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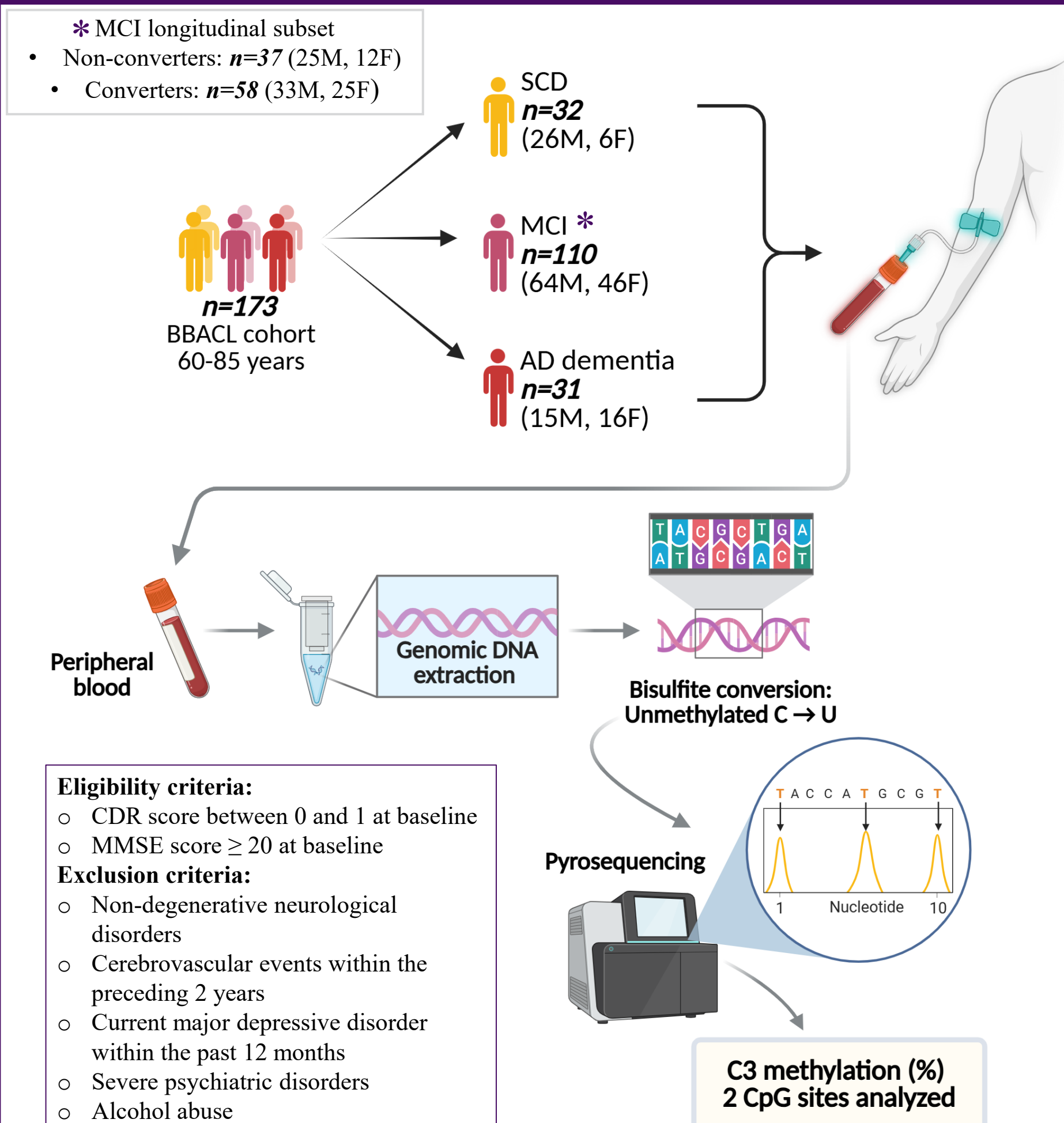
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C3 methylation was shown to decrease with aging and did not differ across clinical diagnosis, suggesting that C3 methylation primarily reflects aging rather than clinical disease stage.

INTRODUCTION

- Complement component C3 has emerged as a key player in Alzheimer's disease (AD) pathogenesis.
- Increasing evidence supports the regulation of complement gene expression via **epigenetic mechanisms**.
- Altered C3 methylation has been previously reported in AD brains (Lardenoije et al., 2019).

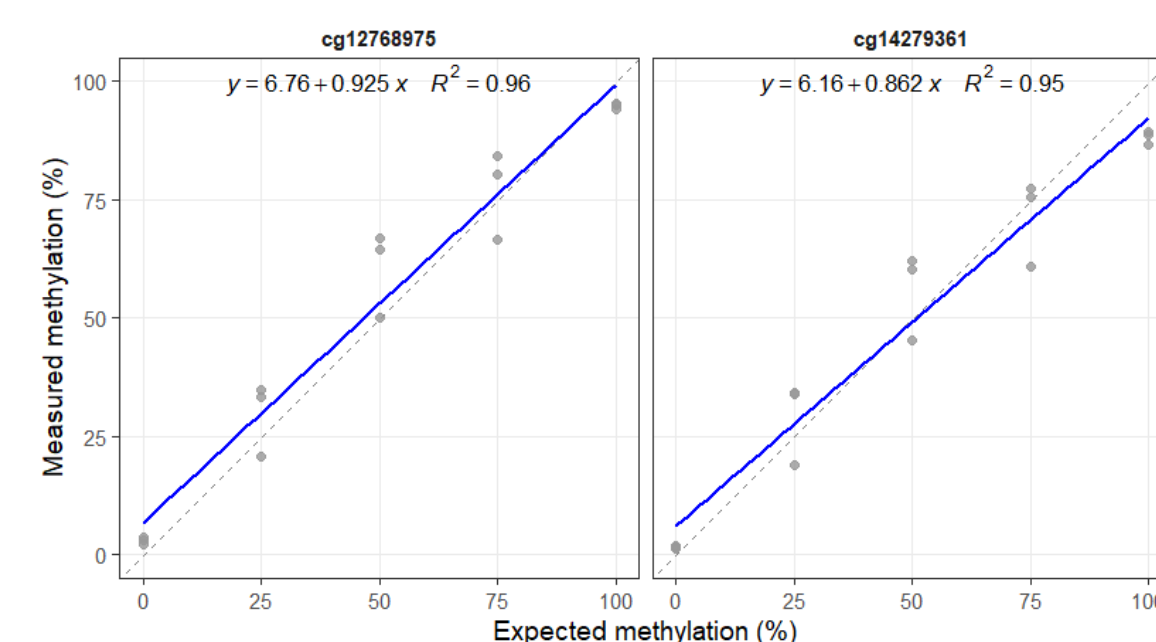
STUDY DESIGN



CDR: clinical dementia rating; MMSE: mini-mental state examination; SCD: subjective cognitive decline; MCI: mild cognitive impairment; AD dementia: Alzheimer's disease dementia; M: males; F: females; CpG: cytosine-phosphate-guanine. Illustration created with BioRender.com

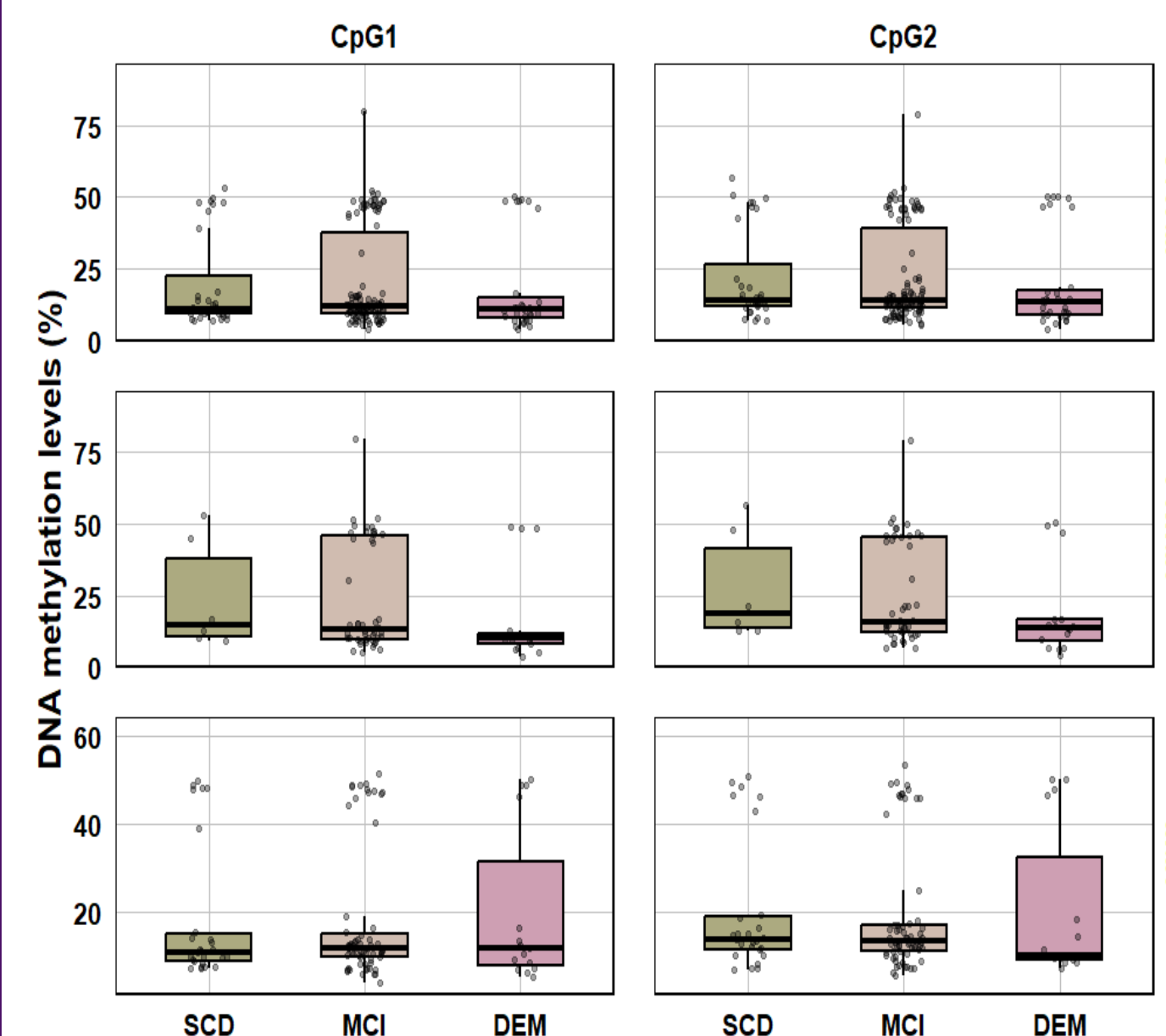
RESULTS

Fig. 1. Assay validation



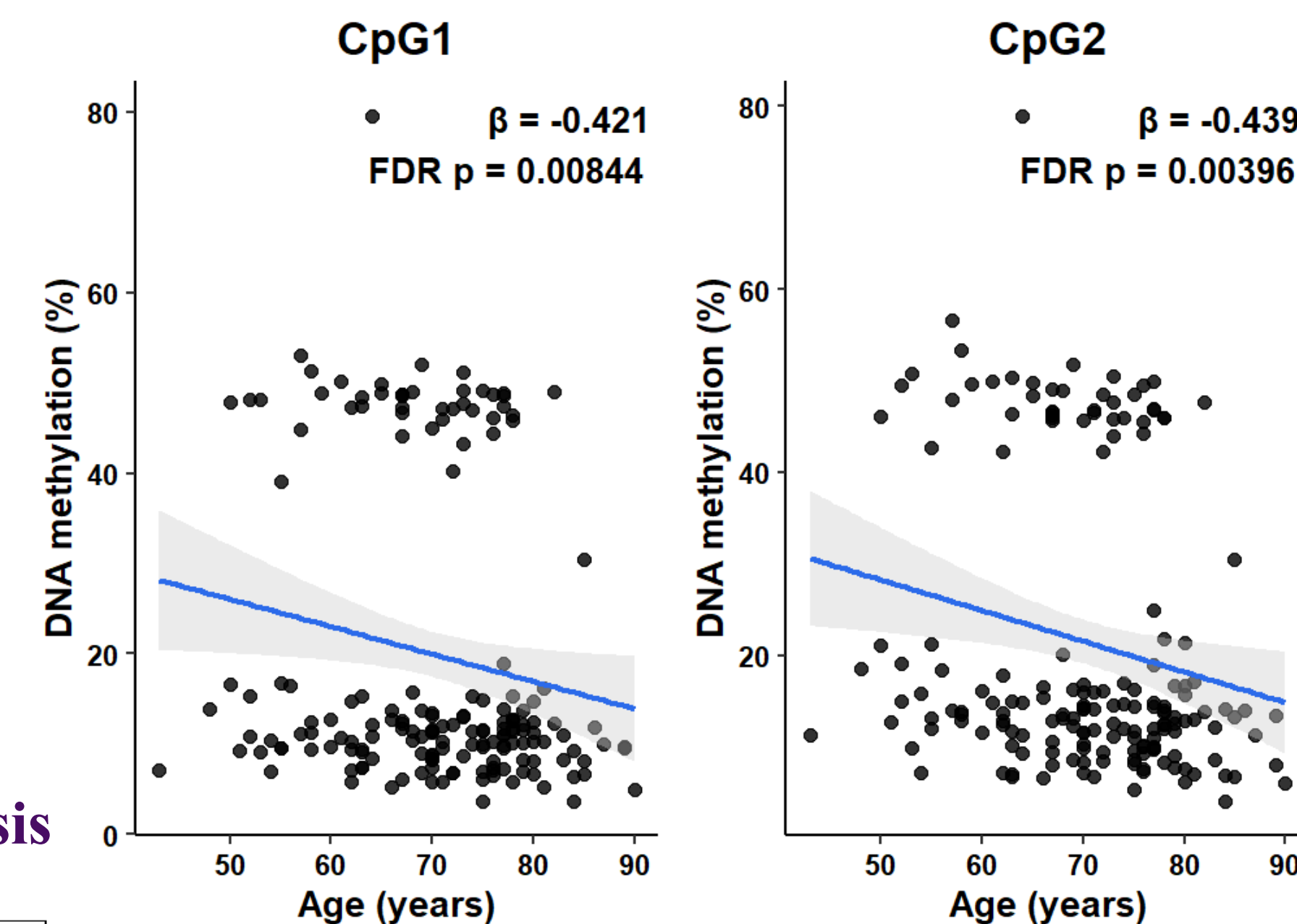
Expected versus measured methylation for CpG1 and CpG2 standards demonstrated excellent assay linearity ($R^2 = 0.96$ and 0.95), confirming robust pyrosequencing assay performance.

Fig. 3. No differences in C3 methylation across clinical diagnosis



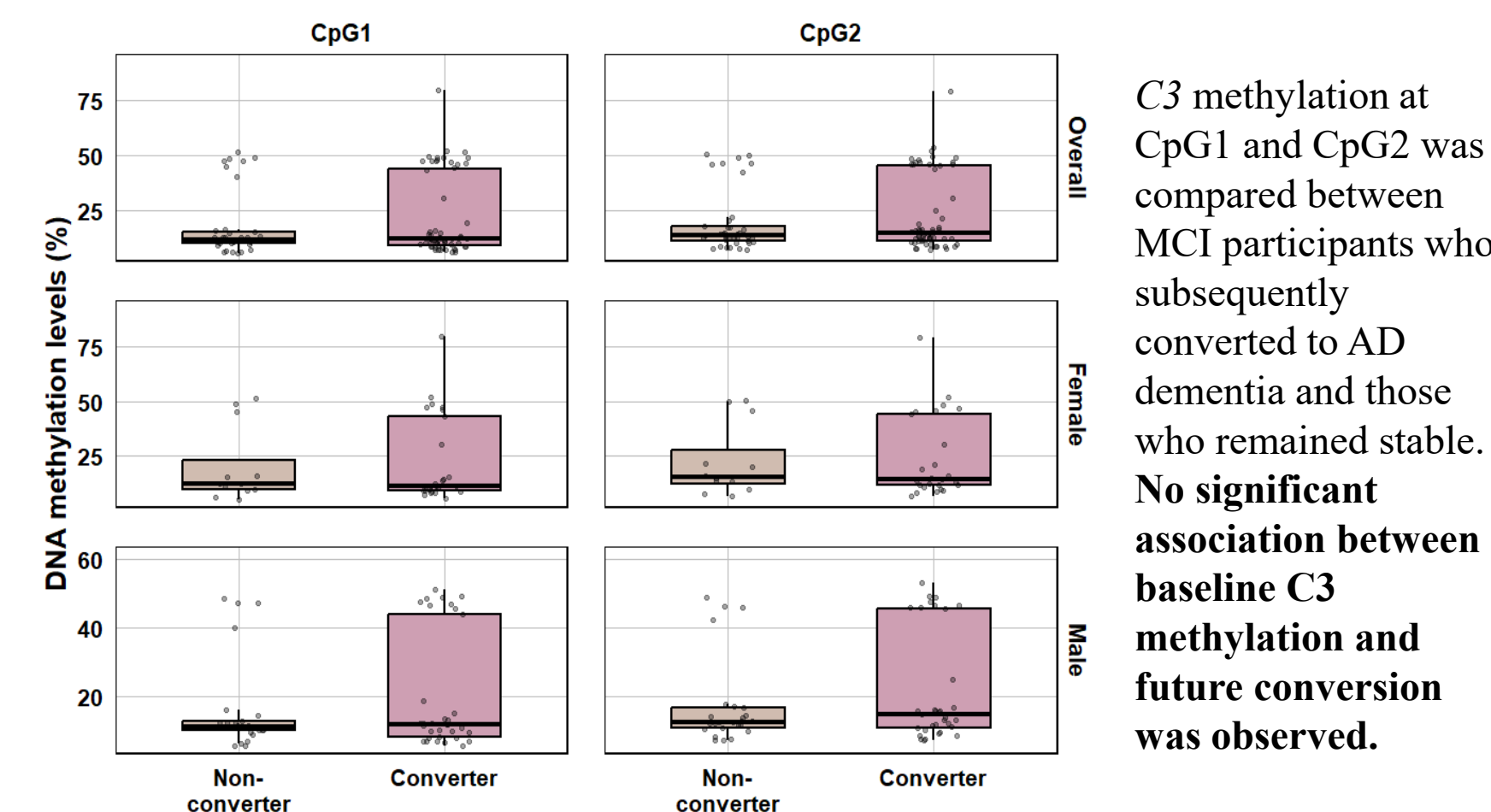
C3 methylation at CpG1 and CpG2 was compared among participants with SCD, MCI, and AD dementia. No significant differences were observed after adjustment for age, sex, smoking, alcohol use, and education.

Fig. 2. C3 methylation decreases with aging



C3 methylation at both CpG sites decreased significantly with age after adjustment for sex, smoking, alcohol use, and education. False Discovery Rate (FDR)-adjusted.

Fig. 4. C3 methylation is not associated with MCI conversion



C3 methylation at CpG1 and CpG2 was compared between MCI participants who subsequently converted to AD dementia and those who remained stable. No significant association between baseline C3 methylation and future conversion was observed.

CONCLUSIONS

- C3 methylation at two CpG sites decreased significantly with age.
- No association was observed with clinical diagnosis or future MCI conversion.
- C3 methylation may represent an age-related epigenetic signature rather than a marker of Alzheimer's disease progression.**

FUTURE DIRECTIONS

Investigate the biological basis of the observed methylation clustering, including potential contributions of cell type composition, genetic variation, and inflammatory status.

REFERENCES

- Papavergi MT, Bathini P, Singh B, Lemere CA. The complement cascade in Alzheimer's disease: modern implications of an ancient immune protagonist. *Mol Neurodegener.* 2025;21(1):5.
- Lardenoije R, Roubroeks JAY, Pishva E, et al. Alzheimer's disease-associated (hydroxy)methylomic changes in the brain and blood. *Clin Epigenetics.* 2019;11(1):164.
- Smith RG, Pishva E, Kouhsar M, et al. Blood DNA methylomic signatures associated with CSF biomarkers of Alzheimer's disease in the EMIF-AD study. *Alzheimers Dement.* 2024;20(10):6722-6739.

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