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LETTER TO THE EDITOR

Replication of blood DNA methylomic signatures associated with cerebrospinal fluid levels of YKL-40 and NfL biomarkers

We read with great interest the article by Smith et al., published in Alzheimer's & Dementia in October 2024.[1] Using the Illumina Infinium Human Methylation EPIC Array (EPIC), the authors conducted epigenome-wide association studies (EWASs) on 15 cerebrospinal fluid (CSF) biomarkers measured in parallel from 885 participants in the European Medical Information Framework for Alzheimer's Disease (AD) Multimodal Biomarker Discovery study. The EWAS identified five differentially methylated positions (DMPs) associated with YKL-40 levels (n = 664) and seven DMPs associated with neurofilament light chain (NfL) levels (n = 662), all of which remained significant after Bonferroni correction. For YKL-40, the DMPs co-localized with previously reported genetic variants in the *CHI3L1* locus—the gene encoding YKL-40—suggesting that methylation may mediate genetic effects on CSF YKL-40 levels.[2] These findings indicate that blood-based epigenetic changes may, at least in part, reflect brain processes, offering valuable insights into AD pathophysiology.

While these findings are significant, they arise from a single dataset without formal replication. Consequently, we conducted an independent replication in two cohorts: the DELCODE study (Germany, n = 448)[3] and the GR@ACE cohort (Spain, n = 276),[4] where blood DNA EPIC data and corresponding CSF biomarkers data were available. Mean age in DELCODE was 70.89 years (± 5.90) and 72.29 years (± 7.21) in GR@ACE. DELCODE included 47.99% females and GR@ACE 46.52%.

The R-package meffil was used for DNA methylation quality control (QC) and normalization.[5,6] Methylation was expressed as beta values. The EWAS was focused on YKL-40 and NfL, for which Smith et al. reported Bonferroni-significant findings. CSF levels of YKL-40 and NfL were quantified using the Proximity Extension Assay technology from Olink. QC and normalization of CSF data followed our established pipeline.[7] The biomarker levels in CSF were z-scored. A multivariable linear regression was employed for the EWAS. Covariates included age, sex, smoking status (predicted using methylation of cg05575921), cellular composition (meffil function), and surrogate variables were estimated via the SmartSVA algorithm.[6] Additionally, we controlled for CSF amyloid-beta (A) and phosphorylated tau (T) positivity, which defined four AT subgroups: A-T- (n = 203 DELCODE, n = 102 GR@ACE), A+T- (n = 141 DELCODE, n = 38 GR@ACE), A+T+ (n = 80 DELCODE, n = 89 GR@ACE), and A-T+ (n = 24 DELCODE, n = 62 GR@ACE).

EWAS was performed in each cohort individually and then combined in a meta-analysis (for details, see Table 1). For YKL-40, the meta-analysis identified seven DMPs reaching Bonferroni-corrected significance, five of which were also significant in Smith et al. (Table 1). The additional two DMPs showed nominal significance in Smith et al. (Table S16 from Smith et al.[1]). Adjusting for AT status did not change the results. All DMPs were located in a region around exon 1 of *CHI3L1*, near the transcription start site. Results in each dataset are presented in Table S1. Using Comb-p (R-package ENmix[8]) with settings similar to those in Smith et al., we identified a single differentially methylated region (DMR) on chromosome 1 around exon 1 of *CHI3L1* (chr1:203186609-203187657, $p_{Sidak} = 1.54 \times 10^{-6}$, Table S2), overlapping with the region reported by Smith et al. within *CHI3L1* (note: their positions are based on the hg19 genome assembly, while ours use hg38). No additional significant DMRs were found in either dataset (Table S2). Results remained unchanged after adjusting for AT status.

The meta-analysis of NfL did not identify DMPs reaching Bonferroni-corrected significance and failed to replicate the seven DMPs reported by Smith et al. (Table 1). No DMRs showed a consistently significant association across the datasets (Table S2). A DMR on chr5:23507349-23507644, overlapping with a DMR reported by Smith et al., showed significant association in GR@ACE ($p_{Sidak} = 0.02$). However, we do not consider this a formal replication, as the larger DELCODE dataset revealed no association with this DMR.

Our well-powered replication confirms the association between *CHI3L1* methylation and CSF YKL-40 levels, supporting Smith et al. and suggesting that methylation may mediate genetic effects on YKL-40. However, variants on chromosome 1 near *CHI3L1* have not been conclusively linked to AD risk in case-control GWAS,[9] raising questions about their role in disease susceptibility. Still, these variants may influence disease progression—an effect not fully captured by such designs.

Our failure to replicate NfL findings may reflect biological differences in its regulation between blood and brain, limiting statistical power.[10] Supporting this observation, cg16625929 showed a consistent effect direction with Smith et al., though without statistical significance in our data (Table S1).

In summary, our findings highlight the potential of blood-derived methylation to inform AD-related epigenetic changes, while

TABLE 1 . Epigenome-wide association studies performed in each cohort

		Smith et al. (2024)		Meta-analysis (w/ AT status)		Meta-analysis (wo/ AT status)	
Biomarker	**CpG ID**	**Coefficient**	***p*-value**	**Coefficient**	***p*-value**	**Coefficient**	***p*-value**
YKL-40	cg07423149	-2.60×10^{-7}	4.91×10^{-13}	-2.19×10^{-2}	1.45×10^{-17}	-1.77×10^{-2}	6.92×10^{-15}
	cg14085262	$-1{,}49 \times 10^{-7}$	2.62×10^{-12}	-1.40×10^{-2}	3.05×10^{-17}	-1.20×10^{-2}	4.54×10^{-16}
	cg03625911	$-1{,}75 \times 10^{-7}$	2.77×10^{-12}	-1.31×10^{-2}	2.80×10^{-15}	-1.12×10^{-2}	5.24×10^{-14}
	cg17014757	$-4{,}81 \times 10^{-7}$	1.44×10^{-11}	-4.64×10^{-2}	3.69×10^{-17}	-3.68×10^{-2}	3.89×10^{-14}
	cg08768186	$-1{,}27 \times 10^{-7}$	3.99×10^{-10}	-9.88×10^{-3}	4.46×10^{-12}	-7.36×10^{-3}	1.10×10^{-8}
	cg25482438	$-7{,}78 \times 10^{-8}$	1.19×10^{-7}	-1.04×10^{-2}	4.12×10^{-15}	-8.33×10^{-3}	1.05×10^{-12}
	cg02097014	$-4{,}67 \times 10^{-8}$	3.02×10^{-4}	-5.56×10^{-2}	1.32×10^{-8}	-4.61×10^{-3}	1.66×10^{-7}
Neurofilament light chain	cg16073540	-8.37×10^{-6}	1.46×10^{-9}	4.29×10^{-4}	0.27	5.93×10^{-4}	0.11
	cg24329658	-7.58×10^{-6}	6.71×10^{-9}	-1.52×10^{-4}	0.58	-1.22×10^{-4}	0.64
	cg26422266	-9.49×10^{-6}	8.28×10^{-9}	-8.37×10^{-5}	0.89	3.91×10^{-4}	0.51
	cg12817352	-6.98×10^{-6}	2.16×10^{-8}	-1.22×10^{-4}	0.83	2.64×10^{-4}	0.62
	cg16625929	-6.36×10^{-6}	2.45×10^{-8}	-4.53×10^{-4}	0.24	-3.49×10^{-4}	0.34
	cg06064220	-6.70×10^{-8}	3.69×10^{-8}	2.69×10^{-4}	0.43	3.57×10^{-4}	0.27
	cg14894702	1.03×10^{-5}	3.89×10^{-8}	-3.74×10^{-5}	0.78	-4.74×10^{-5}	0.71

Note: Comparison of CpGs identified for YKL-40 and NfL by Smith et al. (2024) with the EWAS meta-analysis in the DELCODE and GR@ACE datasets. The CSF amyloid-beta (A) and phosphorylated tau (T) positivity groups were used as an additional covariate. w/ AT status: meta-analysis with AT status as a covariate. wo/ AT status: meta-analysis without AT status as a covariate. For the meta-analysis, a fixed-effect model with inverse-variance weighting was utilized as implemented in the R package metafor. The *p*-values presented in the table are unadjusted. The Bonferroni-corrected *p*-value threshold is $p_{Bonf} = 5.94 \times 10^{-8}$.

underscoring, as suggested by NfL findings, the need for larger studies to identify additional methylation signals linked to CSF biomarkers and disease progression.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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