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A Meta-analysis Of Cortical DNA Methylomic Variation Associated With Alzheimer's Disease Pathology, And The Influence Of *APOE* Genotype

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Introduction

DNA methylation may help mediate interactions between genetic and environmental risk factors in **Alzheimer's disease (AD)**. However, previous large-scale **AD epigenome-wide association studies (EWASs)** have largely relied on Illumina 450K methylation arrays and have not comprehensively assessed the impact of ***APOE* genotype** on methylation in the human brain.

We performed three coordinated EWAS meta-analyses using Illumina 450K and higher-density EPIC array data from post-mortem prefrontal cortex (PFC):

1. ***APOE* $\epsilon 4$ risk allele¹ EWAS meta-analysis (N = 1334)**
2. ***APOE* $\epsilon 2$ protective allele² EWAS meta-analysis (N = 1334)**
3. **AD Braak stage EWAS meta-analysis – the largest of its kind in post-mortem PFC to date (N = 1978)**

1. *APOE* $\epsilon 4$ Meta Analysis

Five Bonferroni-significant differentially methylated positions (DMPs) found, all in the broad ***APOE*** region. Three have been previously reported¹⁰. Two additional FDR-significant DMPs were detected: a hypomethylated exonic site in ***FEM1C*** (chr5:114,880,330), a gene involved in ubiquitin-dependent protein degradation, and a hypermethylated exonic site in ***ARMC1*** (chr8:66,546,317), a gene encoding a protein involved in mitochondrial distribution¹¹.

DMR analysis (seed $p \leq 1 \times 10^{-4}$; region Šidák-adjusted $p \leq 0.05$) identified a $\epsilon 2$ -associated hypermethylated promoter CpG island at the ***CAT*** locus, a key peroxisomal antioxidant gene. Catalase plays a central role in hydrogen peroxide detoxification and oxidative stress regulation, processes implicated in AD pathophysiology¹³.

Materials & Methods

APOE Genotype Meta Analysis: 1334 samples

Pittsburgh (PITT)³: 210

Brains for Dementia Research (BDR)⁴: 511

UK Brain Bank Network (UKBBN): 243

Medical Research Council (MRC)⁵: 370

Controlled for: Sex, Age, Cell Type, Braak Stage, Brain Bank & Batch

Cohort Specific $\epsilon 4$ EWAS
(Continuous: 0 $\epsilon 4$, 1 $\epsilon 4$ & 2 $\epsilon 4$)

Cohort specific $\epsilon 2$ EWAS
(Case Control: 0 $\epsilon 2$ vs 1 $\epsilon 2$)

Random effect meta-analysis & Bonferroni threshold applied

Differentially methylated regions (DMRs) identified using comb-p⁹

Braak Stage Meta Analysis: 1978 samples

Pittsburgh (PITT)³: 220

Brains for Dementia Research(BDR)⁴: 548

UK Brain Bank Network (UKBBN): 249

London⁶: 109

Mount Sinai⁷: 141

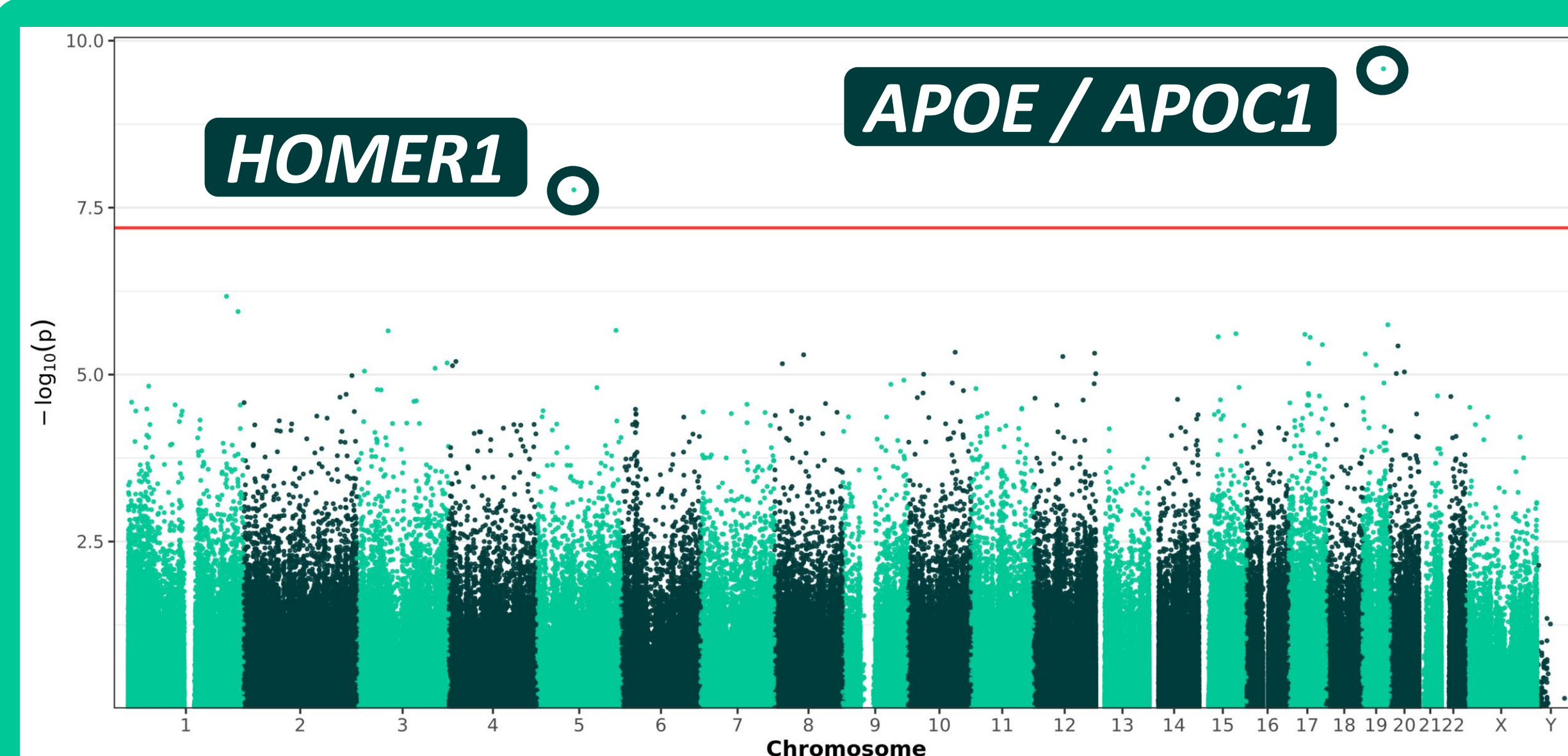
ROSMAP⁸: 711

Controlled for: Sex, Age, Cell Type & Brain Bank

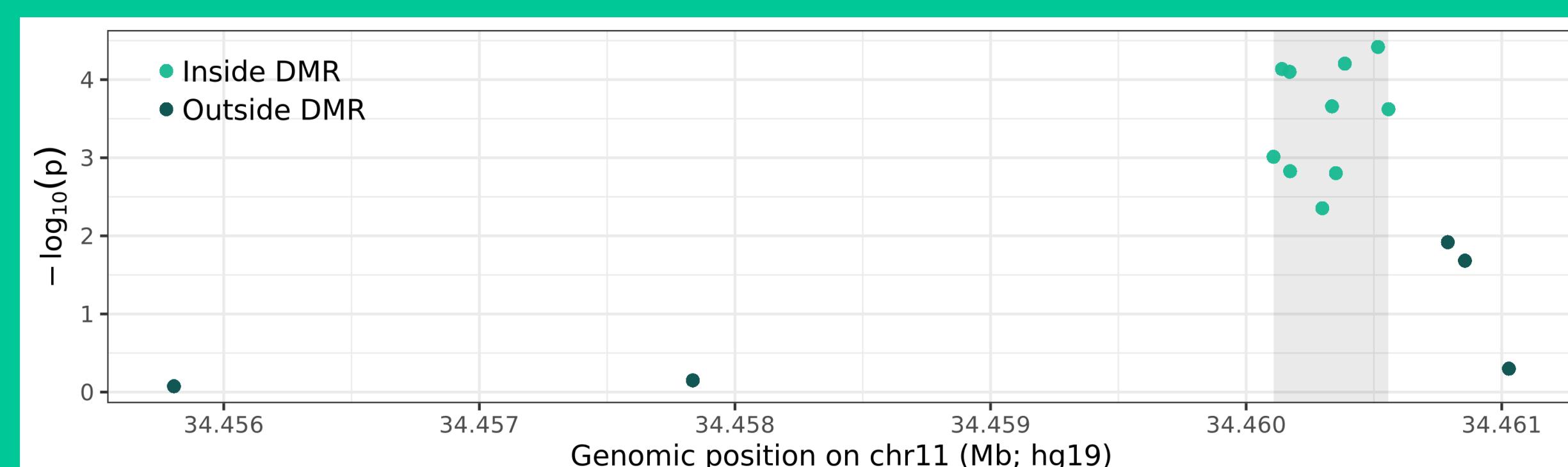
AD Braak Stage EWAS (Continuous: Braak Stages 0-6)

Random effect meta-analysis & Bonferroni threshold applied

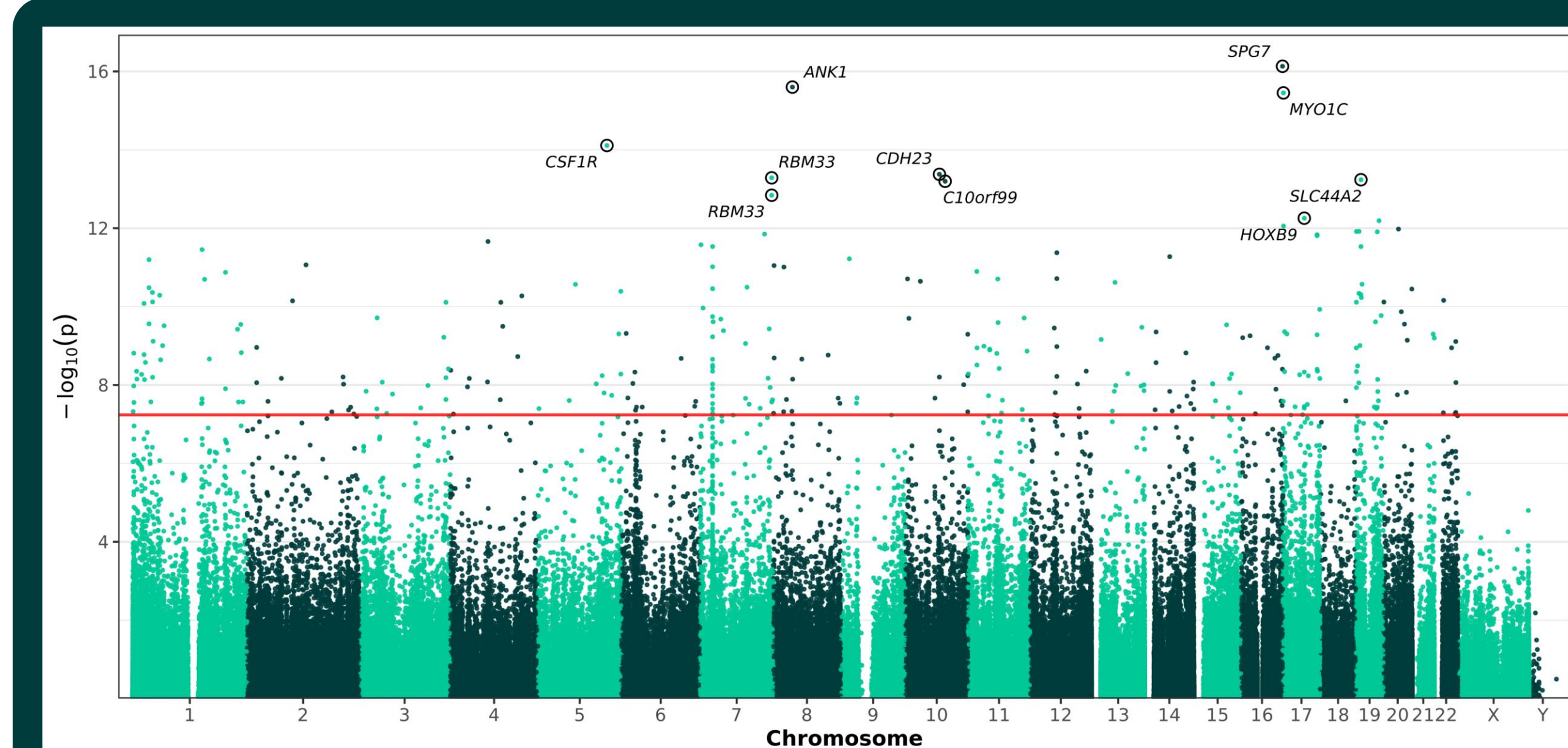
2. *APOE* $\epsilon 2$ Meta Analysis



Two hypermethylated Bonferroni-significant DMPs were identified: one intergenic site between ***APOE*** and ***APOC1***, and one in the ***HOMER1*** promoter, a gene implicated in glutamatergic signalling, synaptic plasticity and AD brain expression changes^{11,12}.



3. Braak Stage Meta Analysis



287 Bonferroni-significant Braak stage-associated DMPs were identified, including known AD methylation loci such as ***SPG7***, ***ANK1***, ***MYO1C***, ***CSF1R***, ***CDH23*** & ***SLC44A2***^{6,8,14-16}. The top DMP was a hypermethylated site in the gene body of ***SPG7***.

Conclusion

This comprehensive meta-analysis links ***APOE* genotype** and **Braak stage** to ***APOE*-proximal** and widespread **AD-associated methylation changes** using 450K and EPIC-array data.

References

[1] Wightman, et al. A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer's disease. *Nat. Genet.* 53, 1276–1282 (2021). <https://doi.org/10.1038/s41588-021-00921-2>. [2] Cengiz-Goldberg, C., et al. *APOE2* enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. *Mol. Psychiatry* 19, 1243–1250 (2014). <https://doi.org/10.1038/mp.2013.94>. [3] Kachhar, M., et al. A brain DNA co-methylation network analysis of psychosis in Alzheimer's disease. *Alzheimer's Disord.* 37(Suppl. 2), S205–S215 (2021). <https://doi.org/10.1093/alzdis/abaa014>. [4] Smith, R., et al. DNA methylation signatures of Alzheimer's disease neuropathology in the cortex are primarily driven by variation in non-neuronal cell types. *Nat. Commun.* 13, 5600 (2022). <https://doi.org/10.1038/s41467-022-27384-1>. [5] Harvey, J., et al. Investigating DNA methylation associated with early onset pathology in a cross-sectional and multi-cohort study. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [6] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [7] Smith, R., et al. Elevated DNA methylation across a 40-kb region spanning the *CD4A* gene cluster is associated with Alzheimer's disease neuropathology. *Alzheimer's Disord.* 37(Suppl. 2), S205–S215 (2021). <https://doi.org/10.1093/alzdis/abaa014>. [8] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [9] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [10] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [11] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [12] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [13] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [14] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [15] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>. [16] Smith, R., et al. *APOE* genotype-specific methylation patterns are linked to Alzheimer's disease pathology and cognitive decline. *Mol. Psychiatry* 2020. <https://doi.org/10.1038/s41380-020-0800-1>.