

1 **Blood-derived DNA methylation clusters associate with adverse social exposures and**
2 **endophenotypes of stress-related psychiatric illness in a trauma-exposed cohort of women**

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56 **Abstract**

57 Adverse social exposures (ASEs) such as low income, low educational attainment, and
58 childhood/adult trauma exposure are associated with variability in brain region measurements of grey
59 matter volume (GMV), surface area (SA), and cortical thickness (CT). These CNS morphometries are
60 associated with stress-related psychiatric illnesses and represent endophenotypes of stress-related
61 psychiatric illness development. Epigenetic mechanisms, such as 5-methyl-cytosine (5mC), may contribute
62 to the biological embedding of the environment but are understudied and not well understood. How 5mC
63 relates to CNS endophenotypes of psychiatric illness is also unclear.

64 In 97 female, African American, trauma-exposed participants from the Grady Trauma Project, we
65 examined the associations of childhood trauma burden (CTQ), adult trauma burden, low income and low
66 education with blood-derived 5mC clusters and variability in brain region measurements in the amygdala,
67 hippocampus and frontal cortex subregions. To elucidate whether peripheral 5mC indexes CNS
68 endophenotypes of psychiatric illness, we tested whether 73 brain/blood correlated 5mC clusters, defined
69 by networks of correlated 5mC probes measured on Illumina's HumanMethylation Epic Beadchip, mediated
70 the relationship between ASEs and brain measurements.

71 CTQ was negatively associated with rostral middle frontal gyrus (RMFG) SA ($\beta = -0.231$, $p = 0.041$). Low income and low education were also associated with SA or CT in a number of brain regions.
72 Seven 5mC clusters were associated with CTQ ($p_{min} = 0.002$), two with low education ($p_{min} = 0.010$), and
73 three with low income ($p_{min} = 0.007$). Two clusters fully mediated the relation between CTQ and RMFG
74 SA, accounting for 47% and 35% of variability respectively. These clusters were enriched for probes falling
75 in DNA regulatory regions, as well as signal transduction and immune signaling gene ontology functions.
76 Methylome-network analyses showed enrichment of macrophage migration ($p = 9 \times 10^{-8}$), T cell receptor
77 complex ($p = 6 \times 10^{-6}$), and chemokine-mediated signaling ($p = 7 \times 10^{-4}$) pathway enrichment in association
78 with CTQ.

80 Our results support prior work highlighting brain region variability associated with ASEs, while
81 informing a peripheral inflammation-based epigenetic mechanism of biological embedding of such
82 exposures. These findings could also serve to potentiate increased investigation of understudied
83 populations at elevated risk for stress-related psychiatric illness development.

84 **Introduction**

85 Adverse social exposures (ASEs) such as low income, low educational attainment, childhood
86 trauma, and adult trauma exposure, are common in the United States (US). Over the past five years, poverty
87 rates in the US have ranged from 12 to 15%[1], and are likely to increase due to the impact of the COVID-
88 19 pandemic[2]. The percentage of US adults aged 18 to 24 that finish high school with a diploma or GED
89 is roughly 88%, meaning that over one million students per year leave high school without completion
90 credentials[3]. Past year exposure to physical abuse in US children ranges from 4% to 16%, whereas past
91 year psychological abuse is reported in 10% of children in the US; sexual abuse is experienced at some
92 point during childhood in 15% to 30% of children[4]. Furthermore, in a US population-based study of 34,653
93 participants, researchers found that depending on self-reported race/ethnicity, respondents' prevalence of
94 lifetime trauma exposure varied between 66% and 84%[5]. Other researchers have observed a lifetime
95 traumatic exposure prevalence ranging from 50% to 90%[6-8]. ASEs are known to have long-term impact
96 on emotional well-being and mental health; however, the mechanistic basis for these effects remain poorly
97 understood.

98 One particularly salient risk following ASE exposure is the development of stress-related psychiatric
99 illness or symptoms of such illnesses. For example, in both adults and children, poverty-related stress is
100 associated with increased internalizing symptoms (e.g., sadness or anxiety), involuntary engagement
101 stress responses (e.g., emotional arousal or intrusive thoughts), externalizing symptoms (e.g., acts of
102 aggression or theft), and involuntary disengagement stress responses (e.g., emotional numbing or cognitive
103 interference) [9]. Relatedly, World Health Organization (WHO) data has shown that rates of anxiety, mood,
104 and substance-use disorders are all positively correlated with measures of socioeconomic disadvantage,
105 including both low income and low educational attainment [10]. Moving a step beyond correlation, a
106 longitudinal study found that higher educational attainment significantly attenuated both the overall risk of
107 adult onset major depressive disorder (MDD), particularly in women, and the conditional risk of MDD as
108 well[11].

109 The effects of childhood maltreatment or trauma on long-term mental health prognoses are also
110 well-documented. In a recent meta-analysis of over 190 studies and 68,000 participants, researchers
111 observed that higher childhood maltreatment (including potential traumas such as emotional abuse,

112 physical abuse, sexual abuse) scores were associated with MDD diagnosis later in life and with depression
113 symptom severity[12]. This built upon previous work showing that exposure to any form of maltreatment
114 during childhood was associated with a two-fold risk of MDD development, and nearly a three-fold risk of
115 generalized anxiety disorder (GAD) development in adulthood[13]. Although childhood and adolescence
116 are regarded as particularly vulnerable periods of development, the impact of trauma on psychiatric
117 illness/symptom development are also notable when trauma is experienced in adulthood, with effects that
118 are known to vary by race/ethnicity and gender. A particularly salient example of this is evident in the
119 potential development of post-traumatic stress disorder (PTSD) after trauma. Recent population-based
120 work highlights race/ethnic differences in psychiatric illness development and PTSD in particular.
121 Researchers found that African Americans are generally more resilient to developing psychiatric illness
122 after a TE, compared to other races/ethnicities[14]; however, this resilience does not apply to PTSD, where
123 African Americans experience elevated conditional PTSD development rates relative to other
124 races/ethnicities[14]. This, in tandem with research showing that females are subject to almost double the
125 risk of developing PTSD and other mood-anxiety disorders throughout their lifetime[6, 15-17], highlights
126 that African American women in particular may be subject to elevated risk of stress-related psychiatric
127 illness development.

128 A great deal of work has been placed in identifying the neural correlates of poverty, low education,
129 and childhood/adult trauma exposure. Researchers have identified specific signatures of central nervous
130 system (CNS) variability in association with ASEs using methods such as structural magnetic resonance
131 imaging (MRI) and functional MRI (fMRI). In rodent [18, 19] and human [20-24] studies, observed neural
132 correlates of ASEs include variability in volumetric, cortical thickness (CT), surface area (SA), excitability,
133 and connectivity measures. Brain regions including frontal cortex, hippocampus, and amygdala, contribute
134 to adaptive behavioral and emotional responses, and are relevant to psychiatric illness development due
135 to their contributions to the generation and regulation of major decision-making, memory formation, and
136 stress-reactivity processes [25]. To this end, a meta-analytic study showed that frontal pole (FP), superior
137 frontal gyrus (SFG), and rostral middle frontal gyrus (RMFG) frontal cortex subregions are heavily involved
138 in attention, working memory, cognitive flexibility, and executive reasoning [26], with added involvement of
139 the frontal pole in introspection and longitudinal thinking [27]. The anterior cingulate cortex (ACC) is

140 regarded as a mediator of the interactions between higher level cognitive processes and emotion/stress
141 reactivity[28], and the orbito-frontal cortex (OFC) contributes to decision making and emotional processing
142 functions[29]. These functions are impaired in numerous mental illnesses, including but not limited to
143 PTSD[30], MDD[31], GAD[32], bipolar disorder[33], and schizophrenia[33]. These neural correlates of
144 ASEs are associated with the development of stress-related psychiatric illnesses and may represent neural
145 endophenotypes of said disorders[34-36].

146 Importantly though, the effects of ASEs do not travel directly from environment to psychiatric illness.
147 The cascade of effect, rather, travel initially through the neuroendocrine system as a response to stressful
148 external stimuli[37], whereby cortisol signaling occurs throughout the body, engaging immune (and other)
149 biological networks[38]. This immune network activation and responses to cortisol signaling result in neural
150 growth, inflammation, metabolism, and stress-related pathway disruption through epigenetic and other
151 molecular mechanisms[39, 40]. The process of DNA methylation, which commonly refers to the addition of
152 a 5-methyl-cytosine (5mC) residue to a cytosine-phosphate-guanine (CpG) base-pair, is one such
153 epigenetic mechanism, and is understood to enhance or reduce mRNA transcription of genes in an
154 experience-dependent, and temporally-stable manner[41]; continued research has pointed to the
155 importance of 5mC in this biological embedding of external stimuli[42, 43]. Because of challenges in taking
156 epigenetic measurements from a living human brain, the primary etiologic tissue of interest in regard to
157 psychiatric illness development, peripheral tissues such as blood or saliva serve as proxies for etiological
158 tissue. It is well-documented that these peripheral epigenetic measures can index changes to the
159 hypothalamic-pituitary-adrenal (HPA)-axis [44, 45], immune system [46, 47], and CNS [48-50], although the
160 direction of effect may be discordant across tissues [49, 51]. Stress-related physiological responses and
161 their accompanying epigenetic alterations are thought to confer the biological embedding of ASEs [52, 53],
162 and can ultimately increase the risk of stress-related psychiatric illness [39, 54]. Peripheral epigenetic
163 measures can also index CNS-relevant endophenotypes of psychiatric illness development, as observed
164 in studies using neuroimaging and peripheral epigenetic measures in tandem. However, these studies have
165 primarily utilized candidate gene approaches, investigating 5mC in the *SLC6A4* [55-57], *NR3C1* [58, 59]],
166 *FKBP5* [60], and *SKA2* [61, 62] genes, and neuroimaging measures of structure and function from the
167 frontal cortex, hippocampus, and amygdala. Findings show that peripheral 5mC can index CNS structural

168 variability[55-62] associated with psychiatric illness development, and that locus-specific peripheral 5mC
169 can mediate ASE-associated CNS structure variability[62].

170 To date, however, limited work has investigated the relationships among ASEs, peripheral 5mC,
171 and CNS endophenotypes of psychiatric illness at a genome-scale. To this end, the current exploratory
172 study applied genome-scale approaches in an all-female, African American sample from the Grady Trauma
173 Project (GTP) to assess whether blood-derived 5mC measurements might index CNS endophenotypes of
174 stress-related psychiatric illness. We focus specifically on the GTP because African American women may
175 be subject to elevated risk of stress-related psychiatric illness development, but the mechanisms underlying
176 that elevated risk are still unclear. In this study, we were specifically interested whether clusters of peripheral
177 5mC measurements statistically mediate the relation between ASEs and fronto-limbic grey matter volume
178 (GMV), SA, and CT measures. Based on previous work, we hypothesized that identified 5mC modules
179 would be enriched with 5mC probes falling in genes with HPA-axis[44, 45], immune system[46, 47], and
180 CNS-relevant [48-50] gene ontology (GO) functions.

181

182 **Materials and Methods**

183

184 *Participants*

185 The current research draws on 97 female, trauma-exposed participants. Participants were part of
186 the GTP, a larger investigation of genetic and environmental factors that predict the response to stressful
187 life events (SLEs) in a predominantly African American, low-income, urban population [15]. Research
188 participants were approached in the waiting rooms of primary care clinics of a large, public hospital. After
189 the subjects provided written informed consent, they participated in a verbal interview and gave a blood
190 sample for genetic and epigenetic analyses. Participants in this cohort also had available demographic,
191 psychosocial, and neuroimaging data. Exclusion criteria included intellectual disability or active psychosis.
192 Participants provided written, informed consent for all parts of the study, and the Institutional Review Boards
193 of Emory University and Grady Memorial Hospital approved the study procedures.

194

195

196 *Exposures of interest*

197 Income, education, adult trauma burden, and childhood trauma burden were considered exposures
198 of interest in current analyses. *Income*: Self-reported approximate household monthly income ("household
199 income") was measured as part of a demographic-focused inventory. Participants indicated total household
200 income within one of the following ranges: \$0 to \$499, \$500 to \$999, or greater than \$1000 per month.
201 *Education*: Participants also reported educational attainment history ("education") and were binned into one
202 of the following categories: did not complete high school, completed high school or GED, or completed
203 more schooling than high school. *Traumatic events inventory*: Adult trauma burden was assessed via a
204 semi-structured interview using the Traumatic Events Inventory (TEI), a scale developed by the GTP
205 researchers during their prior work in the Grady hospital primary care population [63]. TEI responses
206 pertaining to childhood were excluded in favor of the Childhood Trauma Questionnaire (CTQ). In the current
207 study, the continuous TEI score was used ("TEI"); the higher the TEI score, the more traumatic life events
208 one encountered. *Childhood trauma questionnaire*: Childhood trauma burden was assessed using the self-
209 report 28 question CTQ [64]. This inventory consists of five subscales, including sexual abuse, physical
210 abuse, emotional abuse, as well as physical and emotional neglect. In the current study, the total CTQ
211 score was used ("CTQ total"). Information regarding previous usage of exposure of interest variables in
212 GTP studies can be found elsewhere [15, 42, 65-68].

213 *Neuroimaging* Structural MRI study procedures followed the same methods as previously described in the
214 GTP cohort [69-72]. Structural images were acquired on a Siemens 3.0-Tesla Magnetom Trio TIM whole-
215 body MR scanner (Siemens, Malvern, PA, USA) with a 12-channel head coil, and using a gradient-echo,
216 T1-weighted pulse sequence (176 slices, TR = 2600 ms, TE = 3.02 ms, 1 mm³ voxel size). The T1 scan
217 was processed using FreeSurfer (available at <http://surfer.nmr.mgh.harvard.edu>), and a standardized
218 protocol (available at <http://enigma.ini.usc.edu/> protocols/imaging-protocols/) was used to check the
219 quality of the segmentations before further analyses were performed. Hemisphere-specific frontal cortex
220 CT and SA measurements were taken along with amygdala and hippocampal gray matter volume to
221 assess brain morphometry of the fronto-limbic pathway. For the purpose of the current study, within each
222 brain region (hippocampus and amygdala), we took the mean volume of the two hemispheres (e.g.,

223 hemisphere-mean hippocampal volume, hemisphere-mean amygdala volume). CT and SA develop along
224 discordant temporal trajectories[73], and are affected in different manners based on underlying genetic
225 sequence[74]. Based on this rationale, we examined CT and SA measures of frontal cortex subregions,
226 instead of gray matter volume. Frontal cortex subregions included in the current study map to Brodmann's
227 areas 8, 9, 10, 11, 24, 25, 32, and 46[25], and include FP, medial OFC (mOFC), lateral OFC (IOFC),
228 SFG, RMFG, rostral ACC (rACC), and caudal ACC (cACC). For frontal cortex SA and CT, we used an
229 average of values taken across hemispheres (e.g., hemisphere-mean RMFG SA, hemisphere-mean
230 RMFG CT).

231 *Molecular*

232 Whole-blood was collected in EDTA vacuum tubes prior to DNA extraction. Genomic DNA was
233 extracted and purified using the AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA) using manufacturer
234 recommended methods. DNA was denatured and bisulfite converted (BSC) using the EZ DNA Methylation-
235 GoldTM Kit (Zymo Research, Irvine, CA). After conversion, BSC DNA was applied to the Infinium
236 MethylationEPIC BeadChip (Illumina; San Diego, California) (850k) using manufacturer recommended
237 protocol to measure DNA 5mC at ~850,000 loci.

238

239 *5mC pre-processing*

240 Beta-values measured from the 850k platform were background corrected with GenomeStudio,
241 quality controlled (QCed), and filtered according to previously published methods[75]. All quality control and
242 pre-processing was performed in R version 3.6.1[76]. These steps removed low quality and potentially
243 cross-hybridizing probes, quantile-normalized probe Beta-values, and removed technical and batch
244 effects[77-80]. 5mC beta-values were variance stabilized and logit-transformed into M-values[81]. X- and
245 Y-chromosome-mapped probes were removed, along with rs-mapped probes. The remaining ~827k probes
246 were then subset to include only those with observed nominally significant Pearson correlation ($p < 0.05$)
247 between blood and brain tissue from the ImageCpG data repository[82], which focused analysis onto loci
248 with greater prospect for proxy or surrogate status with etiologically-relevant CNS tissue. Following QC and
249 filtering steps, 92,208 probes remained.

250

251 Covariates

252 Age, leukocyte cell estimates, intra-cranial volume (ICV), multi-dimensional scaling (MDS) of
253 genomic ancestry, an epigenetic proxy of smoking[83], and current employment/disability status were
254 included in models as covariates, where pertinent. *Leukocyte cell estimates*: DNAm measurements were
255 used to estimate the proportions of granulocytes, monocytes, B cells, natural killer cells, CD4T and CD8T
256 cells within each sample. Calculations were done according to methodology described in Houseman et
257 al[84]. Estimated proportions of monocytes, B cells, natural killer cells, CD4T and CD8T were included as
258 covariates in multiple regression models, where pertinent. *Genomic ancestry*: To avoid potential
259 inaccuracies/confounding effects of self-reported race/ethnicity, genetic ancestry was modeled using MDS
260 measures extracted from participant genome-wide association study (GWAS) data using PLINK[85]. The
261 first four MDS genetic ancestry measures were chosen to be used as covariates based on visual inspection
262 of scree plots, in line with previous work[86]. *Smoking*: Cigarette smoking is known to exert significant
263 effects across the methylome[87]. Using a method discussed in detail elsewhere[88], the current study
264 corrected for the methylomic effects of smoking by calculating effect size estimates of the top twenty-six
265 probes from a recent smoking epigenome-wide association study (EWAS) performed in participants with
266 African American genomic ancestry[83]. *Employment and disability*: As applied previously in the GTP [66],
267 employment/disability status was considered as: unemployed; unemployed receiving disability support; or
268 employed, with or without disability support.

269

270 Probe clustering

271 To remove non-desired effects from probe M-values, we composed linear models in R using age,
272 leukocyte cell esimates, genomic ancestry, and the smoking proxy as predictors of probe-wise 5mC M-
273 values. For each probe, residual values (“residualized M-values”) were extracted for clustering. Taking the
274 92,208 residualized M-values, the “WGCNA” package was then used in R to build a co-methylation
275 network[89]. First, scale-free topology model fit was analyzed. As recommended, a soft-threshold value of
276 ten was chosen based on the lowest power at which adjusted $R^2 > 0.90$. Adjacency and dissimilarity
277 matrices were generated, and unsupervised hierarchical clustering was used to generate a clustered
278 residual M-value network. Setting a minimum cluster size of ten generated 73 clusters of 5mC probes,

279 where each cluster was identified by a unique color. After clustering, we extracted the first principal
280 component of each cluster, referred to from here on as a “module eigengene” (ME). Compared to EWAS-
281 type analyses, which assess differential methylation on the level of individual 5mC loci, network-based
282 methods, as used in the current research, utilize dimension-reduction techniques to create networks of
283 related 5mC probe clusters. This reduces the burden of multiple hypothesis testing across hundreds of
284 thousands of probes, while enabling investigation into the biological significance of clustered probes, and
285 provides the potential for increased statistical power in circumstances with a small number of biological
286 replicates[90].

287

288 *Statistical analyses*

289 To understand the relationships between variables used throughout the current analyses, we
290 carried out Pearson correlations and mapped their correlation coefficients. We then conducted a set of
291 multi-variate linear regression analyses, as shown in Figure 1. Figure 1 Arm A analyses included
292 hemisphere-mean hippocampus and amygdala volumes as dependent variables (in separate models).
293 Income, education, CTQ total, and TEI were included as independent variables of interest, whereas
294 genomic ancestry, age, employment/disability and ICV were included as covariates. The same predictors
295 and covariates were included, minus ICV, to predict frontal cortex subregions SA and CT measures. Overall
296 CT and/or SA were not included as covariates based on prior work with this cohort[91]. Figure 1 Arm B
297 analyses included 5mC MEs as dependent variables (in separate models). Income, education, CTQ total,
298 and TEI were included as independent variables of interest, and employment/disability was included as a
299 covariate. Covariates were limited in this stage of analysis due to the removal of confounding effects
300 through residual extraction in prior pre-processing steps. Figure 1 Arm C analyses included individual
301 fronto-limbic morphometry measures as dependent variables (in separate models). Individual 5mC MEs
302 were included as independent variables of interest, whereas age, genomic ancestry, employment, and ICV
303 (ICV was only included in hippocampus and amygdala models) were included as covariates. Within each
304 phase of the analyses, including mediation analyses, continuous dependent and independent variables
305 were standardized, resulting in standardized effect estimates. Nominal p-values were corrected for multiple
306 hypothesis testing by controlling the false discovery rate (FDR=0.10) using the Benjamini Hochberg (BH)

307 procedure(85). Briefly, for each nominal p-value, a BH critical value was calculated where nominal p-value's
308 assigned rank over the number of tests was multiplied by the accepted FDR. Nominal p-values less than
309 this threshold were deemed BH-significant. Due to the exploratory nature of the current work, both nominal
310 and BH-significant terms were considered for interpretation; and nominal p-values are presented within the
311 results section of the current text.

312

313 *Mediation analyses*

314 MEs were tested for statistical mediating status between independent variables of interest (ASEs)
315 and dependent variables (hemisphere-mean fronto-limbic brain morphometry) using the "mediation"
316 package in R (Figure 1). Importantly, only fronto-limbic brain morphometry measures nominally associated
317 with income, education, CTQ total or TEI (Figure 1, Arm A) were considered. Similarly, MEs tested for
318 mediation included only those nominally associated with an exposure (Figure 1, Arm B) and a fronto-limbic
319 brain morphometry measure (Figure 1, Arm C). For each ME, average indirect effect (IDE), direct effect
320 (DE), and total effect estimates and confidence intervals were calculated as a result of 10,000 quasi-
321 Bayesian Monte Carlo approximations, applied with the mediation package. Consistent with methodology
322 employed in the field [92, 93], we considered an ME a full mediator if the DE=0 while the IDE and total
323 effect ≠ 0, or a partial mediator if the DE, IDE, and total effect (TE) ≠ 0. Individual probes from full mediator
324 modules were assessed for mediation status, in order to gain insight into potential locus-specific mediation
325 effects.

326

327 *Gene set enrichment analyses*

328 To assess underlying methylomic network enrichment, all four ASEs of interest were included in
329 linear models as either independent variables or covariates, where individual residualized probe M-values
330 were included as dependent variables, while controlling for employment/disability status. Importantly, only
331 exposures with a fully-mediating 5mC cluster were considered independent variables of interest. Other
332 exposures which did not exhibit a fully-mediating 5mC cluster were considered as covariates. For
333 probes/ASEs with nominally significant relationships ($p < 0.05$), we extracted probe p-values and probe
334 names and used them as input to gene set enrichment analyses (GSEA) with the "methylGSA" package[94].

335 Importantly, ASE-associated probe p-values were only captured for ASEs in which a full mediator was
336 previously observed, which limited analyses to CTQ only. GO sets of 25 to 1,000 genes were allowed,
337 eliminating high-level GO-terms such as “biological process”, which facilitated testing of 5,478 gene sets.
338 To produce a condensed summary of non-redundant GO-terms, the web-based tool “Revigo” was used[95].
339

340 **Results**

341 *Study participants*

342 Descriptive statistics for demographic and psychosocial measures in study participants are shown
343 in Table 1. Thirty percent of the sample reported household income in the range of \$0 to \$499 per month,
344 while 34% of the sample reported household income above \$1000 per month. Fifteen percent of the sample
345 reported not having finished high school, whereas 55% of the sample reported having more schooling than
346 high school. Mean CTQ total value for participants was 40.5 (+/- 15.4), whereas mean TEI value for
347 participants was 4.2 (+/- 2.3). Pearson correlations between variables used in the current study are shown
348 in Figure 2.

349

350 *ASEs predict fronto-limbic brain morphometry*

351 At nominal significance, CTQ total was negatively associated with RMFG SA ($\beta = -0.231$, SE =
352 0.111, $t = -2.079$, $p = 0.041$), but was not associated with other outcomes of interest ($p > 0.05$). Relative to
353 high educational attainment (completed more schooling than high school), low educational attainment (no
354 high school certificate) was negatively associated with cACC SA ($\beta = -0.855$, SE = 0.280, $t = -3.049$, $p =$
355 0.003), and positively associated with FP CT ($\beta = 0.684$, SE = 0.302, $t = 2.267$, $p = 0.026$) and SFG CT (β
356 = 0.553, SE = 0.256, $t = 2.162$, $p = 0.034$) at the nominal significance threshold, but no other brain
357 morphometry outcomes of interest ($p > 0.05$). On the other hand, relative to high income (\$1000+/month),
358 low income (\$0-\$499/month) was nominally negatively associated with amygdala GMV ($\beta = -0.649$, SE =
359 0.283, $t = -2.299$, $p = 0.024$), but no other neuroimaging outcome of interest ($p > 0.05$). TEI was not
360 associated with any neuroimaging measures ($p > 0.05$). Nominally significant model outcomes can be found
361 in Table 2. In controlling for 64 total tests at FDR = 0.10, no outcomes were BH-significant. Brain
362 morphometry outcomes nominally associated with ASEs were carried into downstream analyses.

363

364 ASEs predict 5mC ME

365 CTQ total was nominally associated with seven MEs, the strongest of which were Darkolivegreen
366 (Darkolivegreen $\beta = 0.354$, SE = 0.110, t = 3.214, p = 0.002) and Steelblue (Steelblue $\beta = -0.306$, SE =
367 0.111, t = -2.748, p = 0.007). Compared to high educational attainment, low educational attainment was
368 positively associated with one ME (Coral1 $\beta = 0.750$, SE = 0.286, t = 2.618, p = 0.010), and negatively
369 associated with one ME (Lightcoral $\beta = -0.615$, SE = 0.296, t = -2.079, p = 0.040) at nominal levels.
370 Relative to high income (\$1000+/month), low income (\$0-\$499/month) was positively associated with three
371 MEs (Antiquewhite4 $\beta = 0.770$, SE = 0.277, t = 2.781, p = 0.007; Indianred4 $\beta = 0.717$, SE = 0.276, t =
372 2.602, p = 0.011; Orange $\beta = 0.571$, SE = 0.274, t = 2.084, p = 0.040) at nominal levels. Because TEI was
373 not associated with any fronto-limbic brain morphometric measures (Figure 1, Arm A), we do not report any
374 results for this exposure. Results from all Arm B analyses can be found in Supplementary Table 1. In
375 controlling for 73 tests at FDR = 0.10, no outcomes were BH-significant. 5mC MEs nominally associated
376 with ASEs were carried into downstream analyses.

377

378 5mC MEs predict fronto-limbic brain morphometry

379 Four out of seven CTQ-associated MEs showed a nominally significant relationship with
380 hemisphere-mean RMFG SA (Maroon, White, Tan, Yellow). The three strongest relationships were the
381 Maroon ME ($\beta = -0.451$, SE = 0.091, t = -4.966, p = 3×10^{-6}) (Figure 3a), White ME ($\beta = -0.392$, SE =
382 0.093, t = -4.231, p = 6×10^{-5}) (Figure 3b), and Tan ME ($\beta = 0.233$, SE = 0.100, t = 2.322, p = 0.023) (Figure
383 3c). Two low educational attainment-associated MEs were tested independently for associations with
384 hemisphere-mean cACC SA, frontal pole CT, and SFG CT. Of these, the Lightcoral ME was nominally
385 associated with cACC SA ($\beta = 0.215$, SE = 0.099, t = 2.182, p = 0.032) (Figure 3d). Finally, of the three low
386 income-associated MEs, none showed a nominally significant relationship with hemisphere-mean
387 amygdala volume (Supplementary Table 2). In controlling for 16 tests at FDR = 0.10, the Maroon and White
388 ME relationships with hemisphere-mean RMFG SA were BH-significant. 5mC MEs nominally associated
389 with fronto-limbic brain morphometry measures were carried into downstream analyses.

390

391 *ME mediation*

392 Four CTQ-associated MEs that also showed a nominally significant relationship with hemisphere-
393 mean RMFG SA were tested for their mediating status between CTQ total and hemisphere-mean RMFG
394 SA (Maroon, White, Tan, Yellow). Both the Maroon ME (TE $\beta = -0.983$, $p = 0.047$; IDE $\beta = -0.466$, $p = 0.008$;
395 DE $\beta = -0.517$, $p = 0.277$) and the White ME (TE $\beta = -1.014$, $p = 0.034$; IDE $\beta = -0.348$, $p = 0.032$; DE $\beta =$
396 -0.667 , $p = 0.164$) were full mediators of this relationship. TE results show that RMFG SA was an estimated
397 $\sim 589 \text{ mm}^2$ lower at the highest levels of CTQ exposure compared to the lowest levels of CTQ exposure.
398 Independently, the Maroon ME accounted for 275 mm^2 (47%) of that effect, whereas the White ME
399 accounted for 204 mm^2 (34%). Neither the Tan nor Yellow MEs were partial or full mediators of the
400 relationship between CTQ and hemisphere-mean RMFG SA (TE $p < 0.05$; IDE $p > 0.05$; DE $p > 0.05$),
401 although the direction of TEs in these analyses closely mirrored those in the Maroon and White ME
402 mediation analyses. The Lightcoral ME, which we previously observed to be associated with low education
403 attainment and cACC SA, was neither a partial nor full mediator of the relationship between low education
404 attainment and hemisphere-mean cACC SA (TE $\beta = -0.844$, $p = 0.003$; IDE $\beta = -0.107$, $p = 0.100$; DE $\beta =$
405 -0.737 , $p = 0.010$). However, the DE indicates that low educational attainment status accounted for cACC
406 SA that is 84 mm^2 smaller than high educational attainment status. In controlling for ten tests of the IDE
407 and DE p-values for the five relationships above at FDR = 0.10, all nominally significant ME IDEs were BH-
408 significant and all DEs remained non-significant (Table 3). Follow-up mediation analyses were then
409 performed on individual probes from the Maroon and White modules.

410

411 *Probe-wise mediation: Maroon module*

412 Six out of the 11 Maroon ME probes were full mediators of the relationship between CTQ total and
413 RMFG SA (Table 4). The top three strongest mediators based on IDE β coefficients were: cg21622733 (TE
414 $\beta = -0.983$, $p = 0.044$; IDE $\beta = -0.512$, $p = 0.003$; DE $\beta = -0.471$, $p = 0.320$), cg19805668 (TE $\beta = -1.003$, p
415 $= 0.042$; IDE $\beta = -0.411$, $p = 0.009$; DE $\beta = -0.592$, $p = 0.217$), and cg03710029 (TE $\beta = -0.990$, $p = 0.048$;
416 IDE $\beta = -0.321$, $p = 0.019$; DE $\beta = -0.670$, $p = 0.182$). Respectively, these probes mapped to the long
417 intergenic non-coding (LINC) RNA gene *LINC01531*, the placental growth factor gene *PGF*, and the solute
418 carrier family 38 member 10 gene *SLC38A10*. The other three full mediating probes mapped to myosin

419 heavy chain 14 (*MYH14*), DNA methyltransferase 3 alpha (*DNMT3A*), and neuralized E3 ubiquitin protein
420 ligase 4 (*NEURL4*). In controlling for 22 tests of Maroon probe IDE and DE p-values, only two nominally
421 significant probes were also BH-significant (cg21622733: *LINC01531* and cg19805668: *PGF*).
422 Comprehensive probe-wise mediation results are found in Supplementary Table 3.

423 In all, probes from the Maroon module map to eight known protein coding genes (*MYH14*,
424 *HGSNAT*, *SFTPA1*, *PGF*, *NEURL4*, *DNMT3A*, *SLC38A10*, and *PEBP4*) and two non-coding RNAs
425 (*LINC01531* and *MIR3659HG*). GO-terms associated with these loci include: *MYH14* (neuronal action
426 potential GO:0019228, actomyosin structure organization GO:0031032), *HGSNAT* (lysosomal transport
427 GO:0007041, neutrophil degranulation GO:0043312), *SFTPA1* (toll-like receptor signaling pathway
428 GO:0002224), *PGF* (chemoattractant activity GO:0042056, vascular endothelial growth factor receptor
429 binding GO:0005172), *NEURL4* (ubiquitin protein ligase activity GO:0061630), *DNMT3A* (DNA (cytosine-
430 5-methyltransferase activity GO:0003886), *SLC38A10* (amino acid transmembrane transporter activity
431 GO:0015171), and *PEBP4* (protein binding GO:0005515), among others.

432

433 *Probe-wise mediation: White module*

434 Three out of 19 White ME probes were full mediators of the relationship between CTQ total and
435 RMFG SA (Table 4): cg01544227 (TE $\beta = -0.994$, $p = 0.044$; IDE $\beta = -0.305$, $p = 0.036$; DE $\beta = -0.689$, $p =$
436 0.175), cg00686169 (TE $\beta = -1.017$, $p = 0.037$; IDE $\beta = -0.277$, $p = 0.043$; DE $\beta = -0.740$, $p = 0.126$), and
437 cg08002107 (TE $\beta = -1.006$, $p = 0.040$; IDE $\beta = -0.268$, $p = 0.047$; DE $\beta = -0.738$, $p = 0.122$). These three
438 probes mapped to *AC092745.5*, *FAM90A24P*, and *USP17L7* loci, respectively. In controlling for 38 tests of
439 White probe IDE and DE p-values, no nominally significant probes were BH-significant. Comprehensive
440 probe-wise mediation results are found in Supplementary Table 3.

441 In all, only one probe from the White module maps to a known protein coding gene, *USP17L7*,
442 which is associated with the thiol-dependent de-ubiquitinase GO term (GO:0004843). One other probe
443 maps to a LINC RNA, *FAM66D*. The remaining probes map to a pseudogene family, named family with
444 sequence similarity 90. Members include: *FAM90A10*, *FAM90A11P*, *FAM90A20*, *FAM90A24P*,
445 *FAM90A25P*, *FAM90A5*, *FAM90A6P*, and *FAM90A7*. The only GO term associated with these
446 pseudogenes is nucleic acid binding (GO:0003676).

447

448 *Gene set enrichment analysis*

449 CTQ total was included as the independent variable of interest in linear models with probe
450 residualized M-values as dependent variables. Income, education, and TEI were included and considered
451 as covariates, as no 5mC clusters mediated the relationships between our exposures and outcomes of
452 interest. We used the resultant 6,335 nominally significant p-values and probe names as GSEA input,
453 facilitating the testing of 5,478 GO-terms. After Revigo redundancy reduction, 30 BH-significant GO-terms
454 remained for interpretation. These included immune-related GO-terms: macrophage migration
455 (GO:1905517, $p = 9 \times 10^{-8}$, rank = 10), T cell receptor complex (GO:0042101, $p = 6 \times 10^{-6}$, rank = 13), and
456 chemokine-mediated signaling pathway (GO:0070098, $p = 7 \times 10^{-4}$, rank = 28), among four others. Two
457 CNS-related GO-terms were present: negative regulation of smoothened signaling pathway (GO:0045879,
458 $p = 2 \times 10^{-6}$, rank = 12), and glutamine family amino acid biosynthetic process (GO:0009084, $p = 1 \times 10^{-4}$, rank
459 = 19), along with one HPA-axis related term: thyroid hormone metabolic process (GO:0042403, $p = 2 \times 10^{-15}$, rank = 5). Additionally, numerous signal transduction and membrane transport GO-terms were present:
460 positive regulation of phospholipase activity (GO:0010518, $p = 5 \times 10^{-25}$, rank = 1), intrinsic component of
461 peroxisomal membrane (GO:0031231, $p = 7 \times 10^{-18}$, rank = 2), and RNA polymerase II carboxy-terminal
462 domain kinase activity (GO:0008353, $p = 8 \times 10^{-15}$, rank = 6), among others (Supplementary Table 4).

464

465 **Discussion**

466 Here we applied genome-scale approaches to a sample of African American women from a low
467 income, highly trauma exposed environment, representing an understudied population, where our main
468 goal was to assess whether blood-derived 5mC cluster MEs might index CNS endophenotypes of stress-
469 related psychiatric illness. Our study findings indicate that high childhood trauma burden, low educational
470 attainment, and low income are each associated with CNS endophenotypes of stress-related psychiatric
471 illness, and that a subset of blood-derived 5mC MEs statistically mediate the relationship between childhood
472 trauma burden and RMFG SA. We further found that the individual probes from mediating 5mC MEs fell in
473 genes with CNS-relevant and immune system GO-functions. Finally, we found that the underlying childhood
474 trauma burden-associated methylomic network was enriched with HPA-axis, immune, and CNS-related

475 gene sets, in addition to signal transduction and membrane transport functions. Overall, we posit that the
476 peripheral epigenetic signatures mediating our relationships of interest are consistent with previously
477 observed patterns in which stress-related psychiatric illness is accompanied by profiles of peripheral
478 inflammation[31, 96-98].

479 In the current study, childhood trauma burden was negatively associated with hemisphere-mean
480 RMFG SA, which mirrors findings from cohorts enriched for stress, trauma, and psychiatric illness[99, 100].
481 We also observed higher CT of both the FP and SFG in participants with low educational attainment, as
482 well as higher cACC SA. Although studies on FP CT and ASEs (besides trauma) are somewhat limited, our
483 findings support past research investigating whether high CT is representative of a predisposition to
484 psychiatric illness development, or whether high CT emerges after psychiatric illness onset. Specifically,
485 prior longitudinal work showed higher cortical thickness in participants at high risk of mood disorder
486 compared to healthy controls at baseline; the high-risk participants who went on to develop MDD then
487 showed increased FP CT over the two-year time period following their diagnosis[101]. This research implies
488 that high FP CT could represent both a predisposition to MDD development risk, and a post-onset emergent
489 phenotype of MDD.

490 Our study also showed that low household income was negatively associated with hemisphere-
491 mean amygdala GMV. This finding aligns with research in children/adolescents showing that low familial
492 income-to-needs ratio is associated with decreased amygdala GMV [92], and with studies in adults showing
493 that past year financial hardship is associated with decreased amygdala GMV [102]. However, the
494 relationships between socioeconomic status-related measures and amygdala GMV are well-studied, and
495 variable conclusions between studies are common[103]. Hemisphere-mean hippocampal volume was not
496 associated with any ASE; as in the case of amygdala GMV, study conclusions are mixed[103].

497 In all, four blood-derived MEs were tested for mediating status between childhood trauma burden
498 and RMFG SA. Both the Maroon and the White ME fully mediated the relationship and accounted for
499 significant proportions of variability (47% and 35% respectively). The Maroon module is composed of 11
500 probes, six of which are also full mediators of the aforementioned relationship. The six probes fully
501 mediating the childhood trauma burden and RMFG SA relationship are located in the *MYH14*, *PGF*,
502 *NEURL4*, *DNMT3A*, *SLC38A10*, and *LINC01531*. GO-terms associated with these loci include: *MYH14*

503 (neuronal action potential GO:0019228, actomyosin structure organization GO:0031032), PGF
504 (chemoattractant activity GO:0042056, vascular endothelial growth factor receptor binding GO:0005172),
505 NEURL4 (ubiquitin protein ligase activity GO:0061630), DNMT3A (DNA (cytosine-5-methyltransferase
506 activity GO:0003886), and SLC38A10 (amino acid transmembrane transporter activity GO:0015171). Of
507 particular interest is the probe falling in the *DNMT3A* gene. Past longitudinal study in a predominantly
508 female African American sample showed differential blood *DNMT3A* 5mC in response to traumatic event
509 exposure[104], and emerging work focused specifically on African ancestry individuals has identified a
510 genome-wide significant association in *DNMT3A* in relation to MDD[105]. What is more, in a rodent model
511 of fear conditioning, *Dnmt3a* mRNA expression is altered in forebrain neurons, leading to differential DNA
512 5mC of synaptic plasticity and memory formation genes[106]. *Dnmt3A* mRNA expression in rodent nucleus
513 accumbens is also altered in response to chronic restraint stress, and targeted inhibition of *Dnmt3A* 5mC
514 potentiates anti-depressant-like effects[107]. Based on the current and prior findings, it appears that the
515 *DNMT3A* gene in humans is a potential locus of stress- or trauma-related biological embedding, and that
516 measurements of *DNMT3A* 5mC taken from peripheral tissues could be indexing fronto-limbic variability
517 associated with these exposures. Overall, our findings in the Maroon module imply that immune signaling,
518 cellular proliferation, neuronal development, and epigenetic regulatory pathways could be potential
519 substrates for the biological embedding of childhood trauma.

520 The second fully mediating ME was the White module comprised of 19 probes, three of which were
521 full mediators of the relation between CTQ total and RMFG SA. Thirteen probes from this module are
522 mapped to the “Family with sequence similarity 90” superfamily of pseudogenes, and all but two probes
523 exist in a one megabase stretch of chromosome eight. The three full mediator probes (cg00686169,
524 cg01544227, and cg08002107) fall in the *FAM90A24P*, *LINC00937*, and *FAM66D* loci, respectively. Little
525 is known of the *FAM90A24P* locus, but the *LINC00937* locus encodes a LINC RNA species that is strongly
526 associated with cutaneous melanoma prognosis[108], as well as endocervical cancer progression[109],
527 suggesting that it plays a role in cellular proliferation. On the other hand, the *FAM66D* locus encodes a
528 LINC RNA species whose expression is significantly upregulated in both Crohn’s disease (CD) and
529 ulcerative colitis (UC), the two most common types of inflammatory bowel disease (IBD)[110]. Efforts made
530 towards elucidating the function of the broader chromosome eight locus highlight its structurally dynamic

531 nature, in addition to its harboring of the defensin genes, the protein products of which play significant roles
532 in the innate immune response, as well as antitumor response[111]. Defensin proteins are also recognized
533 as key contributors to innate immune defense against UC, CD, and IBD in general[112].

534 Taken together, results suggest that probes mediating the childhood trauma burden and RMFG SA
535 relationship interact with endothelial growth and innate immune response regulatory pathways. This notion
536 is supported by repeated observation of increased blood-based inflammation profiles, as measured in
537 adults that had previously experienced childhood trauma exposure [113-115]. More CNS-focused research
538 has also shown significant dysregulation of stress-response and inflammation-related mRNA/protein levels
539 in post-mortem frontal cortex of patients with neurodevelopmental[116] and psychiatric illnesses[117, 118].
540 What is more, decreased CT of medial pre-frontal cortex is observed in MDD patients, a relationship that
541 was partially mediated by inflammatory factors known to influence neuroplasticity[119]. However, the
542 relevant studies informing our current results are sparse, and could be considered underpowered. Thus,
543 while suggestive, more work remains to be done in order to draw robust inferences regarding the
544 relationship between peripheral inflammation and neural endophenotypes of stress-related psychiatric
545 illness.

546 In our GSEA of childhood trauma burden -associated probes, the top enriched term was “positive
547 regulation of phospholipase activity”, meaning that multiple probes fall in genes that increase the frequency,
548 rate, or extent of phospholipase activity. Of close relation is the 11th ranked GO-term, “inositol phosphate
549 phosphatase activity”. Together, these terms represent one of the major mechanisms of neuronal and
550 hormonal signal transduction in mammals, the activation of phospholipase C (PLC) and subsequent
551 activation of inositol signaling pathways[120]. In brief, extracellular stimuli receptor binding activates PLC.
552 PLC then functions to convert phosphatidylinositol-4-5 bisphosphate (P45P2) into inositol-1-4-5
553 trisphosphate (I145P3). From this, the kinase-activating secondary messenger diacylglycerol (DAG) is
554 activated[120]. There are many isoforms of the PLC enzyme family, but PLC-δ1 is strongly implicated in
555 regulating cell-motility and cytoskeletal organization[121], and in inhibiting inflammatory immune
556 responses[122]. Quantities of this PLC isoform, PLC-δ1, are highly correlated with nuclear levels of
557 P45P2[123]; interestingly, P45P2 regulates transcription through binding interaction with the RNA
558 polymerase II C-terminal tail[124]. The aforementioned processes are specifically represented within our

559 GSEA results (Supplementary Table 4): RNA polymerase II carboxy-terminal domain kinase activity,
560 regulation of mitotic spindle organization, and numerous immune signaling and motility terms. Given
561 childhood trauma burden is the ASE implicated most strongly in our results, it stands to reason that in this
562 sample of African American women, peripheral inflammatory signals could be heightened in those with high
563 childhood trauma burden, and phospholipase activity may be upregulated in an attempt to restore
564 homeostatic balance.

565 Overall, we posit that the blood-based epigenetic signatures mediating our relationships of interest
566 are potentially explained by ASE-associated peripheral inflammation. In response to perceived stress or
567 trauma, fronto-limbic brain regions associated with cognitive function, emotional reactivity, and memory are
568 activated, signaling to the HPA-axis' neural hub, and eventually potentiating glucocorticoid (GC) release.
569 Critical to immunological, metabolic, cardiac, and homeostatic functions, GCs act mechanistically in and
570 around the CNS and peripheral nervous system, including serving as a long-range negative feedback
571 mechanism to inhibit further HPA-axis activity. Additionally, the ligands and receptors of cytokines and
572 neurotransmitters are shared throughout the CNS, HPA-axis, and immune systems[125, 126]. Chronic
573 stress signaling can result in neural growth, inflammation, metabolism, and stress-related pathway
574 disruption through molecular and epigenetic mechanisms[40], which are consistent with 5mC-derived
575 patterns observed in peripheral tissue in the current report.

576 The foremost strength of the current research is the inclusion of multi-modal data types
577 (epigenetic/neuroimaging) to characterize the biological embedding of ASEs. Furthermore, the dimension
578 reduction techniques used in the current work, clustering and principal component extraction, were valuable
579 in reducing the burden of multiple hypothesis testing and in describing potentially related networks of 5mC
580 loci. It also focused the analysis onto loci with greater prospect for proxy or surrogate status with
581 etiologically-relevant CNS tissue. The composition of the sample is also a strength of the current work, as
582 it reflects an understudied population at elevated risk for PTSD and for persistence of stress-related
583 psychiatric illness[127]. However, because of the homogenous nature of the sample, we are unable to make
584 confident conclusions about ancestry-specific or sex-specific effects. Limitations of the current study include
585 relatively small sample size and the inability to infer causality from cross-sectional samples. In addition, the
586 current research used peripheral tissue in lieu of etiological CNS tissue for obvious technical reasons.

587 Functional MRI measures such as neurotransmitter specific PET imaging could also provide deeper
588 endophenotypic insights. Future studies on this topic should capture longitudinal data from a larger sample
589 and could investigate genetic factors or tissues of etiological interest.

590 The current study showed that exposure to high childhood trauma burden, low educational
591 attainment, and low income are each associated with neuroimaging endophenotypes of psychiatric illness,
592 and that the relationship between childhood trauma and RFMG SA in particular is mediated by a subset of
593 blood-derived 5mC measurements. In addition, we found that the mediating 5mC MEs were enriched with
594 probes falling in genomic regulatory regions, and in genes with CNS-relevant and immune system GO-
595 functions. Finally, we found that the underlying childhood trauma burden-associated methylomic network
596 was enriched with HPA-axis, immune, and CNS-related gene sets. Together, these results highlight a
597 feasible epigenetic mechanism through which ASEs become embedded in human physiological systems,
598 and through which they contribute to the development of stress-related psychiatric illness. Although these
599 concepts are broadly applicable across race, ethnicity, and biological sex, the current results highlight
600 mechanisms of biological embedding that may be specific to African American women. These epigenetic
601 signatures could be taken as peripheral biomarkers of perturbed underlying neurobiology associated with
602 ASEs, and could further research efforts into etiological tissues of interest such as endocrine, immune, and
603 CNS cell types. These findings could also serve to potentiate increased investigation of understudied
604 populations at significant risk for onset or persistence of certain stress-related psychiatric illnesses.

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606

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612

613 **Conflict of interest**

614 The authors report no conflicts of interest.

615

616 **Data Availability Statement**

617 DNA methylation data analyzed in this paper are available at <https://www.ncbi.nlm.nih.gov/geo/> (dataset
618 GSE132203). Individual-level neuroimaging data will be made available to researchers following an
619 approved analysis proposal through the GTP. For additional information on access to these data,
620 including PI contact information for the GTP, please contact the corresponding author.

621

622

623 **Figure Legends**

624 **Figure 1.** Conceptual model testing individual module eigengenes (MEs) as mediators of the hypothesized
625 associations between adverse social exposures (ASEs) and variability in fronto-limbic brain morphometries.
626 Arm A. Analyses included hemisphere-mean hippocampus and amygdala volumes as dependent variables
627 (in separate models). Income, education, CTQ total, and TEI were included as independent variables of
628 interest, whereas genomic ancestry, age, employment/disability and ICV were included as covariates. The
629 same predictors and covariates were included, minus ICV, to predict frontal cortex subregions SA and CT
630 measures. Arm B. Analyses included 5mC MEs as dependent variables (in separate models). Income,
631 education, CTQ total, and TEI were included as independent variables of interest, and
632 employment/disability was included as a covariate. Arm C. Analyses included individual fronto-limbic
633 morphometry measures as dependent variables (in separate models). Individual 5mC MEs were included
634 as independent variables of interest, whereas age, genomic ancestry, employment, and ICV (ICV was only
635 included in hippocampus and amygdala models) were included as covariates.
636

637 **Figure 2.** Pearson's correlation heatmap of variables used throughout the current study. Age is negatively
638 associated with multiple frontal cortex cortical thickness (CT) measures (Pearson's correlation $r_{range} = -0.15$
639 to -0.47 , $p_{range} = 0.092$ to 6×10^{-7}). Within frontal cortex CT measures, multiple subregions show positive
640 correlations with one another (Pearson's correlation $r_{range} = 0.32$ to 0.84 , $p_{range} = 0.004$ to $< 2 \times 10^{-16}$). The
641 same phenomenon is observed within frontal cortex surface area (SA) measures (Pearson's correlation
642 $r_{range} = 0.16$ to 0.77 , $p_{range} = 0.210$ to $< 2 \times 10^{-16}$). Overall CT is positively correlated with each of the frontal
643 cortex CT measures (Pearson's correlation $r_{range} = 0.43$ to 0.86 , $p_{range} = 1 \times 10^{-7}$ to $< 2 \times 10^{-16}$), and overall SA
644 is positively correlated with each of the frontal cortex SA measures (Pearson's correlation $r_{range} = 0.37$ to
645 0.88 , $p_{range} = 5 \times 10^{-4}$ to $< 2 \times 10^{-16}$).
646

647 **Figure 3.** CTQ total is negatively associated with rostral middle frontal gyrus (RMFG) surface area (SA).
648 Low educational attainment status is associated with low caudal anterior cingulate cortex (cACC) SA. Out
649 of seven CTQ-associated module eigengenes (MEs), the three strongest relationships with hemisphere-
650 mean RMFG SA were the Maroon (A), White (B), and Tan (C) MEs. Out of two low education attainment-
651 associated MEs, the strongest relationship with hemisphere-mean cACC SA was the Lightcoral ME (D). As
652 shown, neuroimaging measures are adjusted by age, ME value, genomic ancestry, employment/disability
653 status, and an adverse social exposure (ASE). Dependent and independent variables were scaled. A. CTQ
654 total was positively associated with the Maroon ME ($\beta = 0.289$, $SE = 0.109$, $t = 2.639$, $p = 9.8 \times 10^{-3}$). In turn,
655 the Maroon ME was negatively associated with RMFG SA ($\beta = -0.451$, $SE = 0.091$, $t = -4.966$, $p = 3 \times 10^{-6}$). B. CTQ total was negatively associated with the Tan ME ($\beta = -0.248$, $SE = 0.112$, $t = -2.220$, $p = 0.029$).
656 The Tan ME was, in turn, positively associated with RMFG SA ($\beta = 0.233$, $SE = 0.100$, $t = 2.322$, $p = 0.023$).
657 C. CTQ total was positively associated with the White ME ($\beta = 0.236$, $SE = 0.110$, $t = 2.151$, $p = 0.034$). In
658 turn, the White ME was negatively associated with RMFG SA ($\beta = -0.392$, $SE = 0.093$, $t = -4.231$, $p = 6 \times 10^{-5}$). D. Low educational attainment (no high school certificate) was associated with the Lightcoral ME
659 ($\beta = -0.615$, $SE = 0.296$, $t = -2.079$, $p = 0.040$). The Lightcoral ME, in turn, was positively associated with
660 cACC SA ($\beta = 0.215$, $SE = 0.099$, $t = 2.182$, $p = 0.032$).
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Table 1. Demographic & psychosocial measure summary statistics (non-scaled) for the current sample (n = 97)

Measure	Description	Value
Age	Mean [SD] (Range)	40 [12.5] (19 - 62)
Sex	Female	100%
Self-reported race/ethnicity	African American	100%
Household income (income)	\$0-499	30%
	\$499-999	36%
	\$1000+	34%
Educational attainment (education)	Some high school	15%
	High school grad or GED	30%
	≥ Undergraduate degree	55%
Employment/disability	Employed	33%
	Disabled (not employed)	7%
	Not employed or disabled	60%
Childhood trauma questionnaire (CTQ total)	Mean [SD] (Range)	40.5 [15.4] (25 - 93)
Traumatic events inventory (TEI)	Mean [SD] (Range)	4.2 [2.3] (0 - 10.7)

Legend: [standard deviation], (range).

Table 2. Adverse social exposures (ASEs) predict hemisphere-mean fronto-limbic brain morphometry

	Low household income				Low educational attainment				Childhood trauma burden				Adult trauma burden			
	β	SE	T	P	β	SE	T	P	β	SE	T	P	β	SE	T	P
Hipp. Volume (mm3)	-0.425	0.267	-1.590	0.116	0.005	0.279	0.019	0.985	0.030	0.115	0.260	0.796	-0.167	0.101	-1.644	0.104
Amyg. Volume (mm3)	-0.649	0.283	-2.299	0.024*	0.091	0.294	0.310	0.757	0.145	0.118	1.228	0.223	-0.146	0.106	-1.380	0.171
FP SA (mm2)	0.556	0.285	1.950	0.055	-0.065	0.294	-0.221	0.826	-0.004	0.114	-0.037	0.971	0.109	0.107	1.017	0.312
Medial OFC SA (mm2)	0.271	0.293	0.926	0.357	-0.374	0.302	-1.237	0.220	-0.052	0.117	-0.440	0.661	-0.112	0.110	-1.021	0.310
Lateral OFC SA (mm2)	-0.033	0.298	-0.111	0.912	-0.244	0.307	-0.796	0.428	-0.211	0.119	-1.769	0.081	-0.093	0.111	-0.834	0.407
SFG SA (mm2)	0.386	0.289	1.336	0.185	-0.435	0.298	-1.460	0.148	-0.223	0.116	-1.926	0.058	0.039	0.108	0.358	0.721
RMFG SA (mm2)	-0.050	0.278	-0.181	0.857	-0.543	0.286	-1.897	0.061	-0.231	0.111	-2.079	0.041*	0.036	0.104	0.346	0.730
Rostral ACC SA (mm2)	-0.176	0.291	-0.602	0.549	-0.511	0.301	-1.700	0.093	-0.229	0.117	-1.961	0.053	0.029	0.109	0.268	0.790
Caudal ACC SA (mm2)	0.121	0.272	0.443	0.659	-0.855	0.280	-3.049	0.003**	-0.110	0.109	-1.006	0.317	0.044	0.102	0.436	0.664
FP CT (mm)	-0.137	0.293	-0.468	0.641	0.684	0.302	2.267	0.026*	-0.079	0.117	-0.671	0.504	-0.120	0.110	-1.098	0.275
Medial OFC CT (mm)	-0.002	0.293	-0.008	0.993	0.346	0.315	1.099	0.275	0.138	0.118	1.172	0.244	0.022	0.111	0.202	0.840
Lateral OFC CT (mm)	-0.023	0.304	-0.074	0.941	0.241	0.314	0.769	0.444	0.055	0.122	0.451	0.653	-0.112	0.114	-0.983	0.328
SFG CT (mm)	-0.088	0.248	-0.356	0.723	0.553	0.256	2.162	0.034*	0.070	0.100	0.699	0.487	-0.170	0.093	-1.831	0.071
RMFG CT (mm)	-0.078	0.281	-0.276	0.783	0.443	0.290	1.530	0.130	0.072	0.113	0.637	0.526	-0.112	0.105	-1.062	0.291
Rostral ACC CT (mm)	-0.063	0.297	-0.211	0.833	0.060	0.318	0.188	0.851	0.019	0.118	0.163	0.871	-0.034	0.112	-0.305	0.761
Caudal ACC CT (mm)	-0.124	0.296	-0.419	0.676	0.187	0.318	0.589	0.558	0.046	0.119	0.388	0.699	0.004	0.112	0.039	0.969

*** P < 0.001, **P < 0.01, * P < 0.05, **P: BH significant (bold)**

Both dependent and independent variables were scaled prior to analysis. Childhood trauma burden was measured with the Childhood Trauma Questionnaire, and adult trauma burden was measured with the Traumatic Events Inventory. In separate linear models including hippocampus and amygdala volumes as dependent variables, income, education, CTQ total, and TEI were included as independent variables of interest, whereas genomic ancestry, age, employment/disability and ICV were included as covariates. The same independent variables and covariates were included, minus ICV, to predict frontal cortex subregions SA and CT measures.

Abbreviations. Hipp: hippocampus, Amyg: amygdala, FP: frontal pole, mOFC: medial orbito-frontal cortex, IOFC: lateral orbito-frontal cortex, SFG: superior frontal gyrus, RMFG: rostral medial frontal gyrus, rACC: rostral anterior cingulate cortex, cACC: caudal anterior cingulate cortex, SA: surface area, CT: cortical thickness, **β**: scaled beta value, SE: standard error, T: t statistic value.

Table 3. Module eigengenes (MEs) mediating observed adverse social exposure (ASE), fronto-limbic brain morphometry relationships

Maroon: Rostral Mid Frontal Gyrus SA	β	95% CI Lower	95% CI Upper
Average indirect effect (IDE)	-0.466**	-0.952	-0.098
Average direct effect (DE)	-0.517	-1.464	0.407
Average total effect (TE)	-0.983*	-1.959	-0.016
Tan: Rostral Mid Frontal Gyrus SA	β	95% CI Lower	95% CI Upper
Average indirect effect (IDE)	-0.124	-0.448	0.100
Average direct effect (DE)	-0.884	-1.869	0.085
Average total effect (TE)	-1.001*	-1.955	-0.040
White: Rostral Mid Frontal Gyrus SA	β	95% CI Lower	95% CI Upper
Average indirect effect (IDE)	-0.348*	-0.781	-0.026
Average direct effect (DE)	-0.667	-1.590	0.251
Average total effect (TE)	-1.014*	-1.983	-0.069
Yellow: Rostral Mid Frontal Gyrus SA	β	95% CI Lower	95% CI Upper
Average indirect effect (IDE)	-0.128	-0.474	0.142
Average direct effect (DE)	-0.888	-1.908	0.148
Average total effect (TE)	-1.016*	-2.003	-0.019
Lightcoral: Caudal ACC SA	β	95% CI Lower	95% CI Upper
Average indirect effect (IDE)	-0.107	-0.307	0.015
Average direct effect (DE)	-0.737*	-1.296	-0.176
Average total effect (TE)	-0.844**	-1.394	-0.283

*** P < 0.001, ** P < 0.01, * P < 0.05, **P: BH-significant (bolded)**

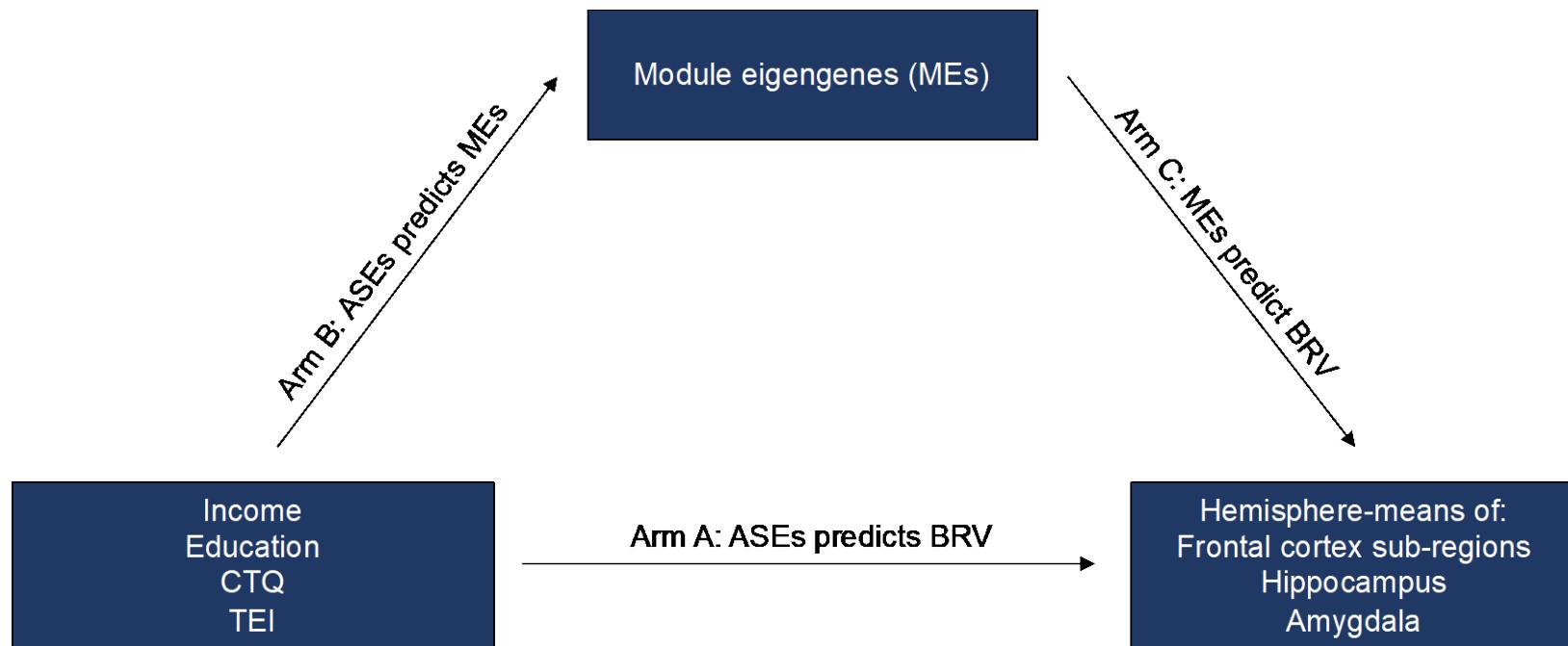
Both dependent and independent variables were scaled prior to analysis. SA: surface area (mm²), CT: cortical thickness (mm), β : coefficient estimate, CI: confidence interval

Table 4. Probe-wise mediation analysis of the relationship between childhood trauma burden (CTQ total) & rostral middle frontal gyrus (RMFG) surface area (SA)

Probe name	Module name	Chr:pos (hg38)	Gene name	IDE β	IDE P	DE β	DE P	TE β	TE P
cg21622733	Maroon	Chr19:35408551	<i>LINC01531</i>	-0.512	0.003**	-0.471	0.320	-0.983	0.044*
cg19805668	Maroon	Chr14:74951972	<i>PGF</i>	-0.411	0.009**	-0.592	0.217	-1.003	0.042*
cg10592766	Maroon	Chr19:50226846	<i>MYH14</i>	-0.321	0.019*	-0.670	0.182	-0.990	0.048*
cg03710029	Maroon	Chr17:81291801	<i>SLC38A10</i>	-0.383	0.025*	-0.613	0.188	-0.996	0.043*
cg15150970	Maroon	Chr2:25250660	<i>DNMT3A</i>	-0.253	0.049*	-0.753	0.125	-1.006	0.040*
cg12219789	Maroon	Chr17:7324180	<i>NEURL4</i>	-0.352	0.049*	-0.622	0.183	-0.974	0.049*
cg01544227	White	Chr12:8323720	AC092745.5	-0.305	0.036*	-0.689	0.175	-0.994	0.044*
cg00686169	White	Chr8:8024026	<i>FAM90A24P</i>	-0.277	0.043*	-0.740	0.126	-1.017	0.037*
cg08002107	White	Chr8:12135255	<i>USP17L7</i>	-0.268	0.047*	-0.738	0.122	-1.006	0.040*

*** P < 0.001, **P < 0.01, * P < 0.05, P: BH-significant (bold)

Both dependent and independent variables were scaled prior to analysis. IDE: indirect effect, DE: direct effect, TE: total effect, β : coefficient estimate, P: nominal p-value, BH: Benjamini-Hochberg



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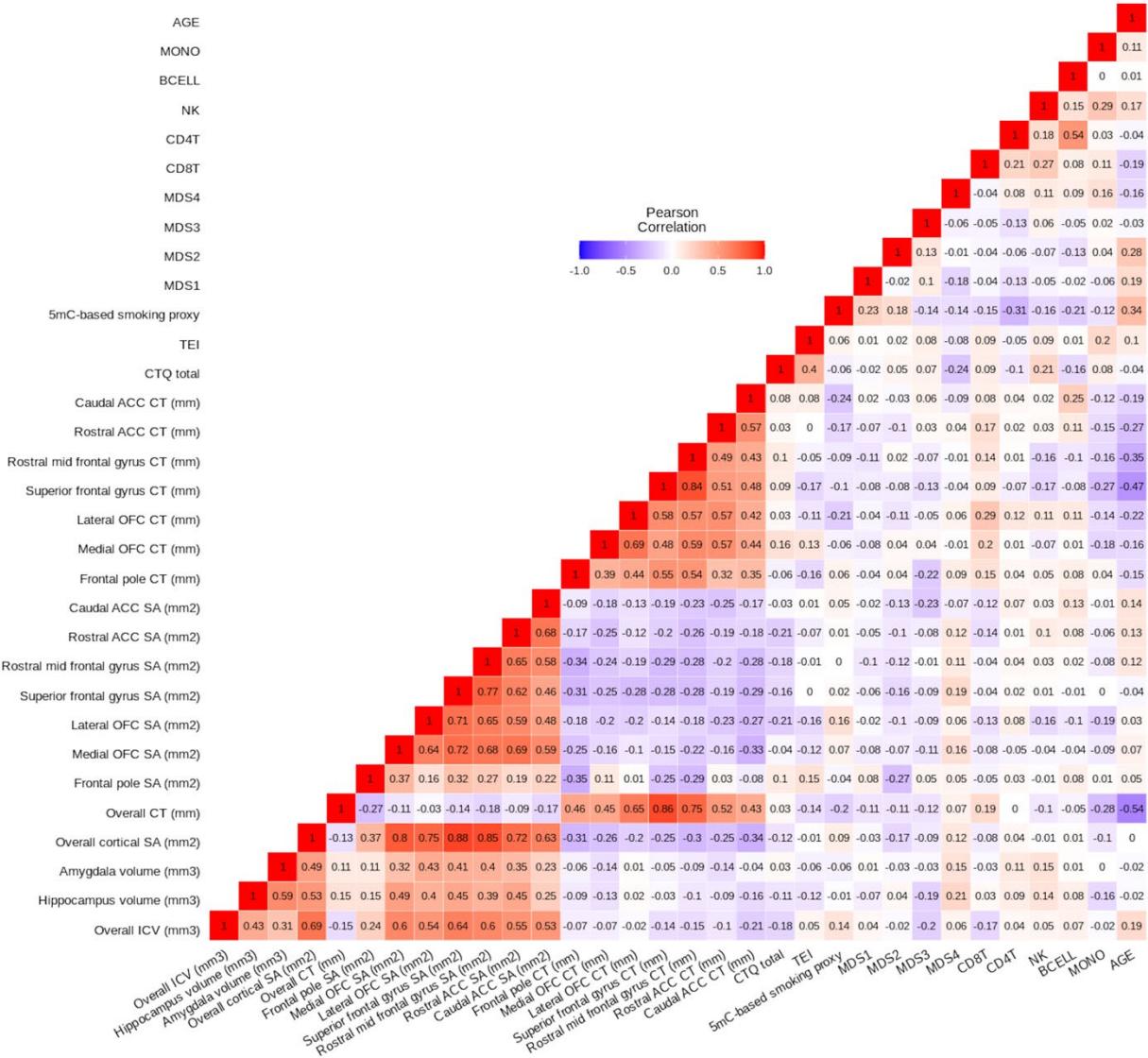
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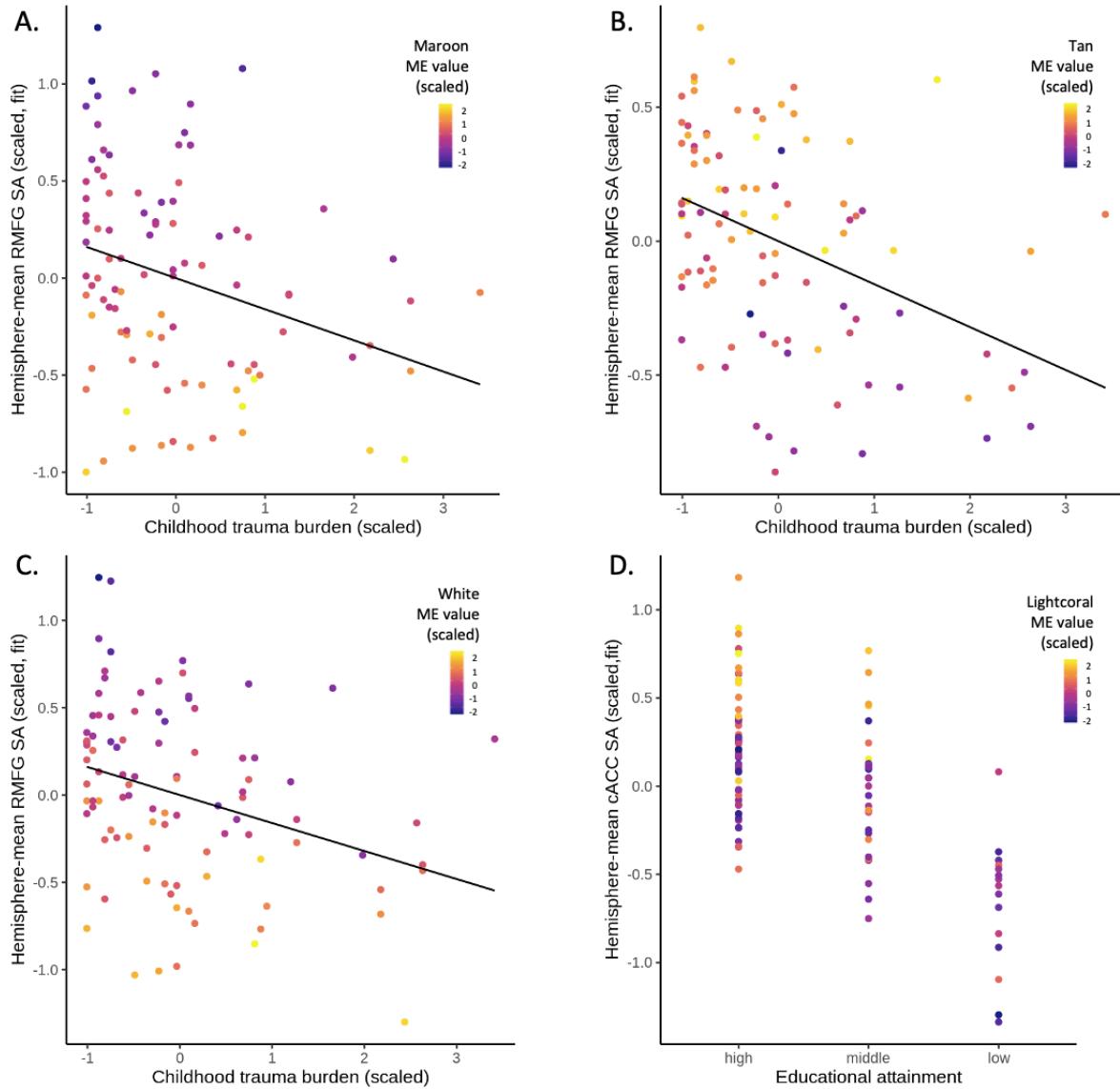
Figure 1. Conceptual model testing individual module eigengenes (MEs) as mediators of the hypothesized associations between adverse social exposures (ASEs) and variability in fronto-limbic brain morphometries. Arm A. Analyses included hemisphere-mean hippocampus and amygdala volumes as dependent variables (in separate models). Income, education, CTQ total, and TEI were included as independent variables of interest, whereas genomic ancestry, age, employment/disability and ICV were included as covariates. The same predictors and covariates were included, minus ICV, to predict frontal cortex subregions SA and CT measures. Arm B. Analyses included 5mC MEs as dependent variables (in separate models). Income, education, CTQ total, and TEI were included as independent variables of interest, and employment/disability was included as a covariate. Arm C. Analyses included individual fronto-limbic morphometry measures as dependent variables (in separate models). Individual 5mC MEs were included as independent variables of interest, whereas age, genomic ancestry, employment, and ICV (ICV was only included in hippocampus and amygdala models) were included as covariates.



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Figure 2. Pearson's correlation heatmap of variables used throughout the current study. Age is negatively associated with multiple frontal cortex cortical thickness (CT) measures (Pearson's correlation $r_{range} = -0.15$ to -0.47 , $p_{range} = 0.092$ to 6×10^{-7}). Within frontal cortex CT measures, multiple subregions show positive correlations with one another (Pearson's correlation $r_{range} = 0.32$ to 0.84 , $p_{range} = 0.004$ to $< 2 \times 10^{-16}$). The same phenomenon is observed within frontal cortex surface area (SA) measures (Pearson's correlation $r_{range} = 0.16$ to 0.77 , $p_{range} = 0.210$ to $< 2 \times 10^{-16}$). Overall CT is positively correlated with each of the frontal cortex CT measures (Pearson's correlation $r_{range} = 0.43$ to 0.86 , $p_{range} = 1 \times 10^{-7}$ to $< 2 \times 10^{-16}$), and overall SA is positively correlated with each of the frontal cortex SA measures (Pearson's correlation $r_{range} = 0.37$ to 0.88 , $p_{range} = 5 \times 10^{-4}$ to $< 2 \times 10^{-16}$).

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Figure 3. CTQ total is negatively associated with rostral middle frontal gyrus (RMFG) surface area (SA). Low educational attainment status is associated with low caudal anterior cingulate cortex (cACC) SA. Out of seven CTQ-associated module eigengenes (MEs), the three strongest relationships with hemisphere-mean RMFG SA were the Maroon (A), White (B), and Tan (C) MEs. Out of two low education attainment-associated MEs, the strongest relationship with hemisphere-mean cACC SA was the Lightcoral ME (D). As shown, neuroimaging measures are adjusted by age, ME value, genomic ancestry, employment/disability status, and an adverse social exposure (ASE). Dependent and independent variables were scaled. A. CTQ total was positively associated with the Maroon ME ($\beta = 0.289$, SE = 0.109, $t = 2.639$, $p = 9.8 \times 10^{-3}$). In turn, the Maroon ME was negatively associated with RMFG SA ($\beta = -0.451$, SE = 0.091, $t = -4.966$, $p = 3 \times 10^{-6}$). B. CTQ total was negatively associated with the Tan ME ($\beta = -0.248$, SE = 0.112, $t = -2.220$, $p = 0.029$). The Tan ME was, in turn, positively associated with RMFG SA ($\beta = 0.233$, SE = 0.100, $t = 2.322$, $p = 0.023$). C. CTQ total was positively associated with the White ME ($\beta = 0.236$, SE = 0.110, $t = 2.151$, $p = 0.034$). In turn, the White ME was negatively associated with RMFG SA ($\beta = -0.392$, SE = 0.093, $t = -4.231$, $p = 6 \times 10^{-5}$). D. Low educational attainment (no high school certificate) was associated with the Lightcoral ME ($\beta = -0.615$, SE = 0.296, $t = -2.079$, $p = 0.040$). The Lightcoral ME, in turn, was positively associated with cACC SA ($\beta = 0.215$, SE = 0.099, $t = 2.182$, $p = 0.032$).

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