 0.6 with the lead SNP are color-coded.

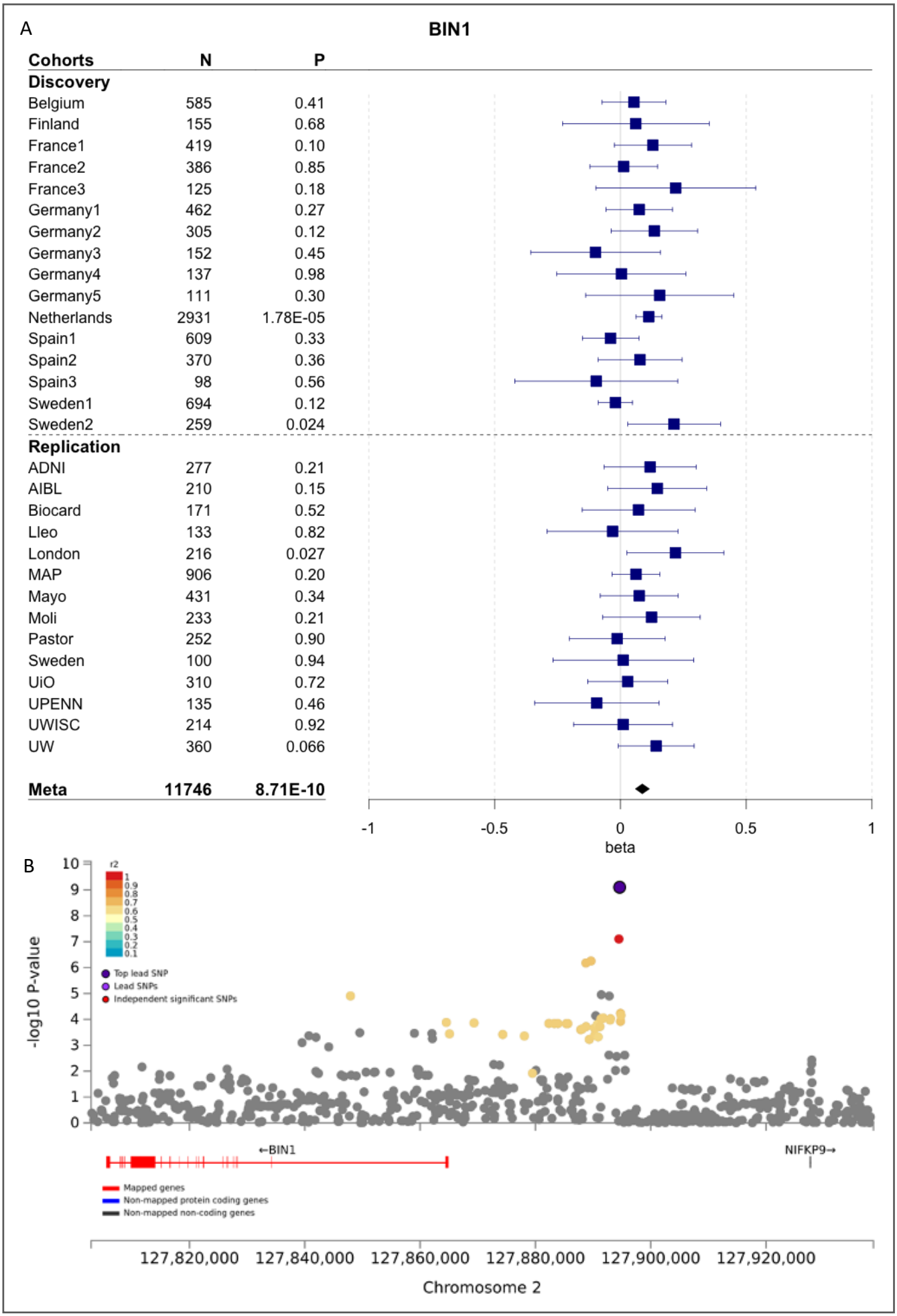


**Figure 17.** Forest (A) and LocusZoom (B) plots of *BIN1* locus associating for pTau. Only the variants with an r2 > 0.6 with the lead SNP are color-coded.

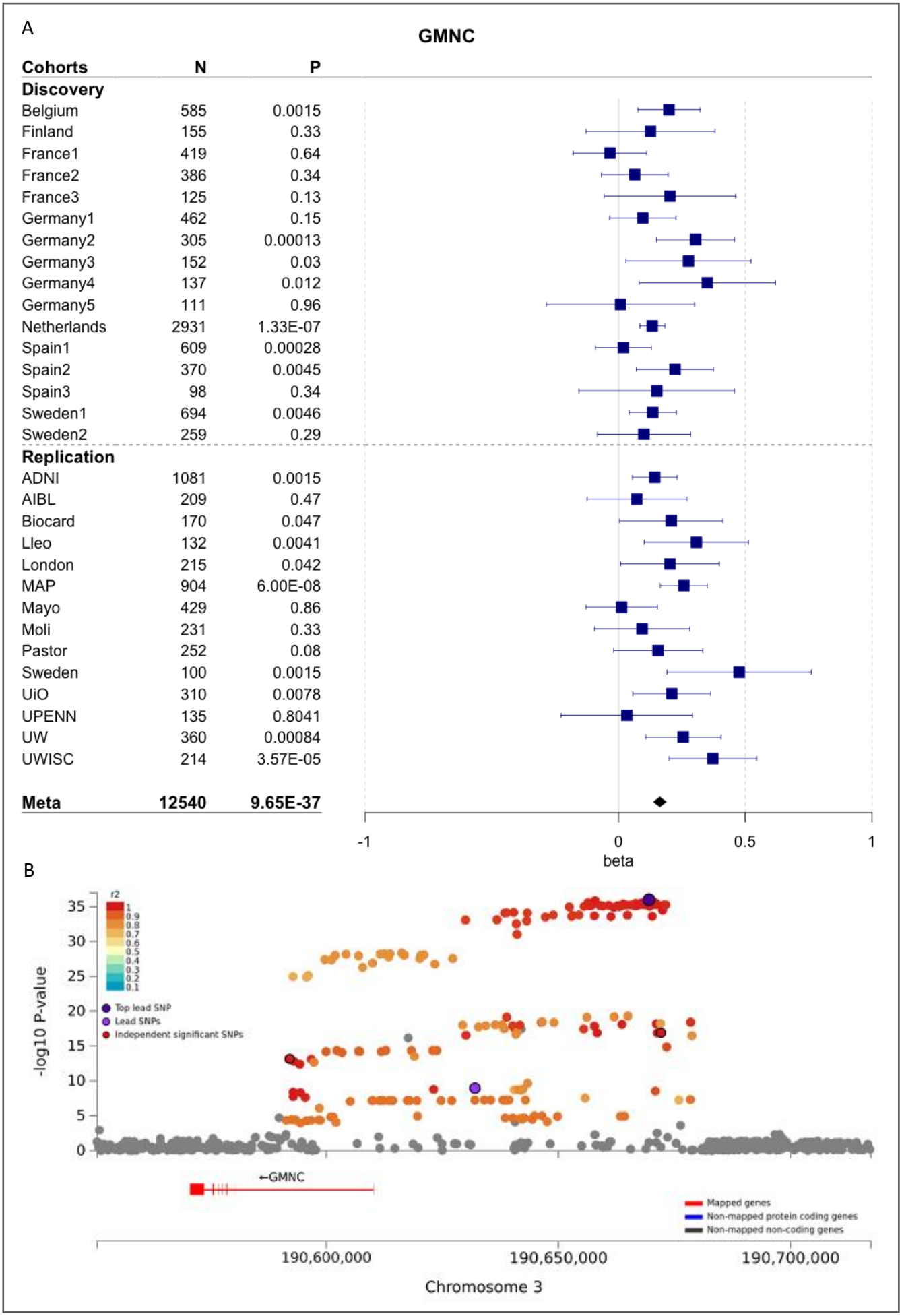


**Figure 18.** Forest (A) and LocusZoom (B) plots of *GMNC* locus associating for pTau. Only the variants with an r2 > 0.6 with the lead SNP are color-coded. The significance level for variants in high LD can vary due to differences in N as a part of the variants were not available in the replication cohorts.

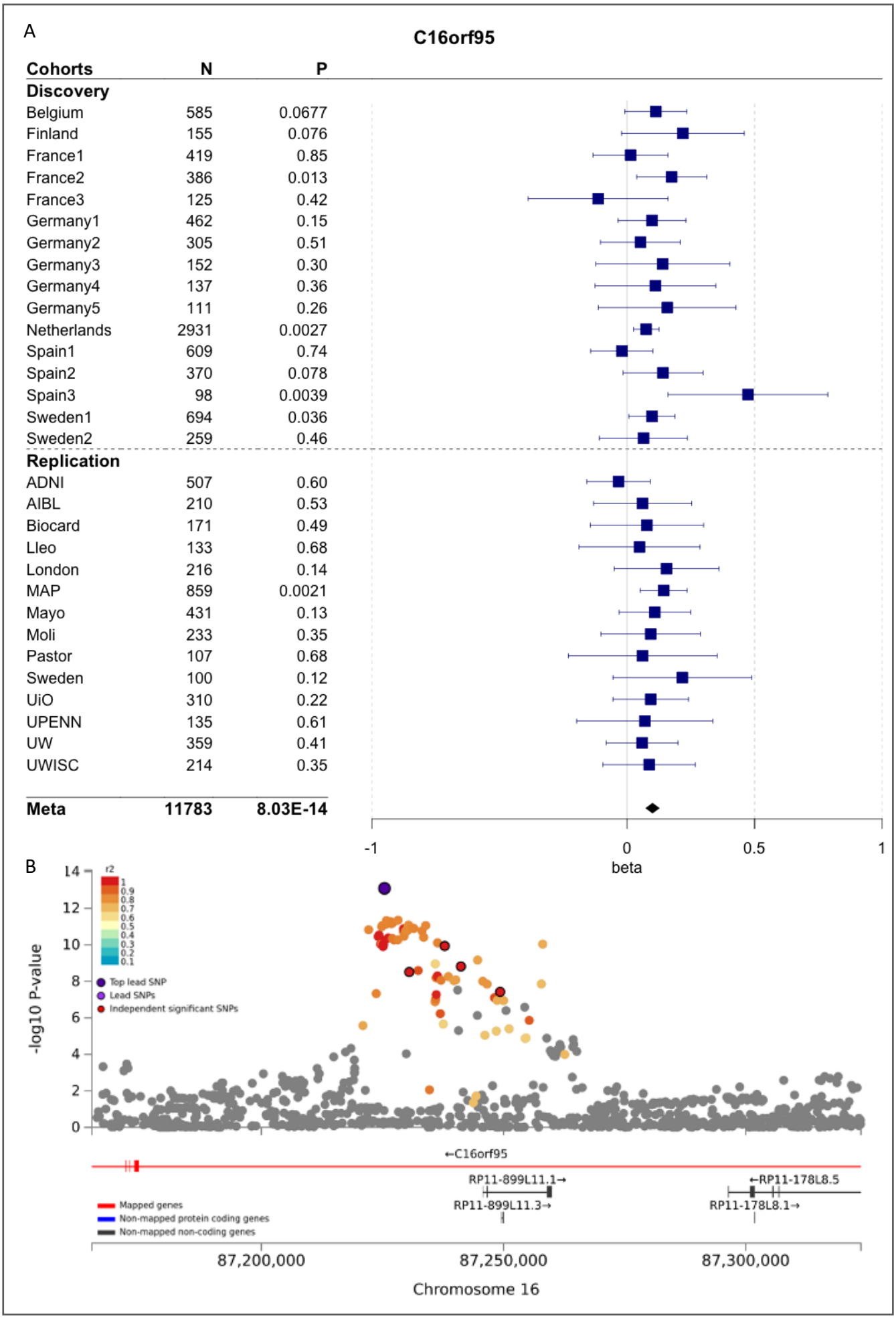


**Figure 19.** Forest (A) and LocusZoom (B) plots of *C16orf95* locus associating for pTau. Only the variants with an r2 > 0.6 with the lead SNP are color-coded.

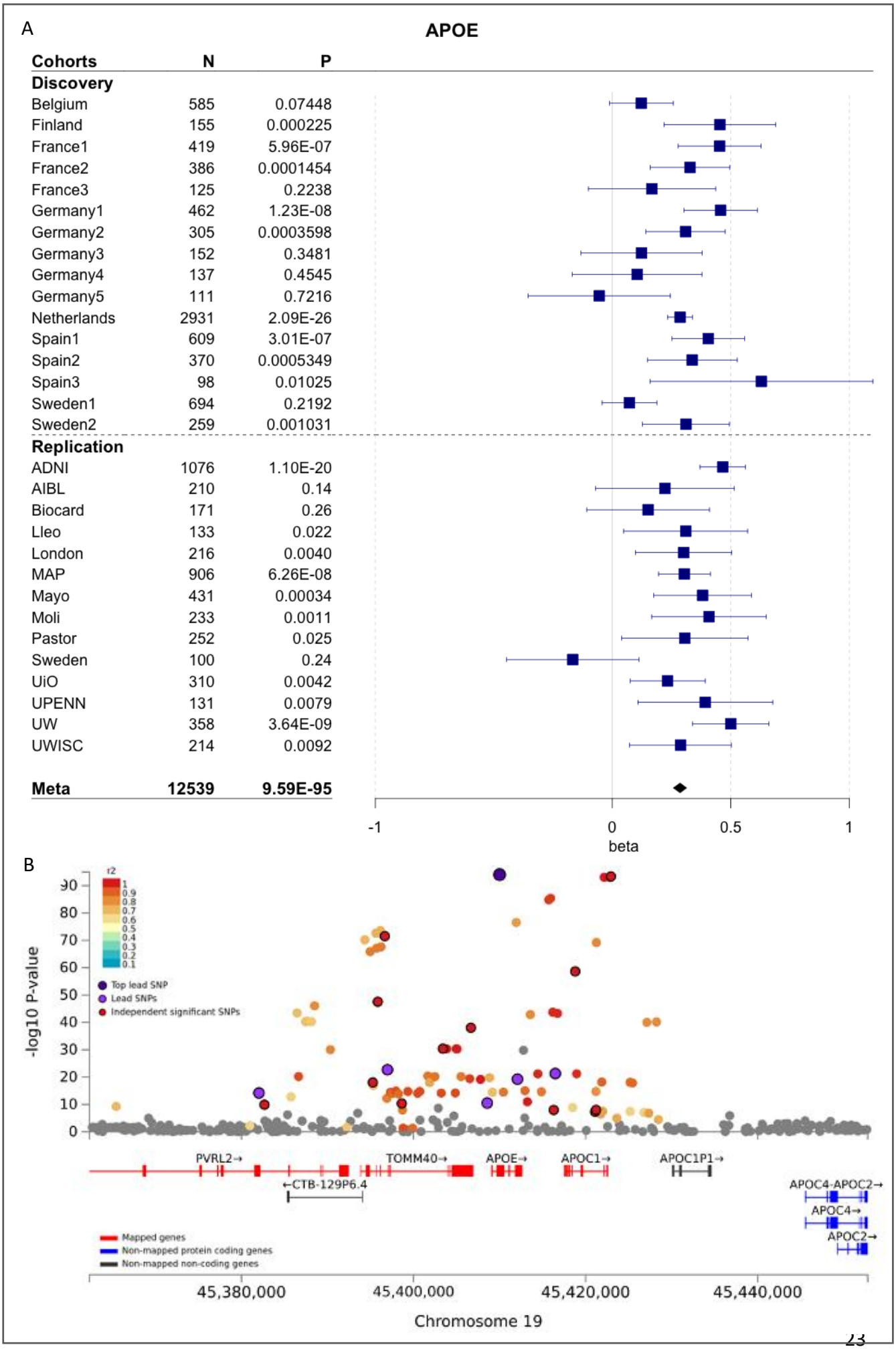




**Figure 20**. Forest (A) and LocusZoom (B) plots of *APOE* locus associating for pTau. Only the variants with an r2 > 0.6 with the lead SNP are color-coded.

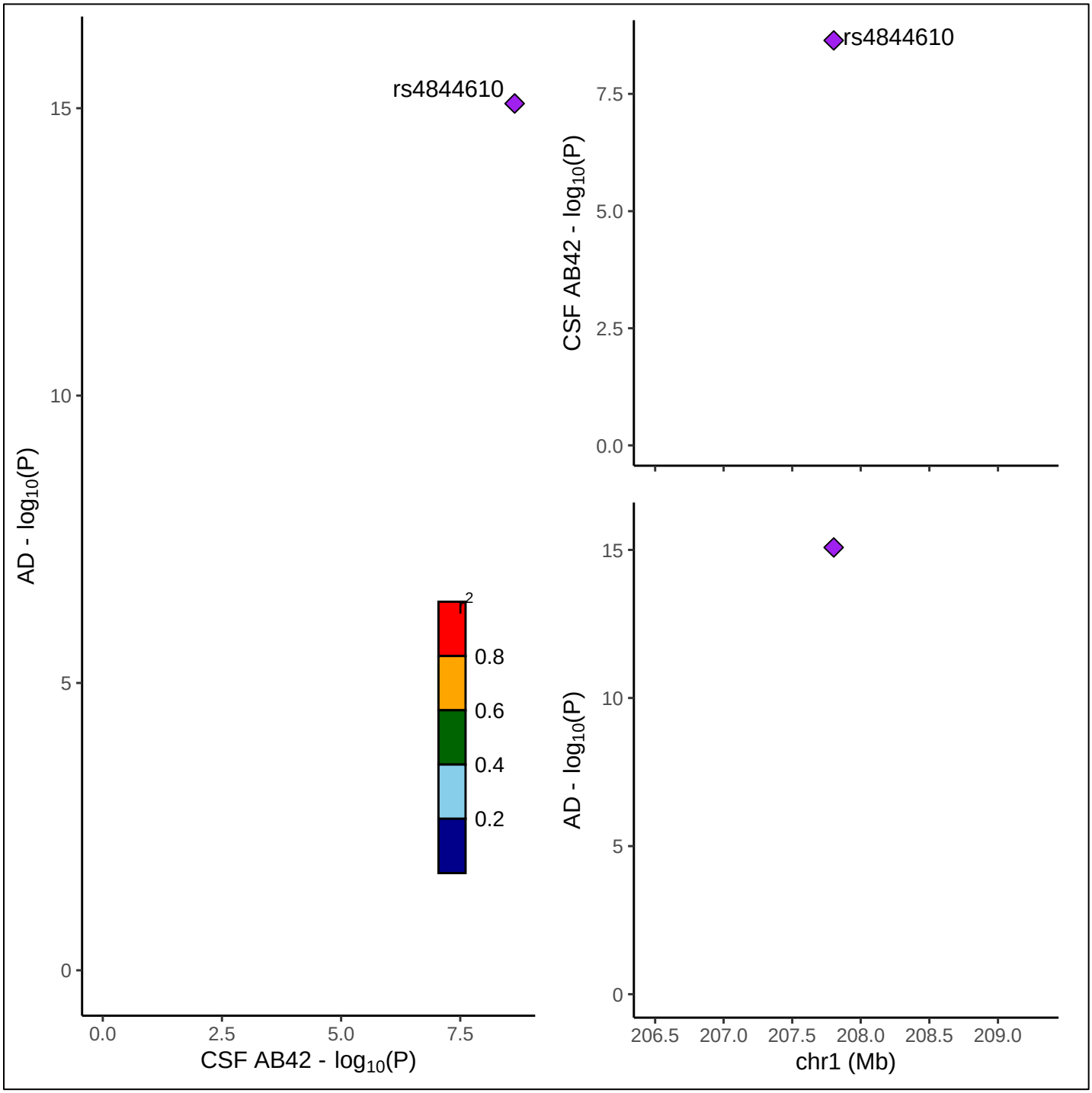


**Figure 21**. Colocalization overview of *CR1* locus for CSF AB42 and AD GWAS.

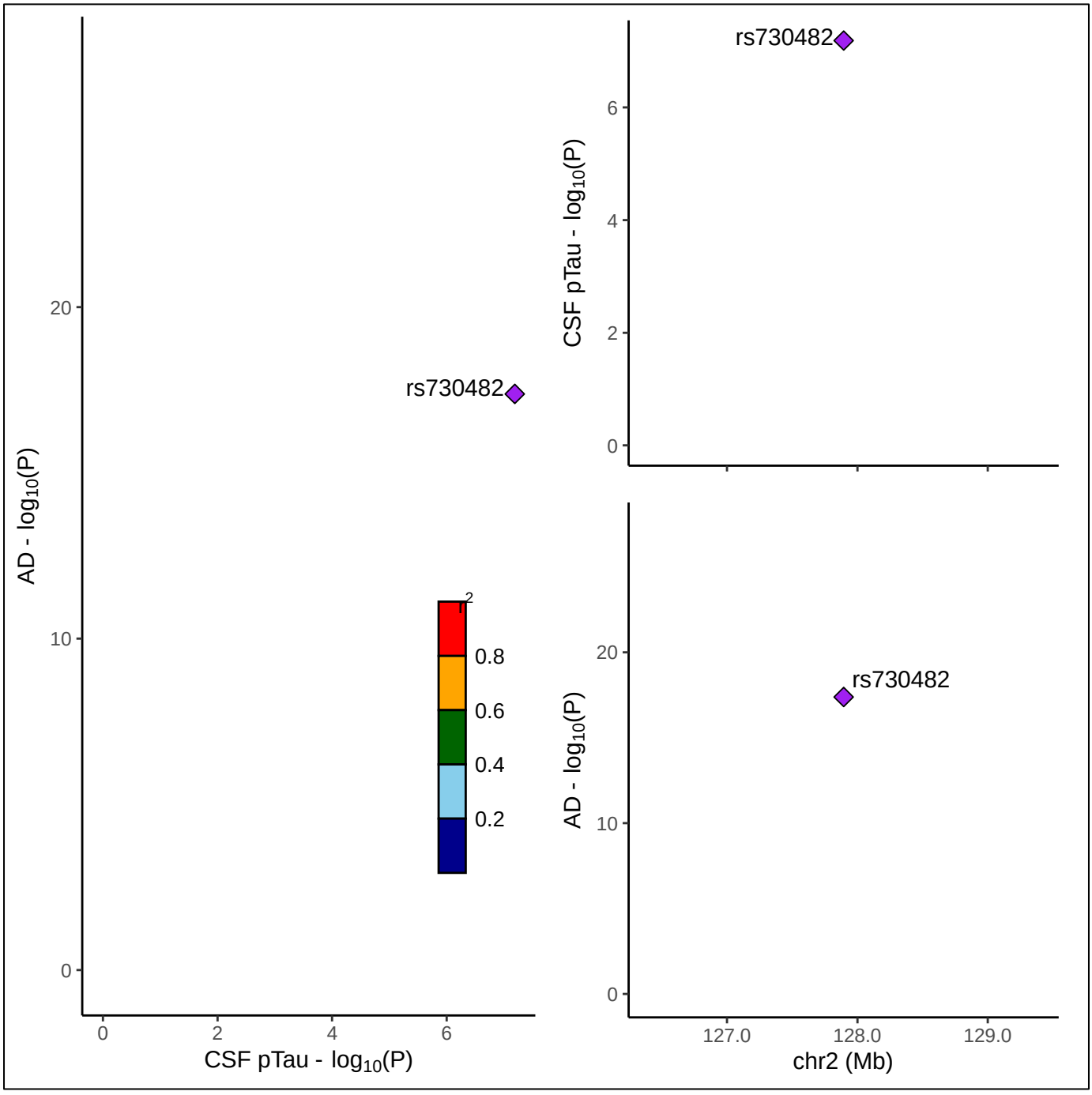


**Figure 22**. Colocalization overview of *BIN1* locus for CSF PTAU and AD GWAS.

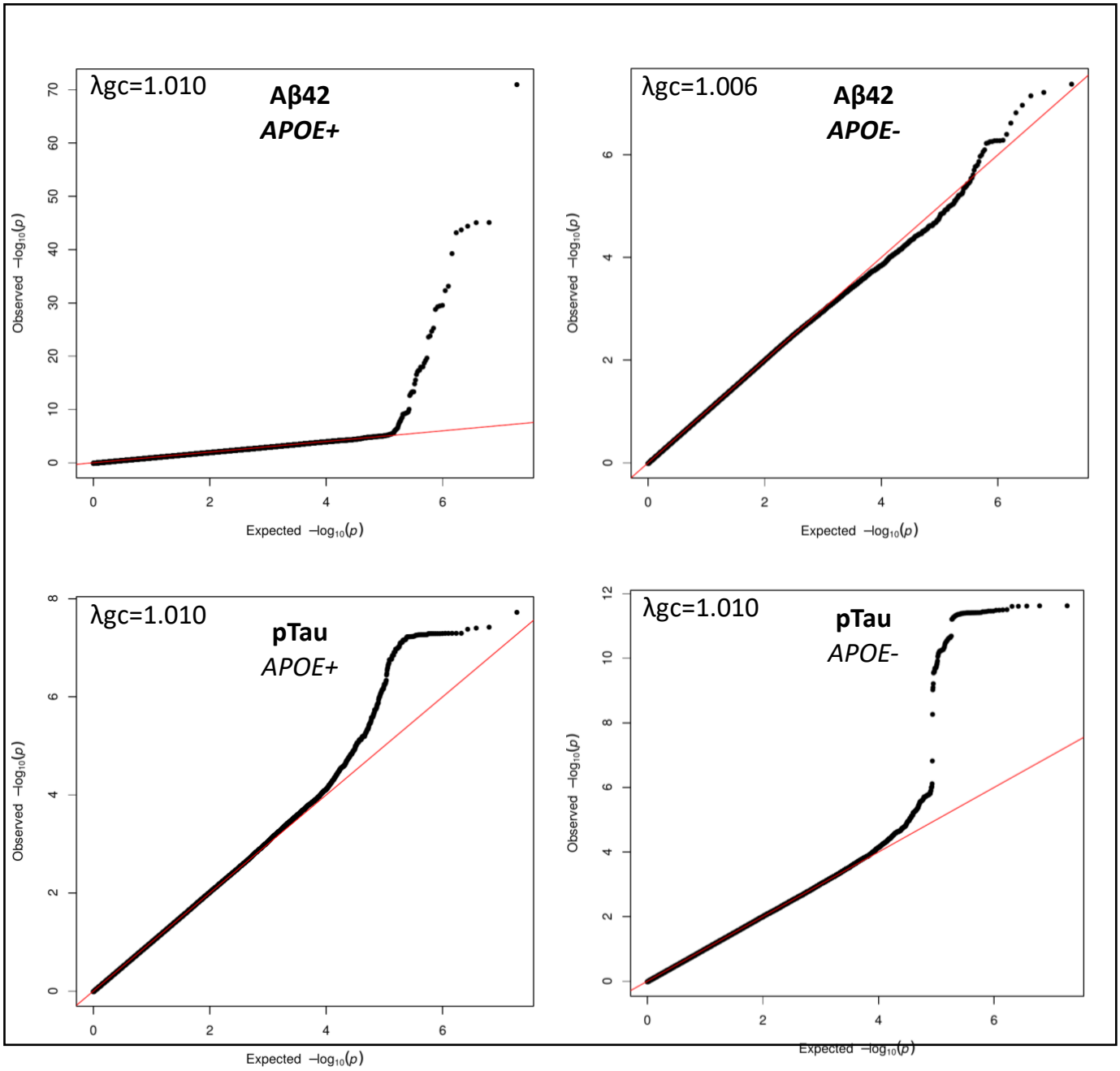


**Figure 23.** QQ plots of Aβ42 and pTau stratified for APOE4 status. A graphical representation of the deviation of the observed P values from the null hypothesis.

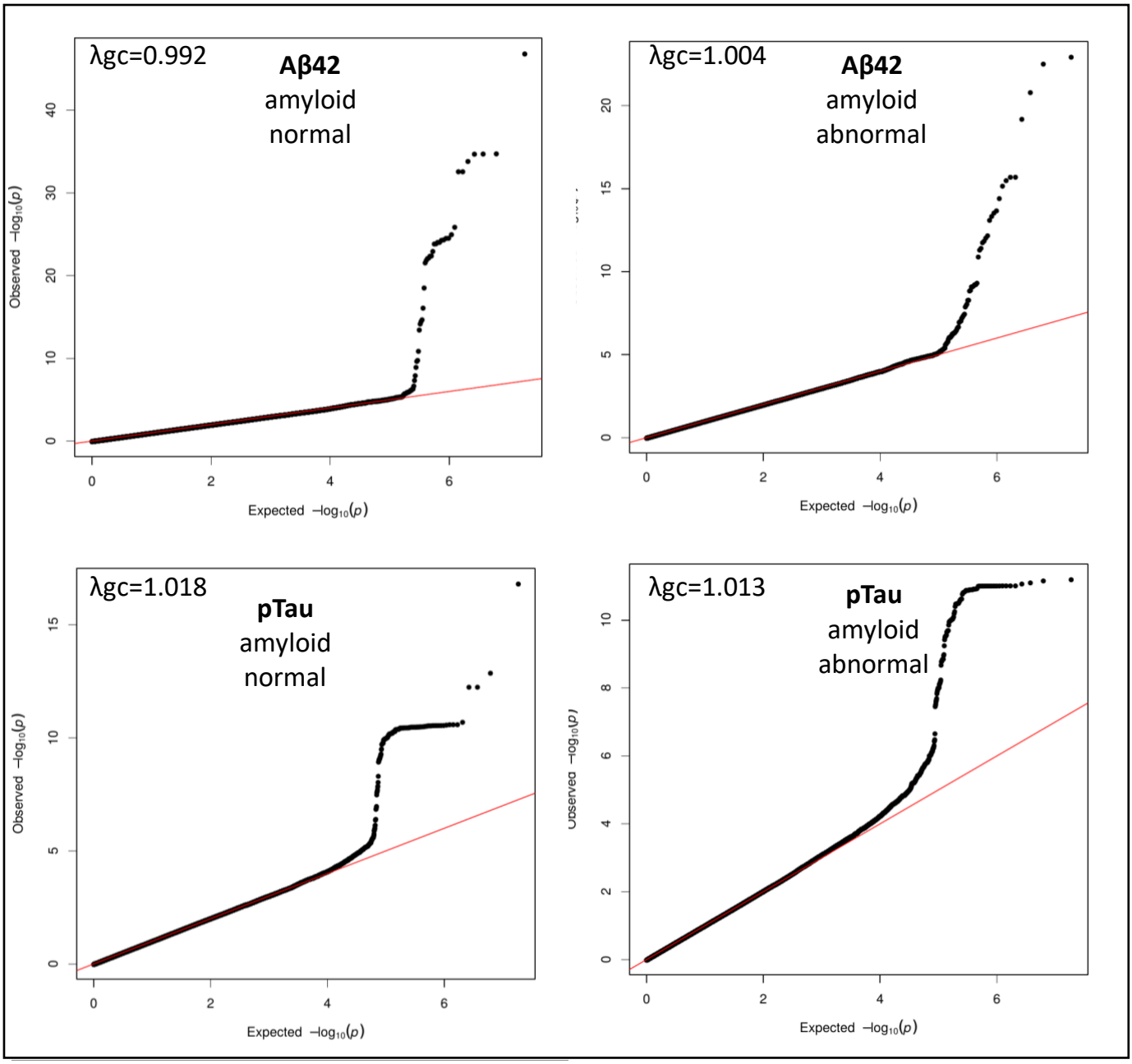


**Figure 24.** QQ plots of Aβ42 and pTau stratified for amyloid status. A graphical representation of the deviation of the observed P values from the null hypothesis.

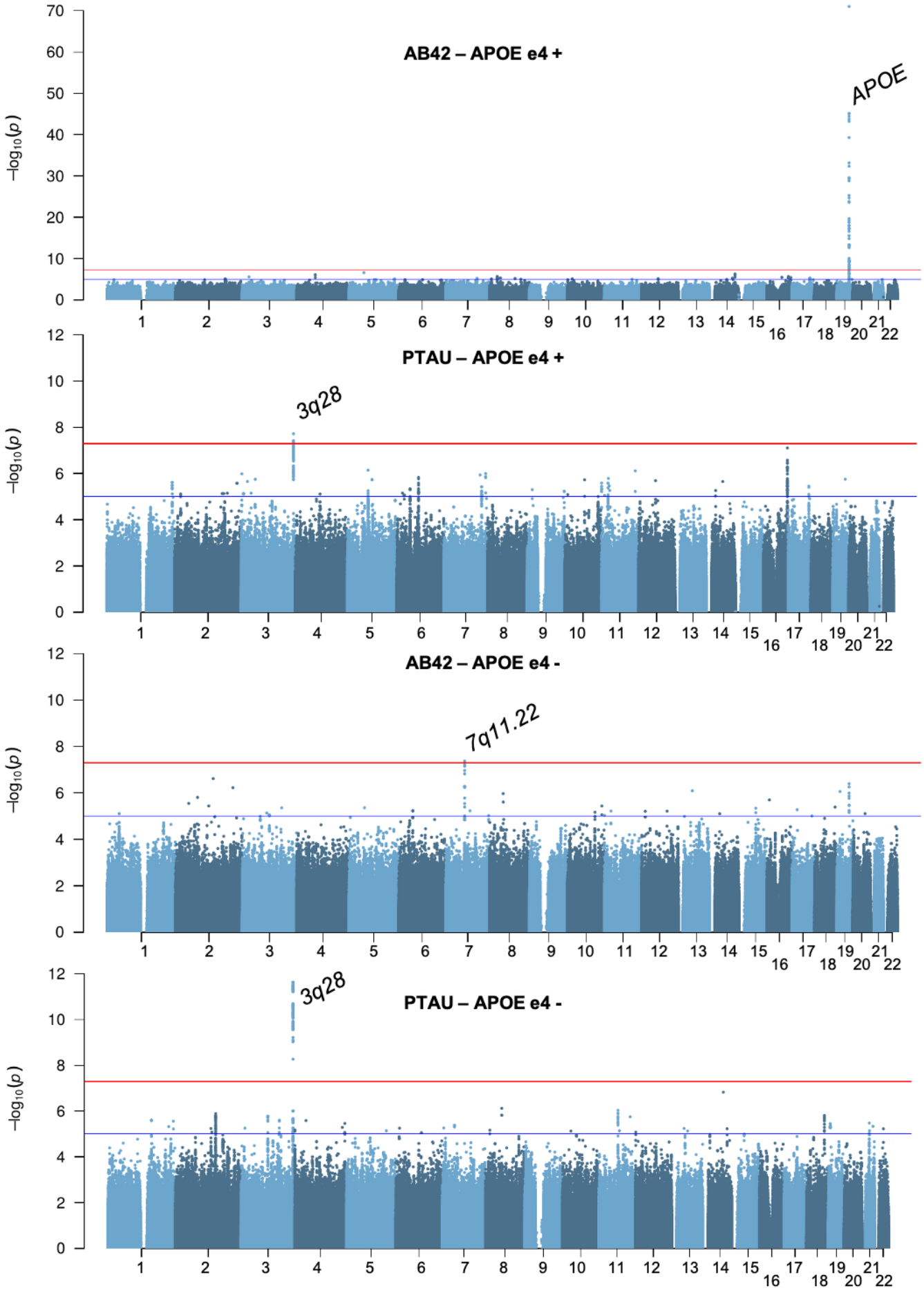


**Figure 25.** Manhattan plots of association results for stratification based on APOE e4 status.

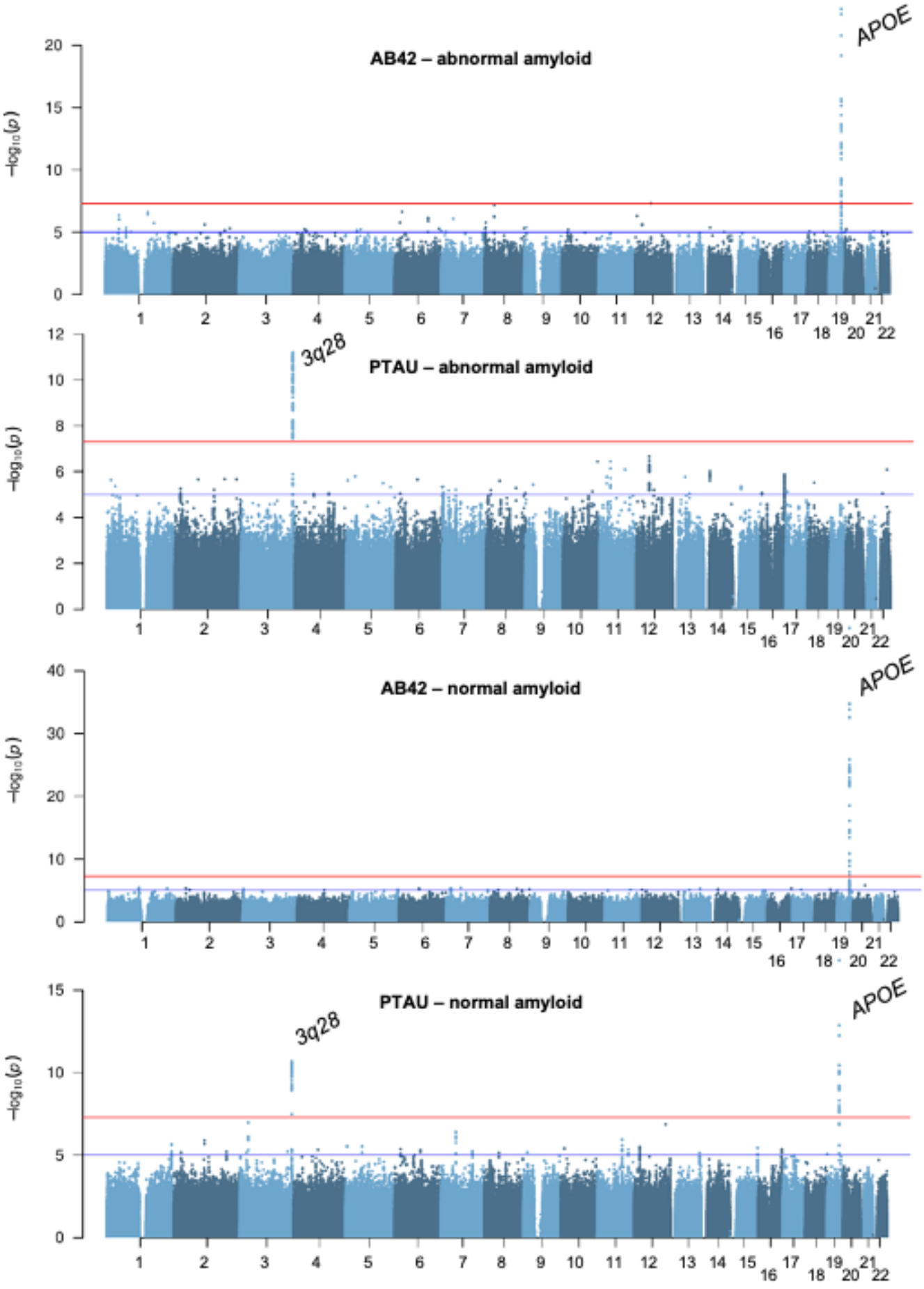


**Figure 26.** Manhattan plots of association results for stratification based on amyloid status in CSF.

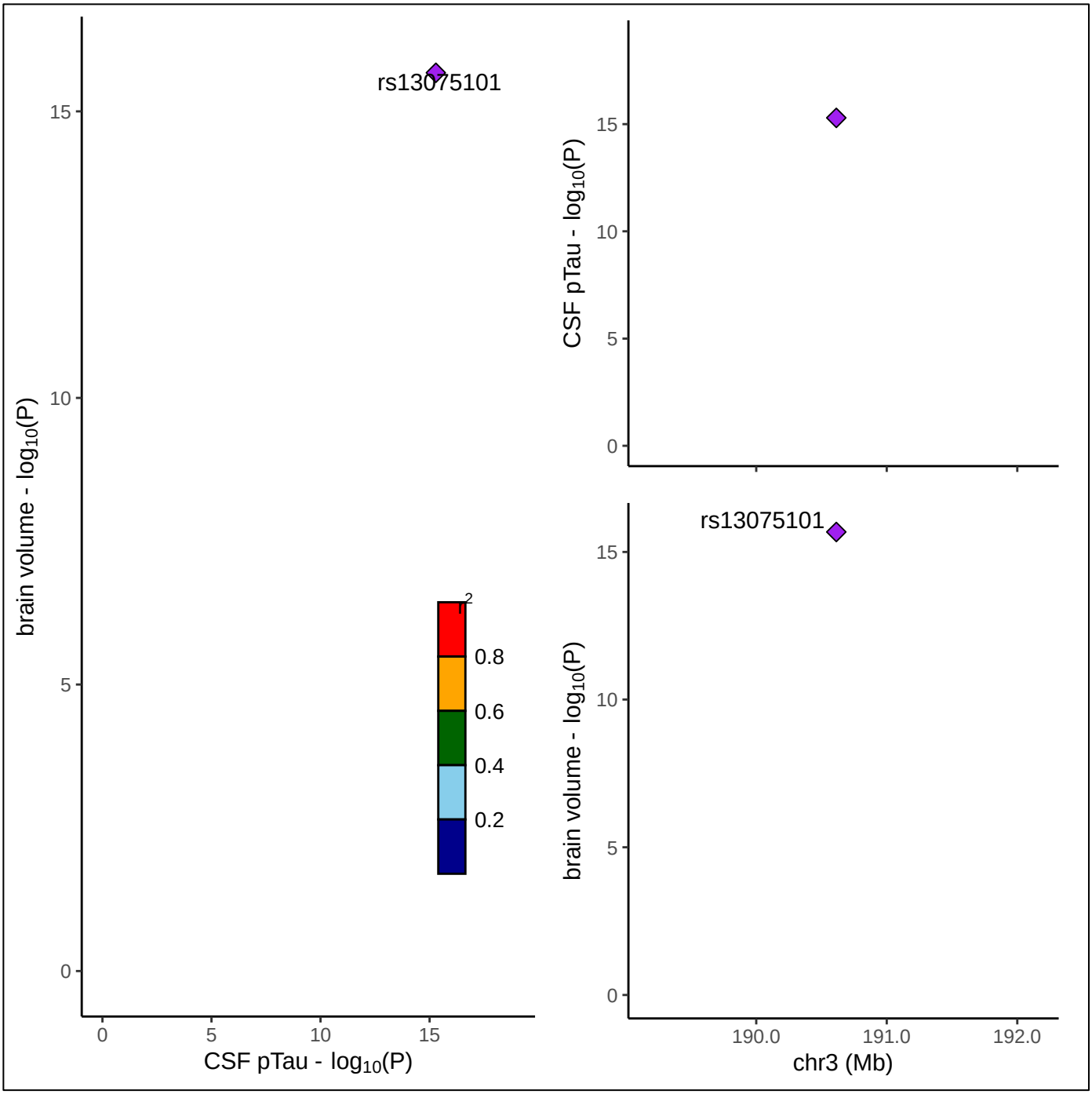


**Figure 27**. Colocalization overview of *GMNC* locus for CSF PTAU and brain volume GWAS.

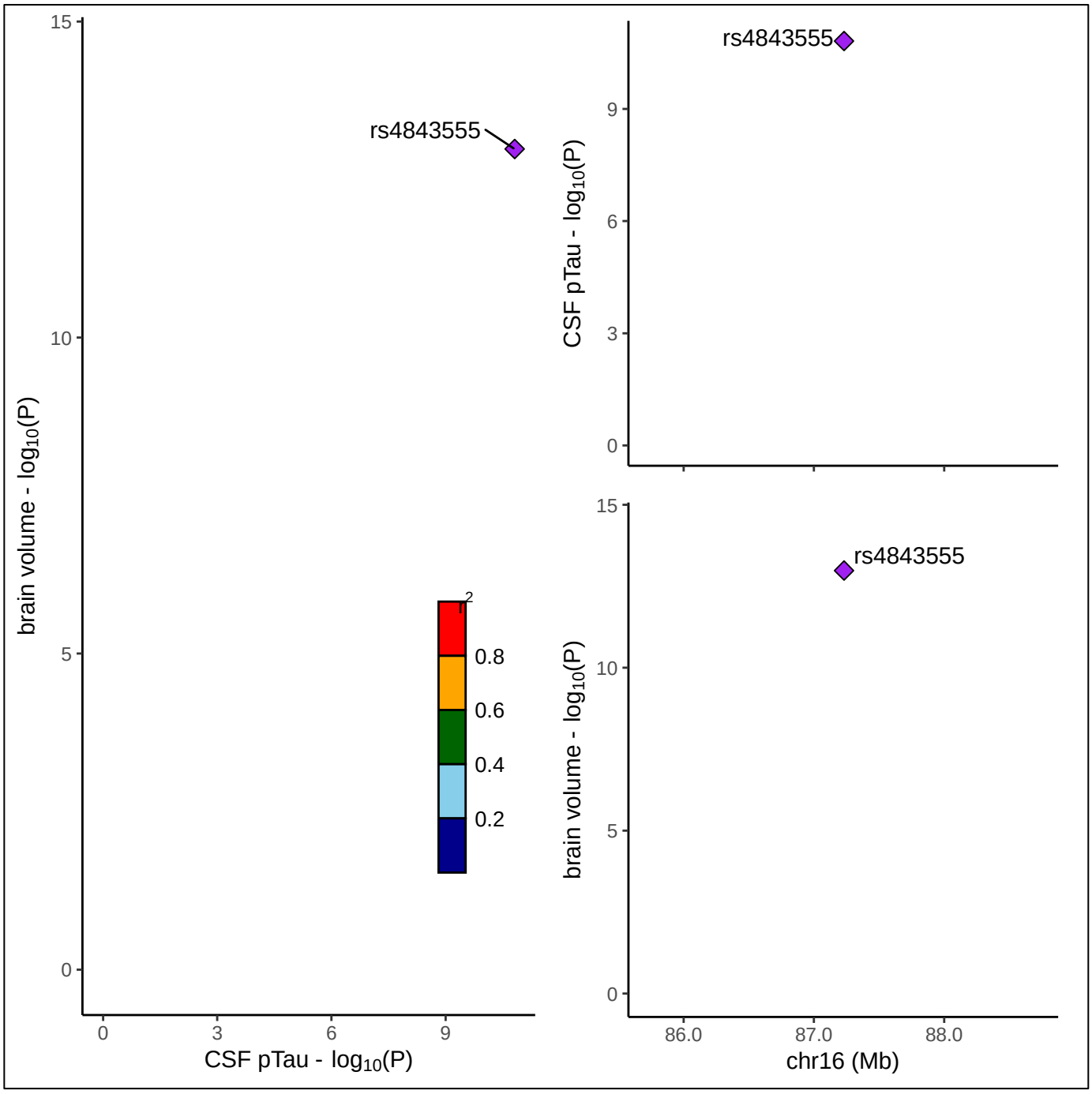


**Figure 28**. Colocalization overview of *C16orf95* locus for CSF PTAU and brain volume GWAS.

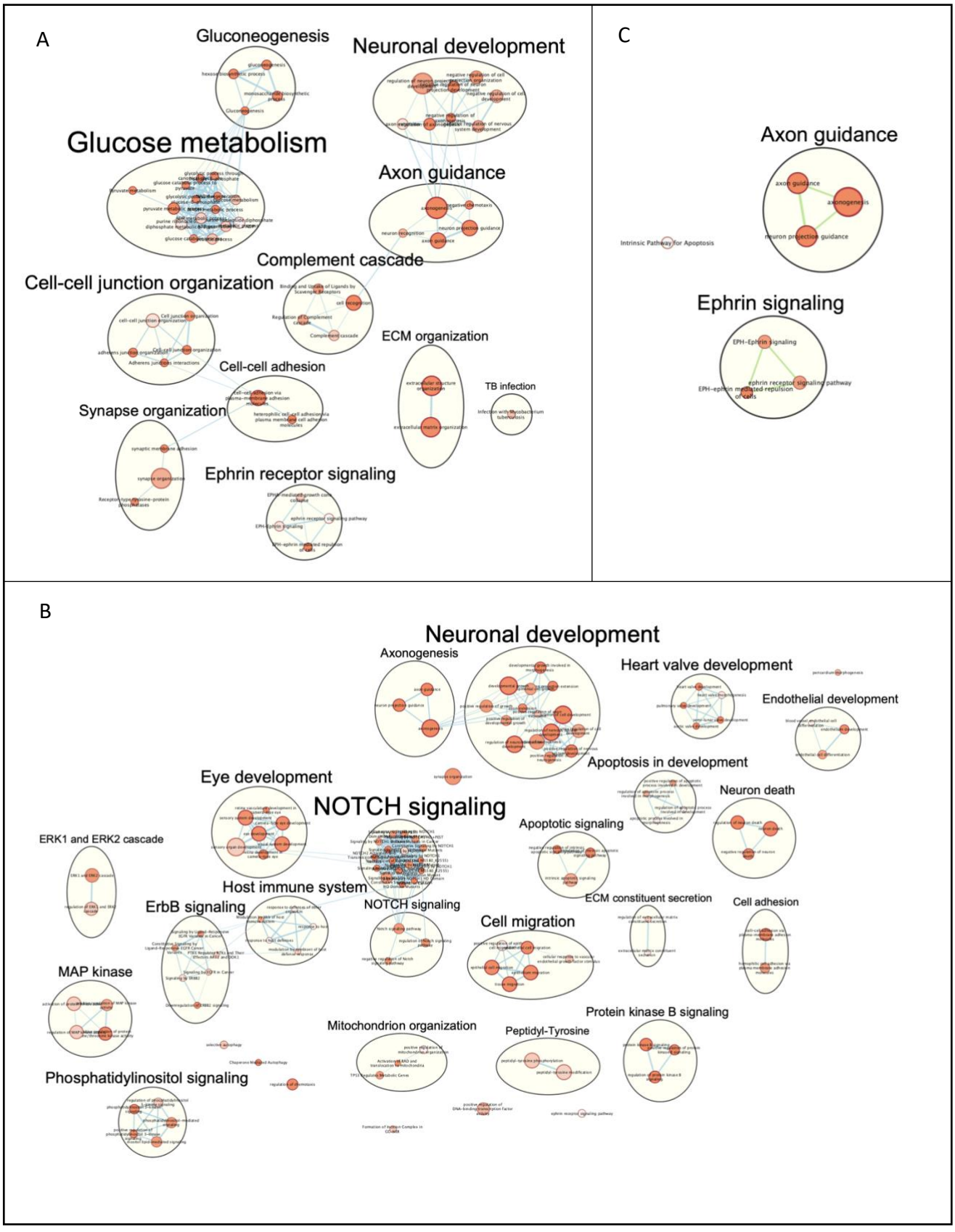


**Figure 29.** Pathway analyses based on CSF-protein analyses of *GMNC* variants within the EMIF dataset (A), the WUSTL dataset (B). (C) displays the overlapping functional pathways between the different datasets.

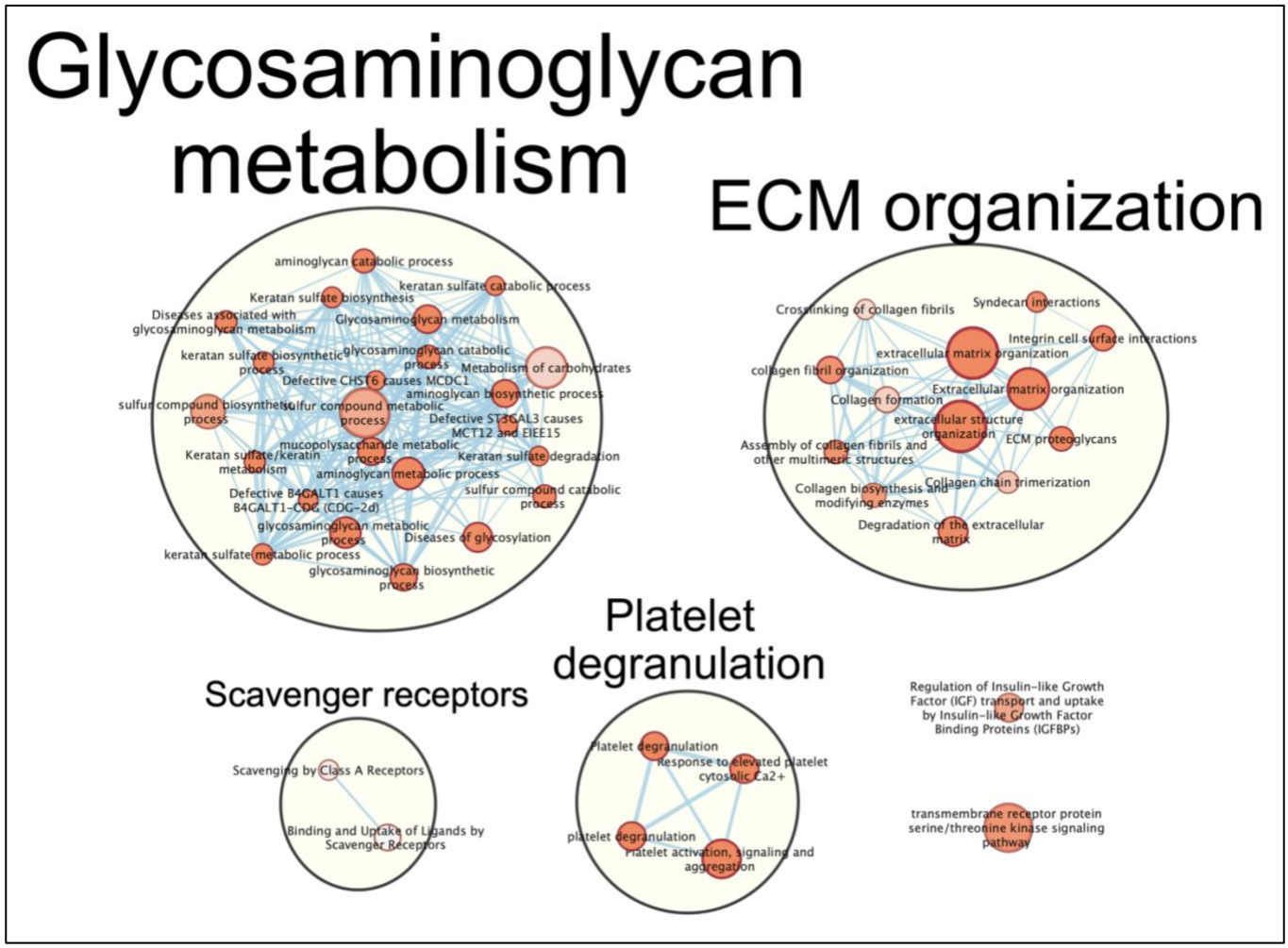


**Figure 30.** Pathway analyses based on CSF-protein analyses of GMNC variants within the EMIF dataset.

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