

Epigenome-wide Analysis Identifies Methylation Marks with Dosage-effect in Peripheral Blood Across the Alzheimer’s Disease Continuum

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Peripheral blood DNA methylation revealed 134 CpGs exhibiting a 'dosage-effect' across the Alzheimer's continuum, offering a non-invasive tool to identify biological changes during the preclinical and prodromal window

INTRODUCTION

RESULTS

CONCLUSIONS

Alzheimer's Disease (AD) is characterized by a long preclinical phase where biological changes precede cognitive decline [1]. Under the AT(N) framework, biomarkers for amyloid (A), tau (T), and neurodegeneration (N) become positive years before symptoms manifest [2]. Changes in DNA methylation have been described in the brain of AD patients [3, 4]. However, the extent to which peripheral epigenetic changes reflect AT(N)-defined stages and contribute to disease pathophysiology remains unknown.

Here, we aimed to identify peripheral blood DNA methylation patterns associated with AT(N) CSF biomarkers at the preclinical and prodromal AD stages.

1| Characterization of Subjects

2| Stage-by-stage Comparison

Table 1. Demographic and Clinical Characteristics					
Characteristic	CU A-T-N- N = 48 ¹	CU A+T-N± N = 49 ¹	CU A+T+N± N = 19 ¹	MCI N = 49 ¹	p-value ²
Sex					0.8
Female	25 (52%)	24 (49%)	12 (63%)	25 (51%)	
Male	23 (48%)	25 (51%)	7 (37%)	24 (49%)	
Age					<0.001
Mean ± SD	68.9 ± 6.3	65.9 ± 5.7	68.4 ± 7.1	71.3 ± 6.0	
APOE ε4					<0.001
0	43 (90%)	33 (67%)	13 (68%)	15 (31%)	
1	5 (10%)	16 (33%)	6 (32%)	34 (69%)	
VascularBurden					0.026
0	38 (79%)	38 (78%)	15 (79%)	27 (55%)	
1	10 (21%)	11 (22%)	4 (21%)	22 (45%)	

¹ n (%)
² Pearson's Chi-squared test; Kruskal-Wallis rank sum test

Analyses revealed 112 differentially methylated loci (DMLs) associated with A+ and 79 DMLs associated with cognitive decline (FDR<0.05, |ΔBeta|≥0.1), enriched in synapsis, neuron-related components, and pathways involved in brain development.

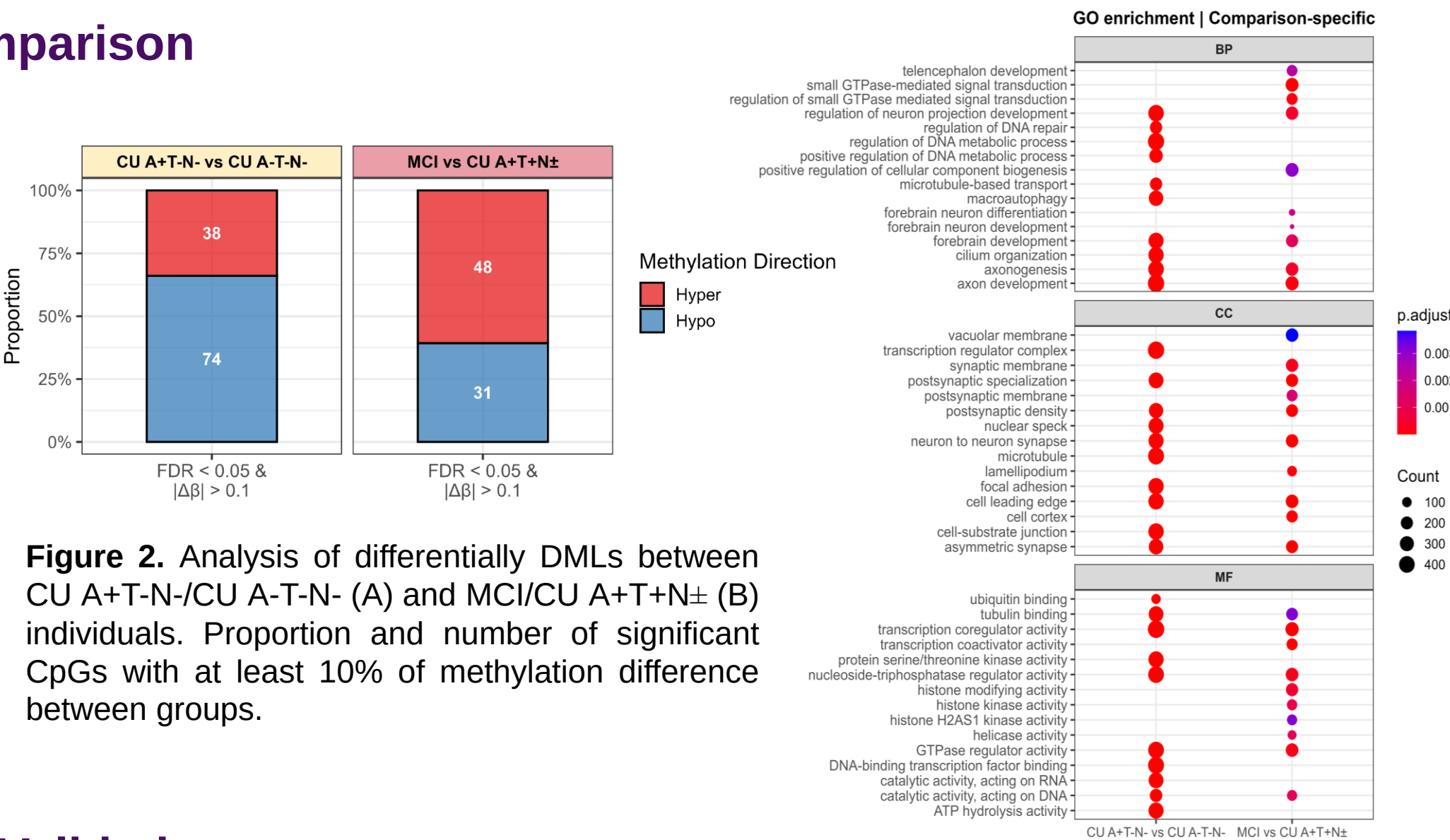


Figure 2. Analysis of differentially DMLs between CU A+T-N-/CU A-T-N- (A) and MCI/CU A+T+N± (B) individuals. Proportion and number of significant CpGs with at least 10% of methylation difference between groups.

2| Identification of “Dosage-effect” CpGs, Selection and Validation

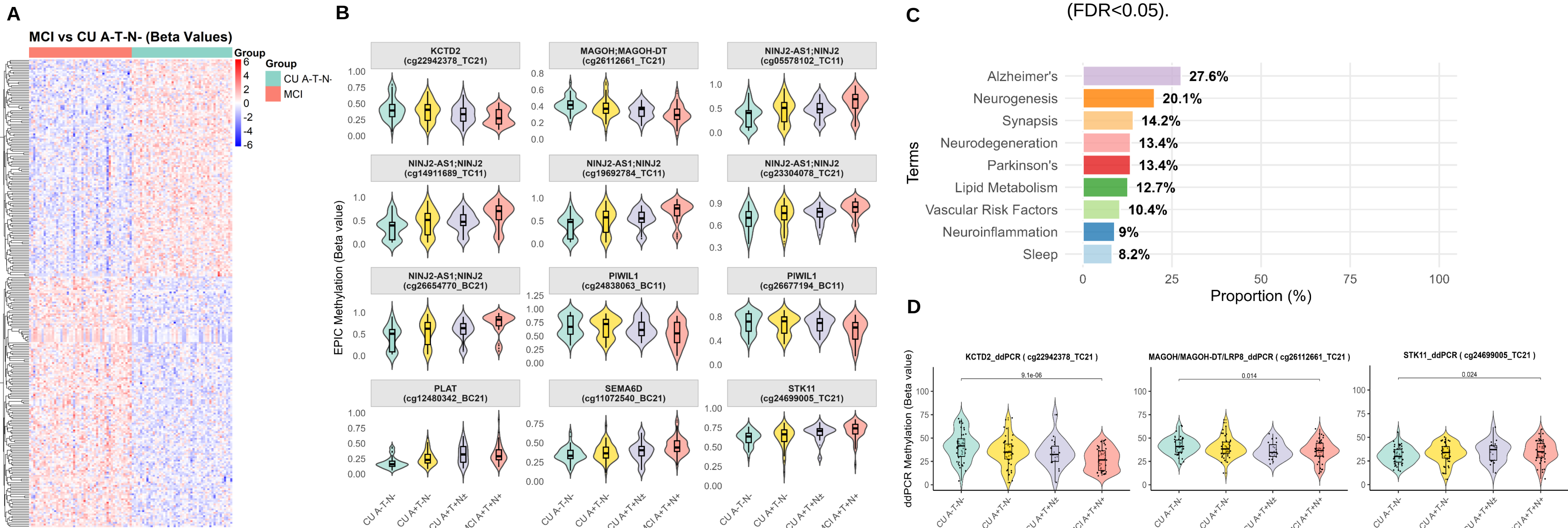


Figure 4. 134 CpGs with “dosage-effect” identified in MCI vs CU A-T-N- analysis. (A) Heatmap of DMPs corrected beta values. Scale “row” is applied. (B) Illustration of 12 CpGs with progressive EPIC methylation levels within groups (C) Proportion of 134 CpGs related to terms of interest. (C) Example of ddPCR methylation levels of three validated CpGs.

Of the 278 DMLs identified between CU A-T-N- and MCI A+T+N+ individuals, 134 CpGs showed progressive methylation changes across AT(N) and clinically defined stages, consistent with a dosage-effect along the AD continuum. Literature curation revealed that up to 24% of annotated genes had been previously linked to AD, 20% to neurogenesis, and 14% to synapsis and neurodegeneration. Three CpGs were independently validated by ddPCR

METHODS

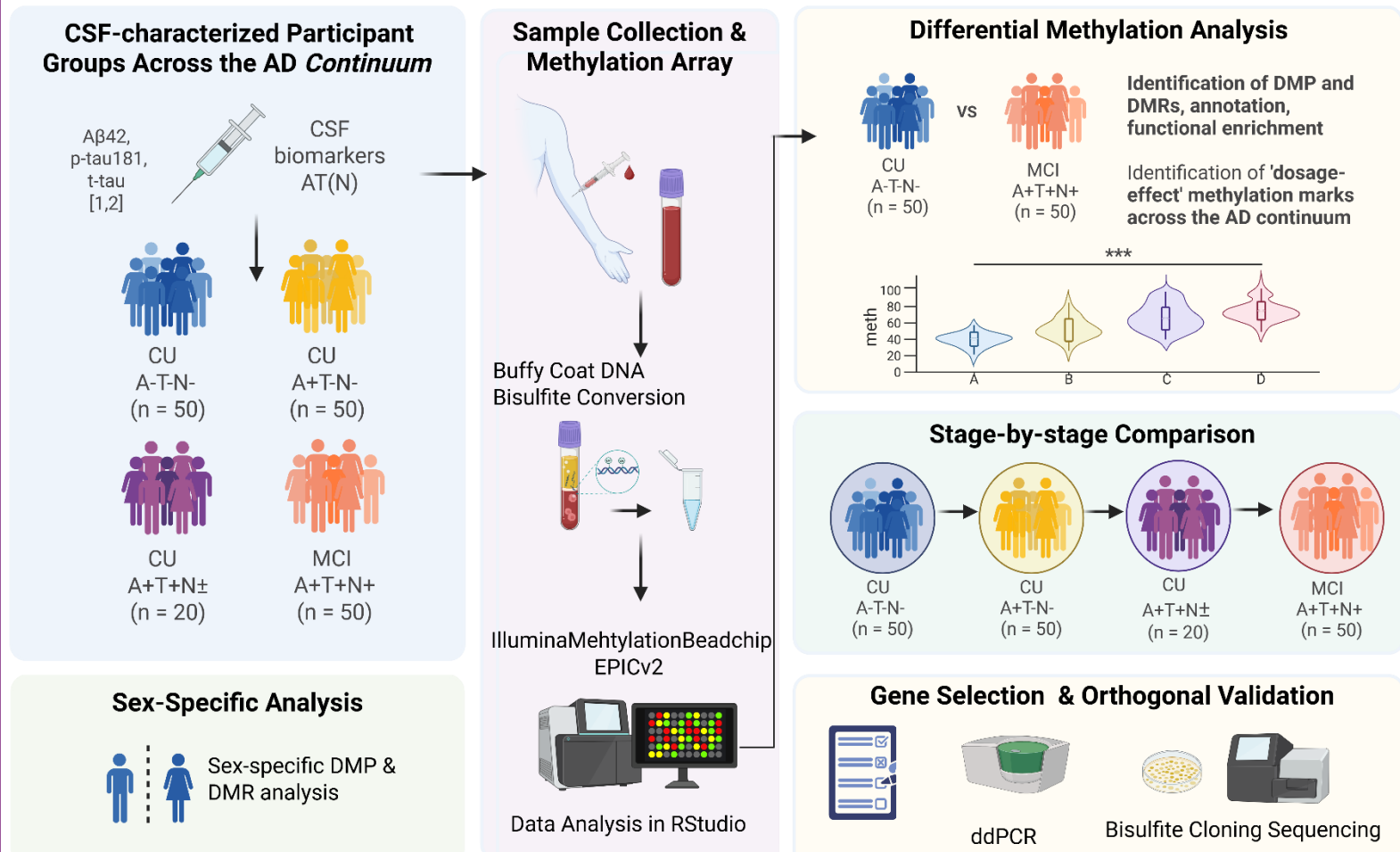


Figure 1. Study Design. Overview of the workflow of the whole study.

- 170 individuals from CITA-Alzheimer Foundation (Basque Country, Spain) and the Sant Pau Memory Unit within the Sant Pau Initiative on Neurodegeneration (SPIN; Barcelona, Spain) [5].
- Infinium MethylationEPICv2 BeadChip Array (Illumina) [6].
- Differential methylation analysis were performed with limma (v3.62.2) and linear models were fitted with batch and cell components- corrected M-values and with age, sex, APOE ε4 status and MRI-derived vascular burden, as covariates.

- Peripheral blood methylation differences are associated with core biological features of AD, particularly amyloid and neurodegeneration, involving mechanisms such as synapsis and neurogenesis.
- Dosage-effect epigenetic alterations across the AD continuum may inform biologically grounded approaches to disease staging, risk and prognostic stratification, contributing to a better understanding of AD-related biological processes.

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