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The association between neighborhood deprivation and DNA methylation in an autopsy cohort

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An abstract of
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Abstract

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By Lindsay Pett

Previous research has found that living in a disadvantaged neighborhood is associated with poor health outcomes. Living in disadvantaged neighborhoods may alter inflammation and immune response in the body, which could be reflected in epigenetic mechanisms such as DNA methylation (DNAm). We used robust linear regression models to conduct an Epigenome-Wide Association Study (EWAS) examining the association between the Area Deprivation Index (ADI), a measure of neighborhood deprivation, and DNAm in brain tissue from 159 participants of the Emory Alzheimer's Disease Research Center cohort, an autopsy cohort of Georgia, USA, residents. We found one CpG site (cg26514961, gene *PLXNC1*) that was significantly associated with the ADI after controlling for covariates and multiple testing (p-value=$5.0e^{-8}$). Furthermore, we found synergistic effects of APOE ε4 and ADI on differential DNAm in cg26514961 and all ten most significant CpG sites in the EWAS of ADI. *PLXNC1* is related to immune response in the body, which may be how neighborhood conditions affect health. We also found concordance between brain tissue and other tissues in four of our top ten CpG sites, making them potential candidates for biomarkers in living individuals. These results could provide novel insights into the etiology of health disparities.

The association between neighborhood deprivation and DNA methylation in an autopsy cohort

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Introduction:

Neighborhood socioeconomic status (SES) is complex and has unique social, cultural, physical, and economic attributes that can impact human health (1). To quantify neighborhood deprivation, most researchers use census indicators describing the SES of a neighborhood (such as income disparity and median home value, for example) and combine them in different ways, typically using principal component and factor analysis (2). The most established measure of neighborhood deprivation is the Area Deprivation Index (ADI). The ADI uses 17 different census indicators to rank neighborhoods by socioeconomic disadvantage, including factors for the domains of income, education, employment, and housing quality. This widely-used measure of neighborhood deprivation can be useful for informing health policy and delivery, especially for the most disadvantaged neighborhood groups. Residing in a deprived neighborhood has been associated with increased incidence of mental health conditions such as depression (3), increased risk of chronic conditions such as cardiovascular disease (3), and increased risk of brain-health diseases including Alzheimer's disease (4,5). Research has demonstrated that living in a disadvantaged neighborhood leads to chronic stress in the body, mainly through the immune and inflammatory response system (6). The specific biological mechanisms that link neighborhood conditions to health outcomes are not fully understood.

A growing body of evidence suggests that epigenetics may explain how neighborhood conditions impact health (7,8). DNA methylation (DNAm) is a well-studied epigenetic mechanism that involves the addition of a methyl group to DNA, typically at the 5-carbon of cytosine at cytosine-phosphate-guanine (CpG) dinucleotides, which can influence gene expression (9). While the link between individual-level socioeconomic factors and differential

DNAm has been well established (10,11), the effect on neighborhood-level socioeconomic factors is less well known.

Existing studies on the relationship between neighborhood deprivation and DNAm are limited due to the novelty of the field of social epigenomics. One study using blood samples and one study using saliva samples both found increased global DNAm among those living in more disadvantaged neighborhoods (12,13). Another study using blood samples found three CpG sites that were associated with neighborhood deprivation, with one being linked to a gene (*MAOB*) that is related to Parkinson's Disease (14). Two other studies using blood samples found increased DNAm in nine genes related to stress and inflammation in the body (7,15). However, none of these identified CpG sites or genes were replicated across different studies. Additionally, the role of many of the CpG sites and genes identified from these studies is not well understood, so little information is available to ascertain their influences on the body. It is also important to note that none of these existing studies have examined the association between neighborhood deprivation and DNAm in brain tissue. DNAm changes in the brain specifically are important to study because they can provide indications of neuropathology outcomes such as Alzheimer's disease (16–22) and depression (23,24). Many of these brain health outcomes have themselves been associated with neighborhood deprivation (3,25,26).

Given this gap in knowledge of how neighborhood conditions impact differential DNAm in the brain, we evaluated this association using DNAm measured from brain tissue samples in a sample of deceased donors from Georgia, USA.

Methods:

Study population

The study population was derived from brain tissue donors recruited by the Emory Alzheimer's Disease Research Center (ADRC). Most of the donors in this study were patients diagnosed as having Alzheimer's Disease (AD) or probable AD and were treated at the Emory University Hospital. In total, 1011 donors enrolled in the study until the third quarter of 2020 (Table S1). The inclusion criteria for our study were the following: 1) residential addresses within Georgia; 2) age at death of at least 55; 3) died after 1999; 4) no missing values in outcomes and key covariates which include race, sex, educational attainment, APOE genotype; 5) DNAm data was available. Based on these criteria, 159 donors remained in the analysis. Written consent was obtained from all donors or their legal guardians. Emory University's Institutional Review Board approved this study.

Assessment of neighborhood deprivation

Neighborhood deprivation was defined using the Area Deprivation Index (ADI), a census-based socioeconomic index developed by Kind et al (27). The ADI is calculated using socioeconomic status domains of income, education, employment, and housing quality indicators obtained from the American Community Survey. Using these domains, the ADI is calculated from 17 census indicators that are multiplied by previously published factor weights and summed for each census block group and then transformed into a standardized index with a mean (SD) of 100 (20). The ADI assigns ranked percentiles that range from 1 to 100, where 100 represents the most deprived neighborhood. A neighborhood is defined as a census block group. A census block is the smallest geographic unit used by the United States Census Bureau to tabulate 100-percent data. A census block group comprises a set of blocks that generally contain 600-3000 people and is the smallest unit with detailed demographic-economic characteristics

(28). We linked the 2020 ADI to each participant's geocoded residential address at the time of their death using Federal Information Processing Standards codes (29).

Assessment of neuropathologic markers

Emory's ADRC conducted neuropathologic evaluations on every donor's brain using diagnostic criteria and established research evaluations. The neuropathologic assessments evaluated the severity of AD-related neuropathology changes, which included a variety of stains and immunohistochemical preparations as well as semi-quantitative scoring of multiple neuropathologic changes in brain regions by experienced neuropathologists using published criteria. AD neuropathology was assessed using the Consortium to Establish a Register for AD (CERAD) score, Braak stage, and a combination of Amyloid, Braak, and CERAD (ABC) score. CERAD score represents the prevalence of neuritic plaques with four levels from zero neuritic plaques to frequent. Braak stage is a staging scheme which represents neurofibrillary tangles (NFTs) and has six stages (Stage I-VI), with higher stages indicating a wider distribution of NFTs in the brain. ABC score combines CERAD and Braak Stage with the prevalence of Amyloid plaques and is converted to one of four levels of AD neuropathologic changes: not, low, intermediate, or high.

Assessment of DNA methylation

Prefrontal cortex samples were collected from participants at autopsy, and DNA was isolated from these samples. Illumina Infinium HumanMethylationEPIC BeadChips arrays were used to assess DNAm in the 159 samples and 6 replicates for quality control (to assess the background technical variation by looking at the root-mean-square error). The raw intensity files

were transformed into a dataset that included beta values for each of the CpG sites, and these beta values were computed as the ratio of the methylated signal to the sum of the methylated and unmethylated signals, ranging from 0 to 1 on a continuous scale. Pre-processing and statistics were completed using R (v4.2.0). We followed a validated quality control and normalization pipeline as previously published (30). All samples passed the quality check and 789,286 CpG sites remained after excluding XY probes and other low-quality probes. The final DNAm beta values were normalized to reduce the probe type differences and corrected by ComBat to remove the batch effect before the downstream analysis (31). We used the estimateCellCounts function in the R package minfi to obtain the cell-type proportions (neuronal vs. non-neuronal cells) for each sample using the most recent prefrontal cortex database (32,33).

Confounder assessment

Confounders were identified from a directed acyclic graph created by examining existing literature (Fig. S1). All models were adjusted for the following covariates: race, sex, educational attainment, age at death, apolipoprotein E (APOE) genotype, cell type, and post-mortem interval. Due to the sample only containing White and Black participants, the race variable was binary. Educational attainment was defined as the highest level of education completed by the participant and classified into high school or less, college degree, and graduate degree. APOE genotype had three levels in the analysis: no ε4 allele, single ε4 allele, and double ε4 allele. Since the APOE ε4 allele is a well-studied risk factor for developing AD, we created a binary APOE genotype variable (ε4 present vs. absent) to examine effect modification.

Statistical analysis

To identify DNAm patterns in brain tissue that are associated with ADI, we conducted an epigenome-wide association study (EWAS) of single CpG sites. For the EWAS, we ran a robust linear regression model using as ADI the independent variable and DNAm beta values at each CpG site as a dependent variable, adjusting for self-reported race, sex, APOE, education, age at death, cell type, and post-mortem interval. We applied a Bonferroni threshold to correct for multiple testing based on the number of tested CpG sites (threshold: $0.05/789889 = 6.33e^{-8}$). We then stratified this analysis by if ε4 was present or absent to examine effect modification since ε4 is a well-known risk factor for developing AD. We also conducted a sensitivity analysis restricting the EWAS to only White participants in our study population.

Next, we investigated whether DNAm patterns in brain tissue that are associated with ADI, are also linked with neuropathology markers. We ran linear regression models using each of the ten most significant CpGs as the independent variables, and three neuropathology outcomes (CERAD, Braak Stage, and ABC) as dependent variables in separate models, adjusting for ADI, self-reported race, sex, APOE, education, age at death, cell type, and post-mortem interval. As before, we stratified this analysis by if ε4 was present or absent.

To further support our findings, we conducted secondary analyses for the ten most significant CpG sites in the EWAS analysis. This included blood–brain concordance analysis using the Blood–Brain Epigenetic Concordance (BECon) tool (34), blood-brain, buccal-brain, and saliva-brain concordance using the Gene Expression Omnibus Database (35,36), methylation quantitative trait loci (mQTL) mapping using the GoDMC database (37), and gene ontology (GO) functional enrichment analysis using the missMethyl R package (38).

Results:

Description of study population

Our study included 159 donors. In the total study population, 89 (56.0%) were male, 142 (89.3%) were white, and the mean age at death was 76.6 years (SD 10.0) (Table 1). Overall, 45.9% were classified as having the highest Braak Stage of 6, 69.2% were classified as having frequent CERAD, and 58.5% were classified as having a high ABC score. Of the total population, 56% had at least one APOE ε4 allele and 95.7% were diagnosed with AD or some other form of dementia. The mean ADI was 36.7 (SD 25.6), which is less deprived than the national average of ADI=50. Overall, 116 (73.0%) were classified into the lower ADI group (ADI<50; less deprived). Compared to those in the high ADI group (ADI≥50; more deprived), those in the low ADI group were more likely to be white (95.7% vs. 72.1% in the high ADI group) and have at least a college degree (79.3% vs. 72.1% in the high ADI group), with the two groups being similar in other demographic categories. Additionally, those in the low ADI group were more likely to be diagnosed with AD or some other form of dementia (97.4.1% vs. 90.7% in the high ADI group) but were similar on other clinical categories including Braak Stage, CERAD, ABC score, and APOE ε4 alleles.

Association between neighborhood deprivation and DNA methylation in the brain

One CpG site (cg26514961, gene *PLXNC1*) was significantly associated with ADI when controlling for self-reported race, sex, APOE ε4, education, age at death, cell type proportions, and post-mortem interval (p-value=$5.0e^{-8}$) (Figure 1, Figure 2). A 20-unit increase in ADI was associated with a -0.0052 decrease in DNAm beta value (Table 2). No other CpG sites were significantly associated with ADI (Figure 1). The other nine most significant CpG sites and their associated genes were cg08087060 (associated with the gene *KLHDC4*), cg01291468 (associated

with the genes *UGT1A10*, *UGT1A7*, *UGT1A9*, and *UGT1A8*), cg16241648 (associated with the gene *ARPC1A*), cg20912923 (associated with the gene *CSMD1*), cg09431774 (associated with the gene *KIAA1671*) and the intergenic CpG sites cg05419854, cg15953452, cg06787422, and cg13521319. We also conducted a sensitivity analysis where we ran this same EWAS only in White participants, and our effect estimates were robust with respect to race (Table S5, Figure S2).

Next, we investigated whether those associations were modified by APOE ε4 carriership. The rightmost column of Table 2 displays the p-values for the effect modification analysis, and all p-values were statistically significant. All associations between the ADI and DNAm observed in the whole study population were driven by associations among donors with at least one APOE ε4 allele, suggesting synergistic effects of neighborhood deprivation and APOE ε4 on DNA methylation patterns in the brain. No CpG sites were found to be significantly associated with ADI in either APOE ε4 group (Figure S3a,b).

We then examined whether any of the ten most significant CpG sites from the EWAS of ADI were associated with AD pathology (CERAD, Braak Stage, and ABC). None of the ten CpG sites were significantly associated with any of the three neuropathology outcomes (Table 3).

Secondary analyses

Of the ten most significant CpG sites from the EWAS of ADI, three were associated with at least one mQTL, namely cg26514961 (*PLXNC1*), cg01291468 (*UGT1A10*, *UGT1A7*, *UGT1A9*, and *UGT1A8*), and cg06787422 (intergenic) (Table S2). Two CpG sites (cg20912923 (*CSMD1*) and cg06787422 (intergenic)) exhibited blood–brain concordance using the BECon

tool in accordance with the criteria listed in the methods (Table S3a). Both of these sites exhibited 75-90% percentile mean correlations between blood and brain samples. Using the Gene Expression Omnibus Database, only cg15953452 (intergenic) exhibited significant blood-brain concordance (Table S3b). Two CpG sites, cg26514961 (*PLXNC1*) and cg16241648 (*ARPC1A)*, exhibited significant buccal cell-brain concordance. Four CpG sites (cg26514961 (*PLXNC1*), cg16241648 (*ARPC1A)*, cg15953452 (intergenic), and cg06787422 (intergenic)) exhibited significant saliva-brain concordance.

After correction for multiple testing (FDR <0.05), we did not identify any GO terms or KEGG pathways with an overrepresentation of genes containing significantly, differentially methylated CpGs that would indicate an enriched biological pathway. The top GO terms and KEGG pathways are included in the supplement (Table S4a,b).

Discussion:

In the ADRC autopsy cohort of 159 participants, we found one CpG site (cg26514961, gene *PLXNC1*) that was significantly associated with the ADI in brain tissue after controlling for covariates and multiple testing. Effect modification by APOE ε4 was found to be statistically significant for the top ten CpG sites from the EWAS, indicating synergistic effects of APOE ε4 and neighborhood deprivation. Four of the ten most significant CpG sites showed a significant concordance between brain tissue and tissues that are easily accessible in living individuals (blood, buccal cells, saliva), including DNAm in cg26514961 (*PLXNC1*). This suggest that differential DNAm in these CpG sites could potentially be detected prior to death. None of the ten most significant CpG sites from the EWAS of ADI were associated with AD pathology in this autopsy cohort.

The EWAS identified cg26514961 as being significantly associated with the ADI, which is associated with the *PLXNC1* gene. This gene is known to be related to the immune response in the body (39). It may also be related to tumor suppression for melanoma (39). Previous research has suggested the immune response as a potential biological pathway of how neighborhood deprivation affects the body (6). This hypothesis is further supported by three additional genes that were among the three most significant CpG sites (cg01291468 [*UGT1A7*, *UGT1A8*, and *UGT1A9*]*)* and which have all been linked to immunosuppression (40–42); thus providing further evidence that neighborhood deprivation impacts physical health through the immune response.

There was little information on the other top 10 CpG sites, but two of the related genes have been associated with brain-related health outcomes and aging. *KLHDC4* (cg08087060) is associated with Huntington's disease (43), and *CSMD1* (cg20912923) is related to learning and memory (44). Both Huntington's disease and learning/memory function are related to aging, thus it seems as if neighborhood deprivation may have an effect on conditions related to aging.

Our study also found concordance between brain and other tissues in four of our top ten CpG sites. It is important to examine the concordance between brain tissue and other tissues (such as blood, saliva, and buccal) because brain tissue samples are not able to be obtained from living donors, whereas these three other tissues are. Differential DNAm in tissues that are easily accessible in living individuals can serve as biomarkers of exposures or to predict related health outcomes. Thus, if DNAm profiles in brain tissue are correlated with other tissues, those profiles can potentially be used for preventative care.

None of the ten most significant CpG sites have been identified in prior studies, most likely due to the different tissues that were used. Two prior studies found increased global DNAm among those living in more disadvantaged neighborhoods (12,13). These studies did not examine

particular CpG sites or genes, so it is unclear which locations experienced increased or decreased DNAm levels. Another study found three CpG sites that were associated with neighborhood deprivation, with one being linked to a gene that is related to Parkinson's Disease (14). None of these three CpG sites were identified in our EWAS (Table S6), but it is interesting to note that their study also identified genes associated with an aging-related disease. Two other studies found increased DNAm in genes related to stress and inflammation in the body (7,15), which is closely linked to the immune response pathway that two of our most significant CpG sites were linked to (45). Overall, our findings align with previous research, but more studies are needed to replicate our findings and to identify other CpG sites and genes with are related to neighborhood deprivation.

We found evidence of effect modification by APOE ε4 in the EWAS of ADI, indicating synergistic effects of APOE ε4 and neighborhood deprivation. This aligns with previous research, which suggests that there are differences in epigenome-wide methylation among APOE ε4 carriers and non-carriers in blood samples (46). Further research is needed to investigate how DNAm differs by APOE ε4 being present or absent, especially in brain tissue.

None of the ten most significant CpG sites from the EWAS of ADI were associated with AD pathology in this autopsy cohort. This finding could be due to most participants in our sample being cognitively impaired, which limits the statistical power to detect differences between impaired and non-impaired individuals. More research on this association with a larger sample of non-impaired individuals is needed to better understand the relationship between these CpG sites and AD.

Lastly, three of our ten significant CpG sites were associated with at least one known mQTL, which is an indicator of the genetic influence on DNAm levels (37). While we are unable to

decompose the effects of the environment and genes on DNAm levels, only a proportion of the variation in DNAm levels is explained by genetic effects. In fact, the joint effects of environmental factors and single nucleotide polymorphisms (SNP) have been found to be larger contributors to DNAm variation than SNPs alone (47).

Our study has several strengths. One major strength is that our study is the first known study on the association between neighborhood deprivation and DNAm in brain tissue. Studying DNAm changes in brain tissue are especially important to study because they can provide insights into neuropathology outcomes such as Alzheimer's disease (16–22) and depression (23,24). These brain health outcomes have themselves been associated with neighborhood deprivation (3,25,26), which is the reason more research on neighborhood deprivation using brain tissue is needed. Another strength of our study is that we had diversity of neighborhood deprivation. In our study, the ADI ranged from 1 to 95, thus including very deprived and very privileged neighborhoods. Another strength of our study is that we used the Infinium methylation EPIC array as opposed to the Illumina Infinium HumanMethylation450 (450K) BeadChip array. The EPIC array covers more than 850,000 methylation sites whereas the 450K array only covers 450,000 methylation sites. Only one of the five previous studies on the association between neighborhood deprivation and DNAm used the EPIC array (13). Of the top ten CpG sites associated with ADI in our cohort, four CpG sites were only available on the EPIC array.

Our study has a few limitations. Our sample size was relatively small (n=159), which limited the statistical power to detect associations. Additionally, our sample was not racially diverse and only contained self-reported race data for White and Black donors. Thus, we are unable to generalize our results to other racial or ethnic groups. Only 10.7% of participants in our sample were Black, limiting our ability to detect racial differences. However, our sensitivity analysis

restricting the EWAS to White participants found similar effect estimates to the EWAS of the total study population, indicating that our main results were not biased due to the racial composition of the sample. Another limitation of our study is that we only had information on the donors' last known address. It is possible that the donors moved around a lot during their life, or only moved to their last address at the end of their life. In these cases, the long-term or even life-term exposure to neighborhood deprivation would not be captured in the data. It is possible that the neighborhood conditions of where someone grew up or lived during most of their life are more relevant to studying the association with DNA methylation as opposed to where they lived at the end of their life, but further research is needed to elucidate these effects throughout the lifespan. Another limitation of our study is that the 2020 ADI measure we used does not correspond with the donors' years of death. This could lead to measurement error in our study, which may result in biased estimates. A final limitation of our study is that very few participants were not cognitively impaired (2.5%). Because the majority of participants had some form of cognitive impairment, the statistical power to detect differences between impaired and non-impaired participants was rather limited. Furthermore, most participants exhibited Braak Stage 6 (45.9%), had frequent CERAD (69.2%), and had a high ABC score (58.5%). These are extreme values as compared to the general US population, demonstrating that our population was not representative of the larger US or Georgia population.

Overall, our study identified one CpG site (cg26514961, *PLXNC1* gene) that was significantly associated with neighborhood deprivation in brain tissue. We also found evidence of effect modification by APOE ε4, suggesting synergistic effects of APOE ε4 and neighborhood deprivation. Our study provides motivation to conduct larger studies on the association between neighborhood deprivation and DNAm in the brain to replicate and expand upon our findings.

The identification of significant CpG sites could provide novel insights into the etiology of health disparities, and the concordance between brain and other tissues for our top CpG sites could make them potential candidates for biomarkers in living individuals.

Author Contributions:

LP wrote the original draft and carried out the statistical analyses. ZL assisted with the main statistical analyses. SA assisted with secondary analyses. AH conceived and supervised the project and helped with the preparation of the original draft. The remaining authors contributed data and participated in editing the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest:

We have no conflicts of interest to declare.

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Tables and Figures

Table 1. Characteristics of individuals from the ADRC cohort, stratified by ADI group (low=ADI<50 corresponding to less deprived than the national average), (high=ADI ≥50 corresponding to more deprived than the national average)

Sample Characteristic n (%) or mean [SD]	**Total** (n=159)		**Low ADI** (n=116)		**High ADI** (n=43)	
ADI	36.7	[25.6]	24.1	[15.5]	70.5	[14.1]
Demographics						
Race						
White	142	(89.3)	111	(95.7)	31	(72.1)
Black	17	(10.7)	5	(4.3)	12	(27.9)
Sex						
Male	89	(56.0)	63	(54.3)	26	(60.5)
Female	70	(44.0)	53	(45.7)	17	(39.5)
Age at death	76.6	[10.0]	76.6	[9.6]	76.6	[11.1]
Education attainment						
High school or less	36	(22.6)	24	(20.7)	12	(27.9)
College degree	76	(47.8)	56	(48.3)	20	(46.5)
Graduate degree	47	(29.6)	36	(31.0)	11	(25.6)
Clinical variables						
Braak Stage						
Stage 1	16	(10.1)	11	(9.5)	5	(11.6)
Stage 2	11	(6.9)	6	(5.2)	5	(11.6)
Stage 3	20	(12.6)	17	(14.7)	3	(7.0)
Stage 4	17	(10.7)	12	(10.3)	5	(11.6)
Stage 5	22	(13.8)	18	(15.5)	4	(9.3)
Stage 6	73	(45.9)	52	(44.8)	21	(48.8)
CERAD						
No	35	(22.0)	26	(22.4)	9	(20.9)
Sparse	4	(2.5)	4	(3.4)	0	(0.0)
Moderate	10	(6.3)	7	(6.0)	3	(7.0)
Frequent	110	(69.2)	79	(68.1)	31	(72.1)
ABC						
Not	15	(9.4)	10	(8.6)	5	(11.6)
Low	29	(18.2)	21	(18.1)	8	(18.6)
Intermediate	22	(13.8)	17	(14.7)	5	(11.6)
High	93	(58.5)	68	(58.6)	25	(58.1)
APOE ε4 Allele(s)						
0	70	(44.0)	51	(44.0)	19	(44.2)
1	68	(42.8)	53	(45.7)	15	(34.9)
2	21	(13.2)	12	(10.3)	9	(20.9)
Cognitive classification						
No dementia	7	(4.4)	3	(2.6)	4	(9.3)
Other dementia	66	(41.5)	51	(44.0)	15	(34.9)
AD	86	(54.2)	62	(53.4)	24	(55.8)

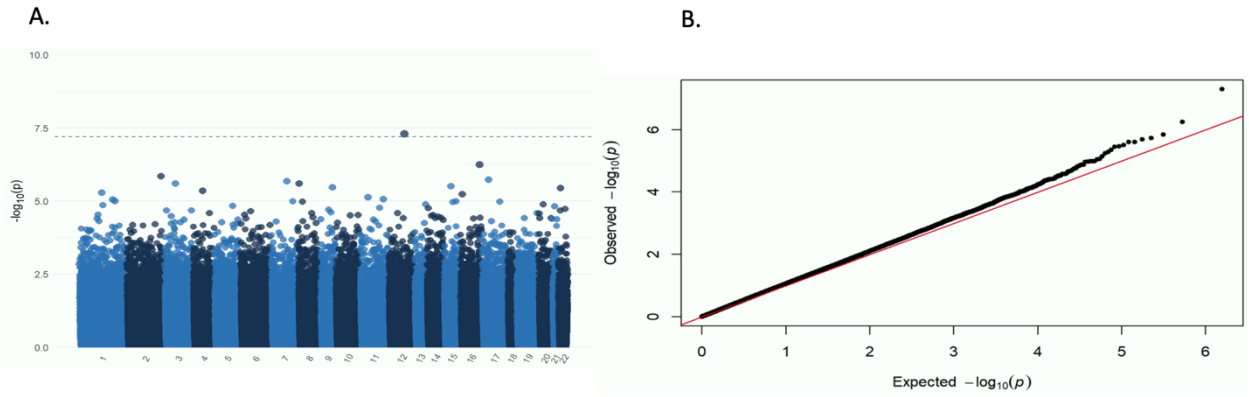

Figure 1. Manhattan and QQ plot from the EWAS of DNAm with the ADI. Adjusted for race, sex, educational attainment, age at death, APOE genotype, cell type, and post-mortem interval. Bonferroni-threshold: $0.05/789889 = 6.33e^{-8}$.

Table 2. Ten most significant CpG sites from the EWAS of DNAm with the Area Deprivation Index, stratified by whether at least one APOE ε4 allele was present or absent.

				Total		ε4 Present		ε4 Absent		Effect Modification
CpG	**Chromosome**	**Position**	**Gene(s)**	**Effect Estimate[1]**	**P-Value**	**Effect Estimate[1]**	**P-Value**	**Effect Estimate[1]**	**P-Value**	**P-Value**
cg26514961	12	94566784	*PLXNC1*	**-0.0052**	**$5.0e^{-8}$**	-0.0050	0.0033	-0.0008	0.0002	$8.7e^{-6}$
cg08087060	16	87795808	*KLHDC4*	-0.0040	$5.7e^{-7}$	-0.0036	0.0030	-0.0007	0.0009	$4.6e^{-5}$
cg01291468	2	234589374	*UGT1A10;UGT1A7; UGT1A9;UGT1A8*	0.0034	$1.4e^{-6}$	0.0027	0.0098	0.0004	0.0019	0.0003
cg05419854	17	19398395	-	-0.0058	$1.8e^{-6}$	-0.0042	0.0190	-0.0008	0.0004	$1.0e^{-5}$
cg16241648	7	98923114	*ARPC1A*	0.0016	$2.1e^{-6}$	0.0018	0.0046	0.0003	0.0020	0.0002
cg20912923	8	28855 16	*CSMD1*	-0.0026	$2.5e^{-6}$	-0.0021	0.0083	-0.0002	0.0008	$3.1e^{-5}$
cg15953452	3	63053400	-	-0.0050	$2.5e^{-6}$	-0.0042	0.0105	0.0008	0.0001	$6.2e^{-6}$
cg06787422	15	63331851	-	-0.0024	$3.1e^{-6}$	-0.0015	0.0411	-0.0002	0.0012	0.0001
cg13521319	9	133423844	-	-0.0018	$3.4e^{-6}$	-0.0020	0.0019	-0.0001	0.0046	0.0007
cg09431774	22	25465561	*KIAA1671*	-0.0028	$3.6e^{-6}$	-0.0020	0.0249	-0.0002	0.0002	$8.6e^{-6}$

[1]Effect estimates presented per 20 unit increase in ADI

Bold: statistically significant at the Bonferroni threshold of $6.33e^{-8}$

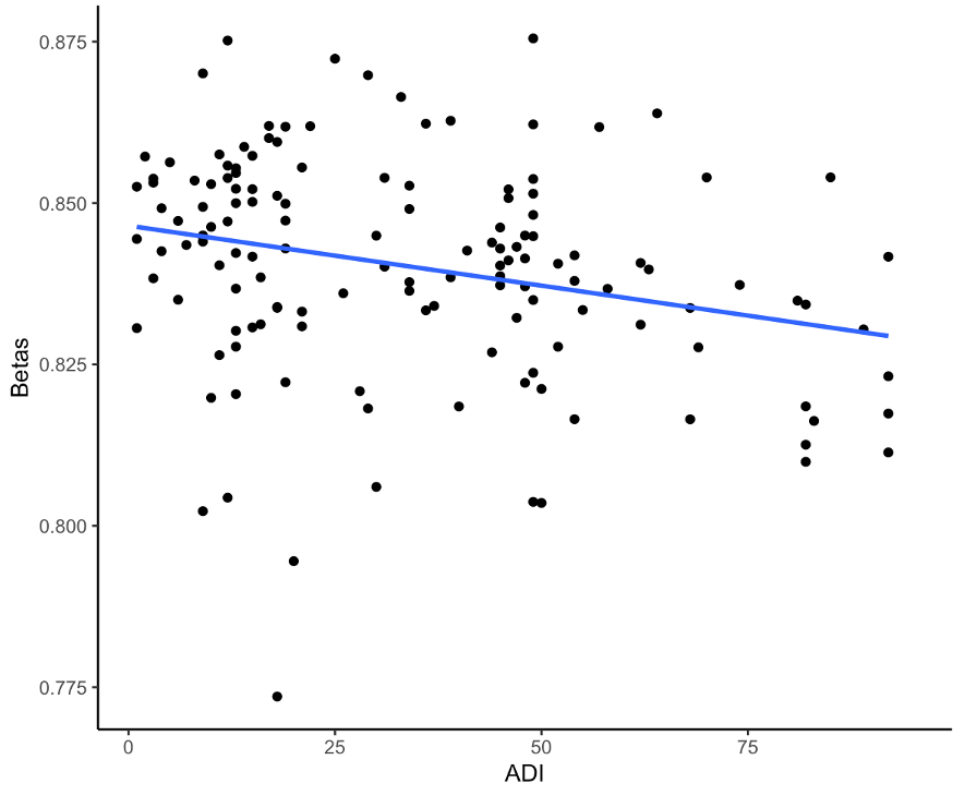

Figure 2: Scatterplot of DNAm beta values and the ADI from the EWAS of DNAm with the ADI for the CpG site cg26514961 (*PLXNC1*). The dots represent the DNAm beta and ADI values for a participant, and the blue line represents the (unadjusted) linear relationship between the DNAm beta values and the ADI.

Table 3. Association between the ten most significant CpG sites from the EWAS of ADI (compare Table 2), and their association with neuropathology markers (CERAD, ABC and Braak stage). Effect estimates can be interpreted per a 0.1-unit increase in DNAm.

				CERAD	**ABC**	**Braak Stage**
CpG	**Chrom-osome**	**Position**	**Gene(s)**	**Effect Estimate (95%-CI)**	**Effect Estimate (95%-CI)**	**Effect Estimate (95%-CI)**
cg26514961	12	94566784	*PLXNC1*	0.41 (-0.85, 1.67)	0.67 (-0.35, 1.70)	0.41 (-0.78, 2.56)
cg08087060	16	87795808	*KLHDC4*	0.57 (-1.17, 2.32)	0.08 (-1.47, 1.63)	0.57 (-2.73, 1.92)
cg01291468	2	234589374	*UGT1A10;UGT1A7; UGT1A9; UGT1A8*	-1.20 (-3.50, 1.09)	-1.14 (-3.13, 0.82)	-1.20 (-4.67, 1.49)
cg05419854	17	19398395	-	-0.89 (-2.17, 0.37)	-0.39 (1.50, 0.71)	-0.89 (-1.05, 2.52)
cg16241648	7	98923114	*ARPC1A*	0.93 (-3.42, 5.30)	-1.08 (-4.96, 2.77)	0.93 (-6.89, 5.12)
cg20912923	8	2885516	*CSMD1*	0.46 (-2.25, 3.18)	1.54 (-0.66, 3.77)	0.46 (-1.82, 5.46)
cg15953452	3	63053400	-	0.11 (-1.33, 1.55)	0.56 (-0.62, 1.75)	0.11 (-0.66, 2.93)
cg06787422	15	63331851	-	-0.13 (-3.33, 3.06)	0.50 (-2.22, 3.22)	-0.13 (-3.20, 4.94)
cg13521319	9	133423844	-	-1.37 (-5.24, 2.46)	-0.69 (-4.24, 2.84)	-1.37 (-6.76, 4.03)
cg09431774	22	25465561	*KIAA1671*	0.37 (-2.17, 2.91)	-0.10 (-2.41, 2.21)	0.37 (-4.31, 2.78)

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