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Epigenetic prediction of 17β-estradiol and relationship to trauma-related outcomes in women

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ABSTRACT

17β-estradiol (E2) levels in women correlate with multiple neuropsychiatric symptoms, including those that are stress-related. Furthermore, prior work from our group has demonstrated that E2 status influences DNA methylation (DNAm) across the genome. We developed and validated a DNAm-based predictor of E2 (one of four naturally occurring estrogens) using a training set of 183 females and a test set of 79 females from the same traumatized cohort. We showed that predicted E2 levels were highly correlated with measured E2 concentrations in our testing set ($r = 0.75$, $p = 1.8e\text{-}15$). We further demonstrated that predicted E2 concentrations, in combination with measured values, negatively correlated with current post-traumatic stress disorder (PTSD) ($\beta = -0.38$, $p = 0.01$) and major depressive disorder (MDD) diagnoses ($\beta = -0.45$, $p = 0.02$), as well as a continuous measure of PTSD symptom severity ($\beta = -2.3$, $p = 0.007$) in females. Finally, we tested our predictor in an independent data set ($n = 85$) also comprised of recently traumatized female subjects to determine if the predictor would generalize to a different population than the one on which it was developed. We found that the correlation between predicted and actual E2 concentrations in the external validation data set was also high ($r = 0.48$, $p = 3.0e\text{-}6$). While further validation is warranted, a DNAm predictor of E2 concentrations will advance our understanding of hormone-epigenetic interactions. Furthermore, such a DNAm predictor may serve as an epigenetic proxy for E2 concentrations and thus provide an important biomarker to better evaluate the contribution of E2 to current and potentially future psychiatric symptoms in samples for which E2 is not measured.

1. Introduction

17β-estradiol (E2) concentrations in women are associated with greater risk for various neuropsychiatric symptoms, including depression [1] and changes in memory [2]. Although the mechanisms by which E2 influences vulnerability to psychiatric symptoms are not fully understood, E2 has effects on multiple neurotransmitter systems, and brain circuits involved in mood and memory regulation are sensitive to variation in E2 concentrations [1]. Studies generally suggest that *lower* concentrations of E2 contribute to higher symptomatology and vulnerability to neuropsychiatric disease, supporting a role for E2 as a neurotrophic, neuroprotective, and psychoprotective steroid hormone [3]. Furthermore, administration of synthetic estrogen to women with low endogenous E2 levels, including those who are in perimenopause, post-menopause, or postpartum, improves affective and cognitive symptoms [4].

While E2 is synthesized and secreted by both sexes, peripheral concentrations are higher overall and fluctuate to a greater extent over the

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course of the menstrual cycle in reproductively-mature women [5]. This fluctuation in E2 is implicated in the higher prevalence, severity, and burden of affective and stressor-related disorders in women as compared to men [3,6]. The relationship between E2 concentrations and stress-related sequelae is a well characterized example of the importance of E2 status on psychiatric symptoms and their underlying neurobiological and molecular substrates. There is evidence that healthy, normally cycling females exposed to a psychosocial stress paradigm have higher subjective distress during periods of low E2 as compared to high E2 [7]. Consistent with this finding, female rats demonstrate higher fear and anxiety-related behaviors during diestrus or metestrous (low E2 phases of their estrous cycle) [8,9], and chronic replacement of E2 in aged, ovariectomized female rats improves anxiety- and depression-like behavior [10]. Similarly, replacement of E2 in ovariectomized female rhesus macaques also improves anxiety-like behavior [11]. These translational findings parallel findings in women showing that low E2 concentrations associate with deficits in fear extinction [12] and inhibition of learned fear responses [13] in both healthy women and those with post-traumatic stress disorder (PTSD). Furthermore, trauma-exposed women experience greater severity of phobic anxiety during a phase of their menstrual cycle characterized by lower E2 (early follicular) as compared to higher E2 (mid luteal) concentrations and this difference is driven by women with PTSD [14]. On a neurobiological level, stress response neural circuitry is more active in women during the low E2, early follicular phase as compared to the high E2, late follicular/midcycle phase [15]. It is important to also note that change in E2 levels has also been found to disrupt affect, although the exact mechanism by which this occurs is unknown [16].

Overall, preclinical and clinical data indicate that E2 status impacts vulnerability to and presentation of psychiatric symptoms in women and highlights the importance of assessing E2 status in studies characterizing female risk for stress- and trauma-related adverse outcomes. However, most studies assessing psychiatric symptoms do not measure E2 concentrations, but an increasing number of studies have genome-wide methylation data available. Because E2 concentrations correlate with DNA methylation (DNAm) in women measured within the same menstrual cycle [17], DNAm signatures may serve as a surrogate of endogenous E2 concentrations within a cycle and potentially across cycles. We note there is conflicting evidence regarding the degree of intra-individual stability of steroid hormones across menstrual cycles. For example [18], showed that E2 and progesterone (P4) levels derived from saliva were stable across two consecutive cycles and [19] demonstrated stability of urinary metabolites of E2 and P4, while there was less consistency in these salivary measures in a study by Ref. [20]. While the literature suggests that there is a relationship between E2 levels and stress- and trauma-related symptoms within a particular menstrual cycle and further studies are necessary to assess the degree to which E2 levels from one cycle can predict symptoms in another cycle, the goals of the present study were to develop a methylation-based predictor of E2 concentrations using epigenome-wide data and to assess the relationship of predicted E2 levels with the presence of current PTSD and MDD diagnoses, and PTSD symptom severity in women. We hypothesized that a DNAm-based prediction of low E2 status would be associated with greater risk for current PTSD and MDD diagnosis and greater PTSD symptom severity in traumatized women.

2. Materials and methods

2.1. DNAm predictor development in women (GTP)

We developed our DNAm-based E2 predictor using data collected as part of the Grady Trauma Project (GTP), a civilian study of trauma exposure in a predominately African American population from a large urban hospital in Atlanta, GA, USA [21]. Briefly, subjects were approached in the waiting rooms of primary care and OBGYN clinics as well as at the hospital outpatient pharmacy. Exclusion criteria included being under age 18, actively psychotic, or having intellectual disability. Participants underwent a screening interview based on screening forms and scales that were read aloud by a volunteer or project staff member, due to the varying literacy of the subjects. Randomly selected subjects were invited to further participate in a clinician-administered structured interview to assess the presence or absence of psychiatric diagnoses on a different day [21]. The overall project was approved by the Institutional Review Board at Emory University and the Grady Health Systems Research Oversight Committee. All participants signed informed consent prior to the start of the screening interview.

In the present study, we used a continuous measure of PTSD symptom severity obtained from the Clinician-Administered PTSD scale (CAPS) [22] for DSM-IV in a subset of participants during an earlier recruitment period and DSM-5 in a later recruitment period. The CAPS is a 30-item self-report scale with good psychometric properties across clinical populations and research settings [23]. Current PTSD diagnosis was based on the CAPS. Presence of each of the 17 DSM-IV or 20 DSM-5 diagnostic criteria for PTSD was determined using a frequency rating of 1 or higher paired with an intensity score of 2 or higher. Current MDD diagnosis was determined using the Structured Clinical Interview for DSM-IV [24].

2.1.1. Blood-based assays in GTP

Study participants provided whole blood in EDTA tubes for biological assays, and serum collected and frozen at −80 °C until time of hormone assay. Total E2 concentrations were assayed at Yerkes Biomarkers Core Laboratory at Emory University using a commercially available radioimmunoassay kit (KE2D1; Siemens Healthcare Diagnostics) as previously described [17]. E2 concentrations were natural log transformed due to non-normal distributions.

DNA methylation was assessed using the HumanMethylation450 BeadChip (Illumina) as described in previous work [25]. Briefly, 1 μg of DNA underwent bisulfite treatment and the >485,000 probes on the array were interrogated for methylation status. Beta values were generated with BeadStudio and set to missing (no call) if detection p-values exceeded 0.001. CpGassoc [26] was used to exclude samples with probe detection call rates <95% and those with an average intensity value of either <50% of the experiment-wide sample mean or <2,000 arbitrary units. In addition, CpG sites with missing data for >10% of samples and probes that cross-hybridize between autosomes and sex chromosomes were removed [27]. Beta Mixture Quantile dilation was utilized to normalize each dataset [28]. The method described by Houseman and colleagues was used to estimate the proportion of granulocytes and lymphocytes in our whole blood DNA samples [29,30].

After quality control steps, we limited probes to transcripts expressed in the blood to correspond with our previous analysis assessing the relationship between E2 concentrations and epigenetic data [17], leaving 87,388 probes corresponding to 15,877 transcripts. To maintain our predictor's compatibility with the Infinium MethylationEPIC BeadChip (Illumina), we further limited the number of probes to those that are overlapping between both arrays (n = 81,637). Additionally, due to variability of correlation of probes between the two chips, we chose to restrict the analysis to a subset of CpGs that correlated at $r > 0.2$ [31] in order to have a sufficient number of starting probes that were at least weakly correlated on both chips (n = 27,335). After excluding probes that had any missingness for 262 female subjects with E2 data, we were left with 23,209 probes to enter into the analysis. Finally, we randomly divided the subjects into training (n = 183, 80%) and testing (n = 79, 20%) sets.

2.1.2. Predictor selection and model building

To select a parsimonious set of predictors, we entered age, cellular proportions (CD4, CD8, CD14, CD19, CD56), and CpGs (n = 23,209) from female participants in our training set into an elastic net regression algorithm using log-transformed E2 concentrations as the outcome. We set the elastic net mixing parameter, alpha, to 0.5 to allow for equal contribution of the ridge and LASSO methods [32], as this has performed

well previously for developing DNAm-based predictors of age in adults [33] and gestational age in neonates [34]. We then used the features identified by the elastic net algorithm to train a random forest (RF) model, as RFs have demonstrated good performance in high-dimensional data in which there are many more predictors than respondents, and they can model non-linear relationships between variables [35]. RFs are a recursive partitioning method in which predictors are divided into decision trees through splits from a parent to child nodes using a random subset of predictors [36]. We used 500 trees and predictors selected at each split. While RFs can be used for feature selection, we elected to use elastic net for this task due to the computational intensity of using RFs for feature selection. The use of a regularized regression method for feature selection and a tree-based algorithm for predictive model building has demonstrated good performance in a prior study in a psychiatric sample [37]. Analyses were performed using R with the packages glmnet and randomForest.

2.2. External DNAm validation sample

We used data from the prospective Grady Predictive Biomarkers Emergency Department (ED) study to externally validate our DNAm-based E2 predictor in women (n = 85). Subjects presenting to the emergency room at Grady Memorial Hospital were recruited to participate if they had experienced a trauma within the past 72 hours and met DSM-IV diagnostic criterion A for PTSD [38]. Exclusion criteria included being <18, having a current episode or past history of mania, schizophrenia, or other psychoses, endorsing prominent suicidal ideation within the past month (having frequent and/or intense thoughts about killing oneself), and experiencing a loss of consciousness for more than five minutes as a result of the traumatic event. Individuals were also excluded if they were currently intoxicated, not alert, oriented, or coherent, or were in active labor, respiratory distress, or hemodynamically compromised. Overall, this ED sample was more ethnically diverse than the GTP cohort [39]. The overall project was also approved by the Institutional Review Board at Emory University and the Grady Health Systems Research Oversight Committee.

2.2.1. Blood-based assays in ED sample

Venous blood samples were collected from participants in the ED following trauma exposure by medical staff using standard techniques [40]. Within six hours of collection, EDTA tubes were centrifuged at 4 °C and plasma was frozen at −80 °C until time of hormone assay. Total E2 concentrations were measured using a commercially available radioimmunoassay kit (DiaMetra) with an inter-assay coefficient of variance (CV) of 2.8% and intra-assay CV of <9%. To help determine how specific the predictor is to E2 concentrations and not other commonly-assessed steroid hormones, we assessed the correlations of the DNAm-based values to the total measured values of three other steroid hormones, including progesterone (P4), cortisol, and cortisone. Total progesterone, cortisol, and cortisone concentrations were assayed using liquid-chromatography-mass-spectrometry (LC-MS) using previously validated protocols [41].

DNA methylation data was generated for 48 female subjects with E2 data using the HumanMethylation450 BeadChip (ED 450K), while the same data was generated for 37 additional subjects with E2 data using the Infinium MethylationEPIC BeadChip (ED EPIC) with the protocol described above. Methylation data for these 85 subjects were combined. Missing CpGs were imputed using k-nearest neighbors in the R package impute using the average values of CpGs in the GTP training cohort as the gold standard.

2.3. Correlation of E2 DNAm with stress-related outcomes

We determined whether the DNAm-based E2 concentrations correlated with stress-related phenotypes in the GTP cohort of women using regression analyses. We assessed the relationship between predicted E2 levels and stress-related outcomes, including current PTSD and MDD diagnoses and PTSD symptom severity, collected on the same day as blood sample collection for the methylation data. In addition to the 79 subjects from the testing set, we also generated DNAm-based E2 levels for an additional 40 subjects with DNA methylation data but no measured estradiol levels (total n = 119). Of all subjects with predicted E2 concentrations, 99 had stress-related outcome measures, including current PTSD and MDD diagnoses and CAPS severity score. We entered age and whether the subjects had measured or predicted E2 levels as covariates. Supplementary Figure 1 shows a CONSORT diagram illustrating the number of GTP subjects in the different analyses.

3. Results

3.1. Predictor development sample (GTP)

3.1.1. Descriptive results for GTP females

Table 1 shows the sociodemographic and hormonal characteristics for GTP females. Age for the total sample of 262 females ranged from 18 to 77 with a mean of 40.3 years old. The range of E2 concentrations in the total GTP sample was 2.85–546 pg/mL with a geometric mean of 29.6 pg/mL, representing women during childbearing years, pregnancy, and menopause. The geometric mean values for measured E2 in the training and testing sets were 31.9 pg/mL and 24.8 pg/mL, respectively. Supplementary Figure 2 shows the distributions of measured E2 in the training (A) and testing (B) data sets, which were comparable (p = 0.22). Of the 262 total women in the GTP sample, 215 had data for stress-related outcomes, including 150 females in the training set and 65 females in the testing set. 34 (23%) and 17 (11%) subjects in the training set met criteria for current PTSD and MDD, while 13 (20%) and 10 (15%) in the testing set met criteria for these diagnoses, respectively (p's > 0.05).

3.1.2. Model performance in GTP females

Elastic net regression selected 35 CpG sites (Supplementary Table 1) and age as predictive of E2 concentrations. The sites were distributed throughout the genome and were located in gene bodies, 5′ and 3' UTRs, and transcriptional start sites of genes. There was no enrichment of CpGs in islands, shores, shelves, or enhancers. 86% of the CpG sites selected by the elastic net algorithm were CpGs associated with E2 concentrations (FDR< 5%) in our previous epigenome-wide study (p = 1.0e-10) [17]. We entered the 35 CpG sites and age selected by elastic net into a RF algorithm to generate the predictor. The correlation between the predicted E2 concentrations generated by the RF model and the measured concentrations was 0.98 (p < 2.2e-16) in the training data, indicating

Table 1
Sociodemographic and hormonal characteristics in the Grady Trauma Project (GTP).

	Total female GTP sample	Training	Testing
Number of subjects	262	183	79
Age (years), mean (SD), range	40.3 (13.3), 18-77	40.1 (13.4), 18-77	40.7 (13.1), 18-70
Measured E2 (pg/mL), geometric mean, range	29.6, 2.85–546	31.9, 2.85–546	24.8, 2.85–546
Measured Childbearing E2, n (%), 30–400 pg/mL	96 (37%)	71 (39%)	25 (32%)
Measured Pregnancy E2, n (%), >400 pg/mL	26 (10%)	18 (10%)	8 (10%)
Measured Postmenopausal E2, n (%), <30 pg/mL	140 (53%)	94 (51%)	46 (58%)
Current PTSD, n (%)	47 (21%), total = 215	34 (23%), total = 150	13 (20%), total = 65
Current MDD, n (%)	27 (13%), total = 215	17 (11%), total = 150	10 (15%), total = 65
CAPS Severity, mean (SD), range	15.8 (14.7), 0–68.2	14.8 (14.7), 4.1–55.9	18.0 (14.7), 0–68.2

strong model fit (Fig. 1A). In the GTP testing data, the correlation between the measured and DNAm predicted E2 concentrations was 0.75 (p = 1.8e-15; Fig. 1B), also indicating a strong fit.

3.2. External validation of DNAm E2 predictor in ED study

Table 2 shows the sociodemographic and hormonal characteristics for the ED data. The range of measured E2 concentrations in the total sample of 85 females was 13.3–2511.4 pg/mL with a geometric mean of 54.2 pg/mL (distribution shown in Supplementary Figure 2C). Age for these subjects ranged from 18 to 63 years old with a mean of 34.8 years old. We found a correlation of 0.48 (p = 3.0e-6) between measured E2 concentrations and those predicted from the RF model generated from GTP data (Fig. 3). The correlation may have been lower in this external validation data set as compared to the GTP testing set due to differences in when the epigenetic data was obtained and the fact that the subjects in the ED study had recently experienced a trauma. A sensitivity analysis excluding the subjects with high E2 and who were likely pregnant (E2 levels > 400 pg/mL) remained significant, although with a lower effect size (r = 0.23, p = 0.04). We were also able to perform a sensitivity analysis for body mass index (BMI) in this cohort, and, using a partial Pearson correlation that corrected for BMI, noted comparable correlation between measured and predicted E2 (r = 0.49, p = 2.3e-06).

Next, we assessed the correlations between model-generated values and three other measured hormones, including progesterone, cortisone, and cortisol to determine the specificity of the predictor. Table 2 shows ranges and geometric means for measured progesterone, cortisone, and cortisol in ng/mL. As shown in Table 3, the correlation between measured E2 and measured progesterone was strong and significant, whereas the correlations between measured E2 and the other measured hormones were weaker (cortisone) or non-significant (cortisol). Consistent with these correlations, the predicted E2 concentrations correlated highly with measured progesterone concentrations (r = 0.52, p = 4.0e-7), but not with measured cortisone (r = 0.11, p = 0.28) or cortisol (r = −0.03, p = 0.72) concentrations.

3.3. Correlation of DNAm predictions with stress-related phenotypes in GTP women

When we examined the association between predicted E2 and stress-related outcomes in 99 GTP females with phenotypic data (24% PTSD) using age as a covariate, there were negative associations with all three stress-related outcomes but they were not significant. A power calculation using a standard power level of 0.8, an alpha of 0.05, and two predictors (E2 concentration and age) indicated that the sample of subjects with predicted E2 concentrations (n = 99) would be insufficient to detect effect sizes in the small range (Cohen's $f^2 < 0.10$) in regression analyses [42]. Thus, we assessed the combination of both predicted E2 (n = 99) and measured E2 (n = 150) concentrations in a total of 249 GTP females with age and type of E2 (predicted or measured) as covariates. These analyses showed significant, negative relationships between E2 values and both current PTSD (β = −0.38, p = 0.01) (Fig. 2A), current MDD (β = −0.45, p = 0.02) (2B) and CAPS severity (β = −2.3, p = 0.007) (2C), showing that the low E2 DNAm levels are associated with greater stress-related outcomes.

4. Discussion

DNA methylation has been used for prediction of several traits, including age [33], gestational age [34], cancer diagnosis and prognosis [43], and smoking status [44]. To our knowledge, the current study is the first to predict E2 concentrations using epigenome-wide methylation data and relate these E2 predictions to stress-related phenotypes in women. Our DNAm-based E2 predictor generated values with strong correlations between measured E2 concentrations in both a holdout testing set and an independent validation cohort of traumatized women. Among the 35 CpGs that can be used to impute E2 concentrations, many associate independently with E2 concentrations in a previous study [17]. For example, cg02187522 is located within *arginine vasopressin*, which encodes a product that is cleaved into arginine vasopressin (AVP). Our finding that higher E2 was associated with lower DNA methylation within *AVP* is consistent with data showing that E2 increases AVP expression [45]. Consistent with the correlation between measured levels of E2 and progesterone, our model's predicted values were highly correlated with both E2 and progesterone. As such, our DNAm predictor may more broadly reflect menstrual phase or more general reproductive status in women, though this study did not have sufficient information to evaluate that possibility.

In the current study, we found that predicted E2 levels correlated negatively with current PTSD diagnosis and PTSD symptom severity in women. Consistent with the present results, both human and animal studies have generally found greater severity of psychological symptoms [14] or fear-related behaviors [8,9,46] during assessment if a female was in a low E2 phase of her cycle. However, one study has found that women with PTSD in the high E2 (luteal) phase had higher levels of a particular PTSD symptom - flashbacks [47]. Furthermore, low E2 concentrations are associated with deficits in fear extinction [12] and inhibition of learned fear responses in women [13]. One possible application for this E2 DNAm predictor is to determine E2 status in publicly available data sets for which epigenome-wide methylation and psychiatric measures

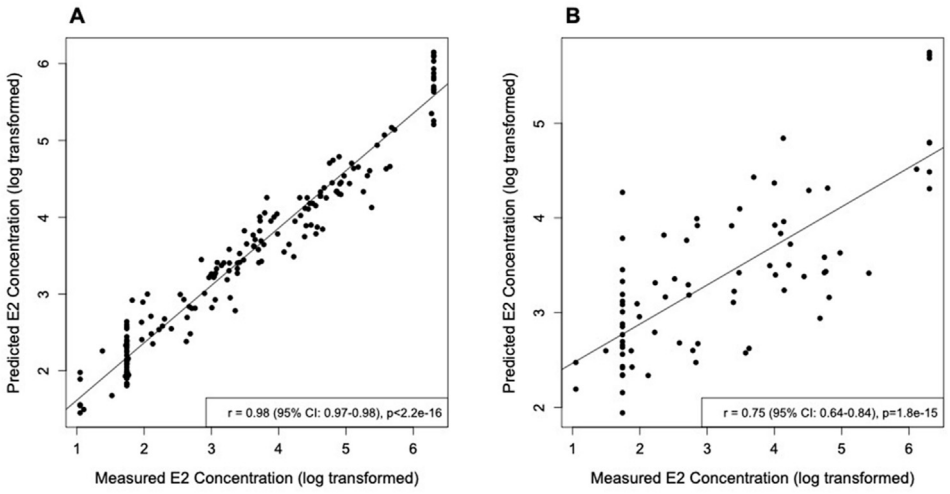

Fig. 1. Correlation between predicted and measured E2 concentrations in (A) GTP training (n = 183) and (B) GTP testing (n = 79) data.

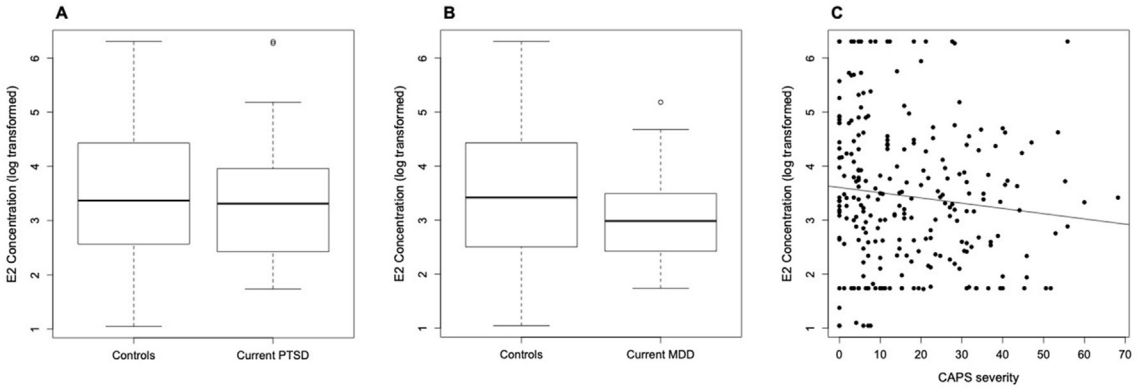

Fig. 2. The combination of predicted (n = 99) and measured (n = 150) E2 concentrations for 249 female GTP subjects were lower in those with (A) current PTSD (p = 0.01) and (B) current MDD (p = 0.02) then those without those diagnoses after correction for covariates. There was also a negative relationship between (C) CAPS severity and the combination of predicted and measured E2 concentrations (p = 0.007) after correction for covariates.

Table 2
Sociodemographic and hormonal characteristics in Grady Predictive Biomarkers ED Study.

	Total female ED sample	ED 450K	ED EPIC
Number of subjects	85	48	37
Age (years), mean (SD), range	34.8 (13.6), 18-63	32.6 (14.0), 18-63	37.5 (12.7), 19-60
Measured E2 (pg/mL), geometric mean, range	54.2, 13.3–2511.4	50.6, 13.3–2511.4	59.2, 21.2–2215.9
Childbearing E2, N (%), 30–400 pg/mL	34 (40%)	22 (46%)	25 (68%)
Pregnancy E2, N (%), >400 pg/mL	4 (5%)	3 (6%)	1 (2%)
Postmenopausal E2, N (%), <30 pg/mL	47 (55%)	23 (48%)	11 (30%)
Measured progesterone (ng/mL), geometric mean, range	0.7, 0.029–163	0.7, 0.029–163	1.2, 0.7–88.8
Measured cortisone (ng/mL), geometric mean, range	26.3, 3.5–56.0	28.9, 3.5–56.0	23.2, 8.5–44.4
Measured cortisol (ng/mL), geometric mean, range	220.8, 35.4–832.0	280.3, 35.4–832.0	159.2, 36.4–630.5

but no E2 assay data exist. DNAm-based predictions of E2 may serve as a biomarker to further assess the relationship between hormone status and psychiatric symptoms. Future studies will be necessary to characterize the degree to which this E2 DNAm predictor changes longitudinally through different menstrual and reproductive phases.

One limitation of the current work is that we used blood-based DNAm data to develop our predictor, and we may have obtained different results if we had used methylation data from other human tissues or cell types. Thus, our predictor will need to be evaluated in different tissues and compartments to assess specificity. Future studies will need to determine whether the model accurately predicts E2 concentrations in urine and saliva, as some research groups have epigenetic data from these sources. Furthermore, our DNAm predictor was modeled on total E2 concentrations, and not free levels of E2. A further limitation is that the predicted range of E2 concentrations in both the holdout testing set and independent validation sets was narrower than the measured values. The limited range of the predicted values restricts the ability of our model to precisely characterize women according to clinical classifications such as childbearing, pregnant, or postmenopausal [40,48]. Similarly, because many of these steroid hormones are correlated, this method does not specifically discriminate between them. As additional samples and data become available, we hope that it can be further refined. An additional limitation is that the ED validation cohort used plasma samples for steroid hormone testing while GTP measures were assayed from serum.

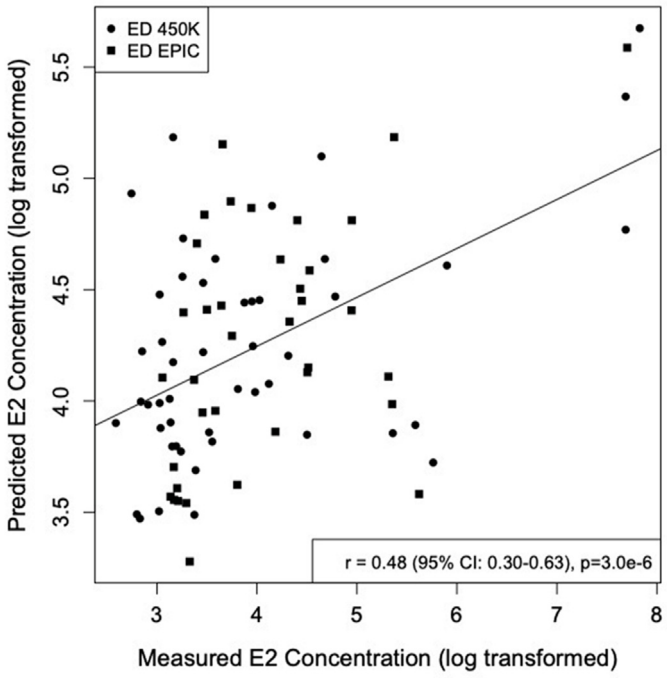

Fig. 3. Correlation between predicted and measured E2 concentrations in the ED Study. Circles show subjects (ED 450K, n = 48) whose methylation data was generated using the HumanMethylation450 BeadChip and squares represent subjects (ED EPIC, n = 37) that were run on the MethylationEPIC BeadChip.

Table 3
Correlations between measured hormone levels in the ED Study. Correlation coefficients (r) are provided for all pairwise comparisons. Significant correlations (<2.2e-16 < p < 9.8e-3) are indicated by bold text.

	E2	Cortisol	Cortisone	Progesterone
E2 (pg/mL)	–	0.15	**0.29**	**0.93**
Cortisol	0.15	–	**0.39**	0.15
Cortisone	**0.29**	**0.39**	–	**0.28**
Progesterone	**0.93**	0.15	**0.28**	–

Number of subjects with data for each hormone level: E2 (n = 85), cortisol (n = 83), cortisone (n = 83), progesterone (n = 84).

While this difference in biological compartment could introduce variability in our predictor, recent data indicates that serum and plasma concentrations of E2 in women are comparable [48]. Additionally,

different radioimmunoassays (KE2D1; Siemens Healthcare Diagnostics vs DiaMetra) were used for the ED and GTP cohorts with the potential for systematic differences in the E2 measurements between the two samples. Future incorporation of additional clinical variables may help to better align the predicted values with measured E2 concentrations. However, we chose to optimize the model for use with minimal demographic data to maximize its potential for use in research studies. Furthermore, the elastic net method we employed chooses a parsimonious subset of features (i.e., CpG sites), selecting only one among highly correlated sites, which introduces an element of chance into CpG selection. Finally, our DNAm predictor will need to be validated in different ethnicities, as methylation of CpG sites can differ by race or ethnicity [49], as well as tested in subjects less than 18 years old. Despite these limitations, this methylation risk score offers the opportunity for researchers to leverage epigenetic data as a surrogate marker of E2 concentrations to evaluate the influence of E2 status on risk and symptom severity of neuropsychiatric conditions and beyond.

Author contributions

LMH conducted all analysis and wrote the initial draft with AKS and VM. TJ, VM, and KJR collected the data and provided clinical expertise for the cohorts. SN and AKS generated methylation data contributed in the quality control of the biological assays. LMH, SN, VK, and AKK conducted the statistical analysis. SAM, AVS, and VM provided critical feedback regarding hormone assays and interpretation of results. All authors reviewed and edited the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2021.100045.

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