Clinical Epigenetics

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Maternal epigenetic index links early neglect to later neglectful care and other psychopathological, cognitive, and bonding effects

Inmaculada León[1,2], Daylín Góngora[3,4], María José Rodrigo[1,2], Silvia Herrero-Roldán[1,5*], Maykel López Rodríguez[6], Colter Mitchell[7], Jonah Fisher[7] and Yasser Iturria-Medina[8,9,10]

Abstract

Background Past experiences of maltreatment and life adversity induce DNA methylation changes in adults, but less is known about their impact on mothers' maladaptive neglectful parenting and its negative effects. We performed an epigenome-wide association study to investigate the role of DNA methylation levels in mothers with neglectful care, who were exposed to childhood maltreatment and neglect, and their current negative effects. Saliva DNA methylation was determined with the Illumina Human Methylation EPIC BeadChip v1. The individual epigenome was the input to a machine learning algorithm for trajectory inference, which assigned a specific state to each mother in the progression from healthy controls to the extreme neglect condition. A compound epigenetic maternal neglect score (EMN) was derived from 138 mothers ($n = 51$ in the neglectful group; $n = 87$ in the control non-neglectful group) having young children. Differential methylation between groups was utilized to derive the EMNs adjusted for education level, age, experimental variables, and blood cell types in saliva samples.

Results Structural equation modeling: X^2 (29) = 37.81; $p = 0.127$; RMSEA = 0.048, confirmed that EMNs link their early experience of physical neglect to current reports of psychopathological symptoms, lower cognitive status, and observed poor mother–child emotional availability. A third of the genes annotated to the CpGs that affect EMNs are related to cognitive impairment and neurodegenerative and psychopathological disorders.

Conclusions EMNs are a novel index to assess the contribution of DNA methylations as a neglected girl to later neglectful caregiving behavior and other negative effects. The evidence provided expands the possibilities for earlier interventions on the neglect condition to prevent and ameliorate the direct or indirect epigenetic impact of maternal adversities on mother–child care, helping to break the cycle of maltreatment.

Keywords DNA methylation, Machine learning, Childhood trauma, Neglectful parenting, Emotional availability

*Correspondence:
Silvia Herrero-Roldán
silvia.herrero@universidadunie.com
Full list of author information is available at the end of the article

Background

Maternal neglect is the most common and severe form of child maltreatment with long-term behavioral, neurobiological, and psychopathological consequences for the child (1–3). Behavioral evidence has shown an intergenerational association between parent and child life experiences of adversity (4). Moreover, mothers with a history of neglect or maltreatment as a daughter and adverse life events are more likely to exhibit neglectful caregiving behavior with their children (5). They exhibit psychopathological vulnerability, early and multiple childbearing, intimate partner violence, often with low educational levels, and residing in economically challenged families (6–8). Additionally, they are more likely to have cognitive impairments indexed by poor cognitive function shown in early adulthood (9). Growing evidence suggests that childhood maltreatment and adverse life events lead to DNA methylation (DNAm) changes (10, 11), establishing a link between life adversity, variations in maternal caregiving quality (12), and also physical and mental health problems (1, 13). Moreover, methylation changes using the PhenoAge clock in neglect contexts reveal increased epigenetic age acceleration in mothers who exhibit neglectful caregiving despite their younger chronological age compared to a control group (14). Similarly, a cumulative score of sexual, emotional, and physical abuse and neglect as a girl was associated with DNAm age acceleration using the Horvath clock in women (15). Finally, DNAm has also been suggested to impact adult susceptibility to complex psychiatric phenotypes (16) and epigenetic-related pathways underlying major mental disorders (17).

While evidence supports the role of DNAm in adverse contexts, the extent to which provides an epigenetic connection between a mother's past adverse experiences and current dysfunctional parenting and consequences remains undefined. To this end, and due to the typical high dimensionality of epigenomic data, compound epigenetic indexes are needed to better explore the antecedent and consequential effects of dysfunctional parenting. Trajectories inference (TI) methods using novel unsupervised machine learning (ML) have emerged as a powerful approach to extract 'progressive' or 'pseudo-temporal' patterns from high-dimensional cross-sectional data (18–20). TI approaches enable the precise identification of biologically defined states within a process of interest and provide an associated quantitative compound index for each individual in a data sample (21). Driven by the need for a better understanding of DNA methylation characteristics in mothers with neglectful care, we apply this novel contrastive TI approach to obtain an individualized compound index of epigenetic maternal neglect (EMN). The advantage of this approach is that it generates a single composite epigenetic score, which helps to ordinate the progression from healthy controls to the extreme neglect condition.

In the first aim, we tested EMNs as an inferential index explaining the role of DNAm as a potential epigenetic link between the mother's past adversity (including childhood physical neglect, other maltreatment, and adverse events) and her current neglectful parenting. According to the hypothesis of behavioral transmission of maltreatment, there is a relationship between having suffered childhood maltreatment and the practice of maltreatment with one's own child (5). Armfield´s et al.'s study provided robust evidence of increased risks of maltreatment among children whose mothers experienced childhood maltreatment, using population-based administrative data of child protection services. As an additional analysis, we performed a comparison between the EMN index with the results of the PhenoAge epigenetic clock obtained in a previous study on mothers with neglectful care (14). In the second aim, we assessed the predictive power of EMNs for both negative and positive outcomes, including psychopathological vulnerability, cognitive status, and the quality of mothers' interactive bonding with their young children, a crucial predictor for secure infant attachment (6, 22, 23). Finally, we tested whether the gene enrichment analysis would show that genes annotated to genomic regions influencing the EMNs are associated with psychological impairments and psychopathological disorders.

Methods

Participants

Recruited from the School Centers, Municipal Social Services, and Primary Health Centers in Tenerife, Spain, 138 mothers (51 neglectful, 87 control) met general inclusion criteria as biological mothers. Their children should not have a history of foster care, premature birth, or perinatal/postnatal complications, according to the pediatricians' reports. Neglectful group criteria included substantiated child neglect without any other report of physical maltreatment or sexual abuse in the last 12 months by Child Protective Services (CPS) meeting Maltreatment Classification System (MCS) indicators for severe neglect (24), according to the pediatrician. Control group criteria involved mothers scoring negatively on all MCS neglect indicators and no CPS/Preventive Services records.

Sociodemographic variables in neglectful and control groups are detailed in Table 1. Given that multiple comparisons were performed in the same dataset, the Bonferroni correction was used. To ensure the robustness of the findings and confirm that the results are consistent when the samples are homogeneous in

Table 1 Sociodemographic profile in neglectful and control groups

Sociodemographic variables	Neglectful group ($n=51$) *M* (*SD*) or n (%)	Control group ($n=87$) *M* (*SD*) or n (%)	*t* (136) /$\chi 2$
Mean age of mother	31.71 (7.65)	34.70 (6.36)	− 2.47
Ancestry of mother (%)			7.8
Caucasian white	50 (98)	77 (88)	
Hispanic	0	10 (12)	
African black	1 (2)	0	
Age at child's birth	29.13 (7.29)	30.99 (6.13)	− 1.60
Number of children	2.49 (1.29)	1.66 (0.73)	4.25 ***
Mean age of target child	2.57 (1.59)	3.71 (2.12)	− 3.31
Child gender male (%)	23 (45)	50 (58)	1.45
Two-parent family (%)	25 (49)	63 (72)	6.63
Level education (%)			17.25 ***
Primary school	41 (80)	37 (43)	
≥ Secondary school	10 (20)	50 (57)	
Rural areas (%)	22 (43)	23 (26)	3.36
Unemployment (%)	36 (71)	51 (59)	1.50
Financial assistance (%)	35 (69)	21 (24)	24.58 ***

$p<0.001$ ($\alpha/11=$ $p<0.004$ Bonferroni). Note: *M*: mean score, SD: standard deviation; *t*: student statistic; $\chi 2$: Chi-Square statistic

both groups ($N=51$), 1,000 bootstrap samples of 51 participants were drawn from the control group and compared with the entire negligent group ($N=51$) and tested in all quantitative variables, thus eliminating potential biases arising from unequal group sizes. The results were similar to those in Table 1, supporting the initial findings: 93% for 'Age at child's birth,' 100% for 'Number of children,' 98.9% for 'Mean age of target child,' and slightly lower (66%) for 'Mean age of mother.' Although not all continuous variables met the normality assumption, the t-test is robust to non-normality according to the Central Limit Theorem, as long as the sample size exceeds $N=50$. Mothers in the neglectful group have higher number of children, lower level of education, and tend to receive financial assistance than those in the control group.

Procedure

Social workers assessed family sociodemographic characteristics, obtained mothers' permission for contact, and informed those interested in the study. Upon written acceptance, a collaborator visited the homes, and in the same visit gathered questionnaire responses from the mothers, recorded mother–child videos, and collected maternal saliva using a Real Saliva DNA Sample Collection Kit (RBMSAL01, Real Laboratory, Valencia, Spain). Mothers received monetary compensation at the session's conclusion.

Psychological and behavioral measures

- *The Childhood Trauma Questionnaire-Short Form* (25, 26) assessed the mothers' abuse and neglect history. Comprising 28 items on a 5-point Likert scale (1 = never; 5 = always), it includes five subtypes: physical neglect ($\alpha=0.71$), emotional abuse ($\alpha=0.92$), physical abuse ($\alpha=0.88$), sexual abuse ($\alpha=0.94$), and emotional neglect ($\alpha=0.93$) in our sample. Subscale scores were obtained by summing the scores for each corresponding item.
- *The Life Stress Scale LSS,* (27) assessed the mothers' adverse life events experienced, making an adaptation of adverse childhood experiences to our risk population. It comprises 16 self-reported adverse life events (e.g., divorce, economic pressure, chronic illness, eviction, unwanted pregnancy). Each item was categorically rated (no/yes occurrence) and its emotional impact with a 3-point Likert scale (0 = no occurrence; 1 = little impact; 3 = very high impact). The total emotional impact was obtained from a cumulative scoring of the intensity of adverse events.
- *The Mini International Neuropsychiatric Interview* (MINI; (28), Spanish version) categorically assesses (no/yes) symptoms of the 16 most common psychopathological disorders in DSM-IV and ICD-10. Mothers' disorder scores result from cumulative symptom scoring, not categorical diagnosis. No mothers in either group were on psychiatric medica-

tion during testing. Average scores, except for Anorexia, Bulimia, and Psychosis (scored as zero), were calculated for internalizing* and externalizing** dimensions (29): Major Depressive Episode*, Dysthymia*, Hypo/Manic Episode*, Generalized Anxiety Disorder*, General Panic Disorder*, Agoraphobia*, Social Phobia*, Obsessive–Compulsive Disorder*, Post-traumatic Stress Disorder*, Alcohol Dependence/Abuse**, Drug Dependence/Abuse**, Suicidality**, and Antisocial Personality**.

- *The Mini-Mental State Examination* (MMSE; (30) Spanish version) previously utilized to assess cognitive status in a population of neglectful mothers (9), assesses cognitive function in 30 questions with a cumulative score. It gauges abilities in spatial–temporal orientation, attention span, concentration, memory, abstraction capacity (calculation), language, visuospatial perception, and ability to follow basic instructions. Higher scores indicate better cognitive status in mothers.
- *The mother–child Emotional Availability* was assessed using the Infancy to Early Childhood Version Scale (31, 32). This measures the ability of the mother and child to read and respond appropriately to each other during a play task with a novel toy. Two external observers, blind to the mothers' grouping, rated the videos, with calculated inter-rater reliability. For the mother's behavior: sensitivity (responsive to child signals and demands, Kappa score $(K)=0.94$); structuring (facilitates child's play, $K=0.90$); non-intrusiveness (supports child's play without being over directive, $K=0.87$); non-hostility (behaves with the child in a non-rejecting manner, $K=0.92$). For the child's behavior: responsiveness (child's ability and interest in exploring and responding to parent's bids, $K=0.92$); involvement (child's ability and willingness to engage the mother, $K=0.86$). Mean scores were calculated for each mother and child dimension.

DNAm assay and methylation analyses

DNA was extracted from the saliva samples, and its concentration and purity were measured by spectrophotometry at the University Hospital N. S. de Candelaria (Tenerife, Spain). Methylation was assayed at the University of Michigan Epigenomics Core in Ann Arbor, Michigan (United States). A total of 250 ng of salivary DNA was bisulfite-converted using Zymo Kits, following the manufacturer's incubation parameters specific for Illumina methylation arrays. The samples were then hybridized using the Illumina Infinium Human Methylation EPIC BeadChip v1. All samples were processed in the same batch, and mother–child pairs (with children being part of other ongoing work) were placed on the same slide. Raw red/green IDAT files were imported into R using the Ewastools package. The ENmix Bioconductor package RELIC dye bias correction was applied to adjust for performance differences between dye types. Probes with an average detection p-value >0.05 were removed ($n=52{,}188$). Samples with an average detection p-value >0.1 or those that failed any of the 17 Illumina quality control metrics were excluded ($n=2$). Cross-reactive probes were removed ($n=41{,}963$). Next, inter-array normalization was performed using the preprocess Quantile function from the minfi package, separately quantile normalizing methylated and unmethylated intensities for Infinium I and II probes (33). Probe-type biases were then corrected using the beta-mixture quantile normalization method (BMIQ) (34), implemented with the bmiq.mc function from the wateRmelon package. After these steps, the dataset consisted of 138 mothers and 771,785 probes. Snakemake was used to manage the bioinformatics workflow in a reproducible manner. Given that DNA derived from saliva shows cellular heterogeneity, the value of the epithelial cells was calculated using the estimated LC function from ewastools *R*-package (35, 36) with the Houseman algorithm. The process (bisulfite conversion, hybridization, methylation value correction, probes, and samples out of range removed) left us with 138 mothers and 771,785 probes of CpGs.

Statistical estimation of EMN scores

First, we statistically adjusted the epigenetic information stored in 771,785 CpG sites for covariates: education level, mother's age, plate position, and leukocyte concentration. The latter covariate included post-outlier imputation with the average, treating values below or above the mean ± 3 * standard deviation as outliers. After pruning residuals by comparing Control and Neglectful groups using t-tests, setting the threshold to $p \leq 0.02138$, the resulting epigenetic data consisted of 7718 CpG sites. The p threshold was defined as the limit of the top 1% p values derived from the comparison of the adjusted CpGs between the neglectful and control groups. (Of note, the EMN estimation was repeated across different p value thresholds [i.e., defined as the limit of the top 0.5% p values and top 0.3%], obtaining consistent results across all thresholds, with strong highly significant correlations with the original EMN values [all $r \sim 0.84$, $p < 10^{-30}$].) Next, taking the 7718 values as data, we used a novel contrastive trajectories inference (cTI) algorithm to aggregate the DNAm data by identifying the trajectories of individuals aligned/ordered with the severity of epigenetic alterations hypothetically associated with maternal neglect. In essence, the cTI algorithm extracts enriched latent information in a target population of interest relative to controls and estimates individual progression scores (EMNs in this context) reflective of the advance in a given process of interest, which has been extensively validated in the context of neurodegenerative diseases (20, 37, 38). Thus, no behavioral/clinical information for the target or control groups is provided for cTI analysis, the algorithm automatically infers biological patterns in the target that are not present in the controls, discarding in such way irrelevant sources of variability while accentuating key components, and subsequently reorders the target individuals based solely on such patterns.

Initially, the CpGs data underwent automatic dimensionality reduction to an enriched space (about five to eight principal components capturing the data's main variability) via a contrastive principal component analysis algorithm (cPCA) (39). This optimized exploration and visualization of the target population's data relative to the control group. In the contrasted principal components (cPC) space, each subject was assigned to an epigenetic trajectory (a concatenation of individuals following aligned enriched patterns). Each subject's position in a trajectory reflects individual proximity to the neglect-free state (controls) or, in the inverse direction, to the extreme neglect state. Individual EMNs were then calculated, and normalized to the interval [0,1], reflecting the distance to these two extremes (control or extreme maternal neglect, respectively). We used the cTI implementation available in the open-access NeuroPM software (https://www.neuropm-lab.com/neuropm-box.html) (37). Before cTI analysis, to reduce the high data dimensionality, 5% of all the CpG sites were initially preselected based on their likelihood to be in a trajectory, comparing global versus neighborhood variance (40).

Design and statistical analyses of EMNs

Figure 1 depicts the sequence of the study's design, variables measured, and analyses performed. Structural equation modeling (SEM) tested EMNs' role in connecting a mother's prior physical neglect experience to neglect toward her child and associated effects.

In the SEM, we introduced three groups of components to test EMNs as a link: (a) Two positive pathways as antecedents of the mothers (early physical neglect and adverse event intensity) on EMNs to determine the optimal fit for the model, considering whether a combination was necessary; (b) one positive pathway relating EMNs with psychopathology; and (c) two negative pathways relating EMNs to cognitive status and emotional availability

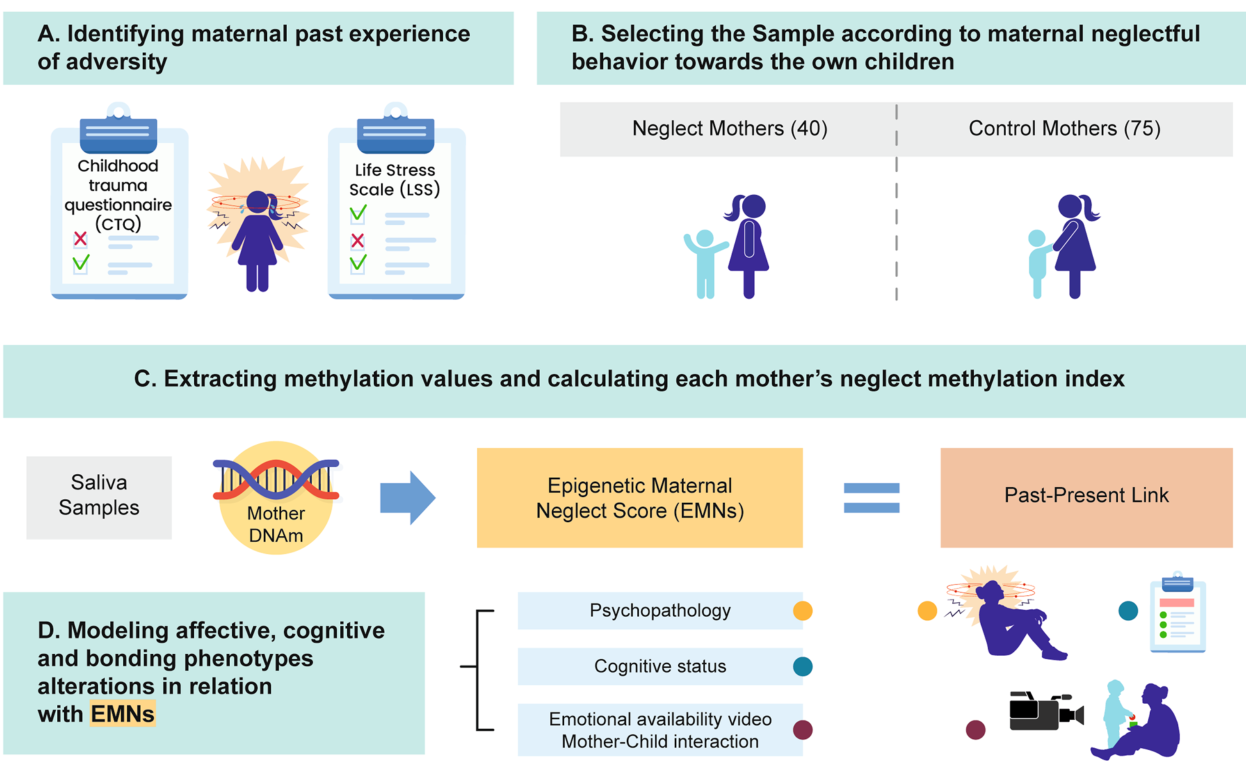

Fig. 1 Design, variables measured, and analyses of the study. **A** Identification of mothers' past adversity, **B** Selection of mothers based on neglectful/control parenting behavior, **C** Generation of the epigenetic maternal neglect score (EMN) from methylation values in saliva samples as an epigenetic link between past experiences and current neglectful behavior. **D** Reporting and observation of associated effects of EMNs

(EA). As the multivariate and univariate normality tests returned significant results (indicating a non-normal distribution of the model variables), MLR estimation (maximum likelihood with robust Huber-White standard errors and scaled test statistic derived from the Yuan-Bentler correction) was selected (41). Model goodness of fit was tested using the following indexes (42, 43): nonsignificant chi-square/degrees of freedom ($\chi 2/df$), comparative fit index ($CFI \geq 0.90$), Tucker–Lewis index ($TLI \geq 0.90$), the standardized root-mean-square residuals ($SRMR < 0{,}08$), and the root-mean-square error of approximation ($RMSA \leq 0.07$, and CI [0–0.1]. Analyses were performed with R and ULLRToolbox (44), using lavaan 0,6–19 package.

Gene enrichment analysis

Gene enrichment analysis was performed with Enrichr tool online (45). We independently analyzed 1022 genes annotated to the first 1500 differentially methylated CpG sites prioritized by order of statistical significance (p-value), and 322 genes annotated to the EMN CpGs. The top 20 relevant categories to our study from the 1022 genes were selected from DisGeNET supra-category from Enrichr ($p < 0.05$); we aimed to highlight the categories, and the genes within them, that are relevant to the mother's deficits (cognitive impairment and/or neurodegenerative and psychopathological disorders), regardless of the number of genes identified in the enrichment analysis. A dot plot was generated with the ggplot2 package in R (46). A logic heatmap displaying genes and their categories (both for the general and the EMN gene lists) was generated with the ComplexHeatmap package in R (47, 48). A network predicting functional relationships between 13 EMN genes overlapping the list of 1022 genes were generated with the online Enrichr-KG tool (accessed in March and April 2023).

Results

Psychological and behavioral group differences

The study variables are listed in Table 2. To address both the increased risk of Type I error associated with non-independent contrasts and the need for a multivariate approach to detect unobservable patterns that may not be captured by univariate analyses alone, we conducted a logistic regression with stepwise forward variable selection. This analysis incorporated the 15 numerical variables along with the group factor as the grouping variable. In this initial analysis, a subset of five variables was selected. To ensure the robustness of these findings, 1,000 bootstrap samples of 51 participants were drawn from the control group and compared with the entire negligent group ($N = 51$), eliminating potential biases arising from unequal group sizes. Each bootstrap sample underwent the same logistic regression analysis as described earlier. This procedure validated the five variables selected by the initial logistic regression, as

Table 2 Descriptive statistics and multivariate selection of the study variables in neglectful and control groups

Study variables	Neglectful group ($n = 51$) *M* (SD)	Control group ($n = 87$) *M* (SD) or %	*B, P(z)*
Mother intensity adverse events	16.76 (8.65)	11.60 (7.71)	
Own childhood maltreatment			
Physical neglect	8.27 (4.01)	5.84 (1.72)	0.352***
Emotional abuse	11.39 (6.70)	7.28 (4.03)	
Physical abuse	8.33 (4.81)	6.15 (2.25)	
Sexual abuse	8.98 (5.95)	5.83 (2.97)	
Emotional neglect	10.98 (5.73)	8.89 (4.27)	
Mother psychopathology			
Internalizing symptoms	0.41 (1.16)	-0.24 (0.80)	
Externalizing symptoms	0.35 (1.41)	-0.20 (0.56)	
Mother cognitive status	26.35 (2.35)	27.52 (2.27)	
Emotional availability			
Mother sensitivity	5.71 (1.27)	6.80 (1.4)	
Mother structuring	3.37 (0.95)	4.15 (0.92)	
Mother non-intrusiveness	3.92 (1.14)	4.70 (0.5)	– 1.429***
Mother non-hostility	4.85 (0.51)	5 (0.24)	
Child responsiveness	4.90 (1.08)	5.93 (0.99)	– 1.560***
Child involvement	4.79 (1.18)	5.14 (0.64)	– 1.550**

M: mean score, *SD*: standard deviation; Logistic Regression parameters (*B*); **p* (z) < .05; ***p*(z) < .01; ****p*(z) < .001. In bold, the variables resulting from the regression logistic analysis and replicated by the bootstrap selection and comparison they appeared in at least 60% of the bootstrap replications. The omnibus test of the original logistic regression was significant: Chi-Square (6) = 80.30, $p < 0.0001$, with 72.5% of the negligent group and 89.4% of the control group correctly classified. The final set of variables selected by this multivariate analysis is those in bold in Table 2, along with their B coefficients and p (z) values. Mothers in the neglectful group reported greater experience of physical neglect as a girl, and a greater likelihood of externalizing symptoms, along with observed lower mothers' non-intrusiveness and lower child responsiveness and child involvement compared to the control group.

EMNs and epigenetic age acceleration

We performed a Pearson correlation to analyze the relationships between the results of the EMNs with those of the epigenetic PhenoAge clock obtained in a previous study with the same sample and distribution of mothers with neglectful care and control (14). The PhenoAge clock is based on age-related DNAm levels at 513 CpG sites to estimate the biological age across multiple tissues and cells. It is measured by the residual scores obtained by regressing DNAm age on chronological age, representing both positive and negative deviations of the epigenetic age from chronological age. The results show a significant relationship between both indexes, $r = 0.24$; $p = 0.0044$.

Group differences and psychological correlates of epigenetic maternal neglect scores (EMNs)

EMNs, reflecting mothers' epigenetic load, were significantly lower in the non-neglectful control group (CG: $M = 0.152$, $SD = 0.05$) compared to the neglectful group (NG: $M = 0.430$, SD = 0.202): t-test (53.668) = −9.614, $p < 0.0001$ (Fig. 2 left). EMNs correlated positively with physical neglect, intense events, internalizing and externalizing symptoms, and negatively with cognitive status, mother sensitivity, mother structuring, mother non-intrusiveness, child responsiveness, and child involvement. Only the correlation with mother non-hostility was not significant (Fig. 2 right). Supplemental Information "see Figure S1 available online" shows correlations and significant values among all study variables.

Testing of the structural equation model

Our model tested the relationships between the mother's past experience of physical neglect and the intensity of adverse events on EMNs, which is related to psychopathology, cognitive status, and emotional availability (EA) effects. We measured past physical neglect and intensity of adverse events, EMNs, and cognitive status in the mothers. Latent variables included two factors of psychopathology (internalizing and externalizing scores) and six emotional availability factors, four corresponding to maternal variables (sensitivity, structuring, non-intrusiveness, non-hostility), and two to child variables (responsiveness and involvement).

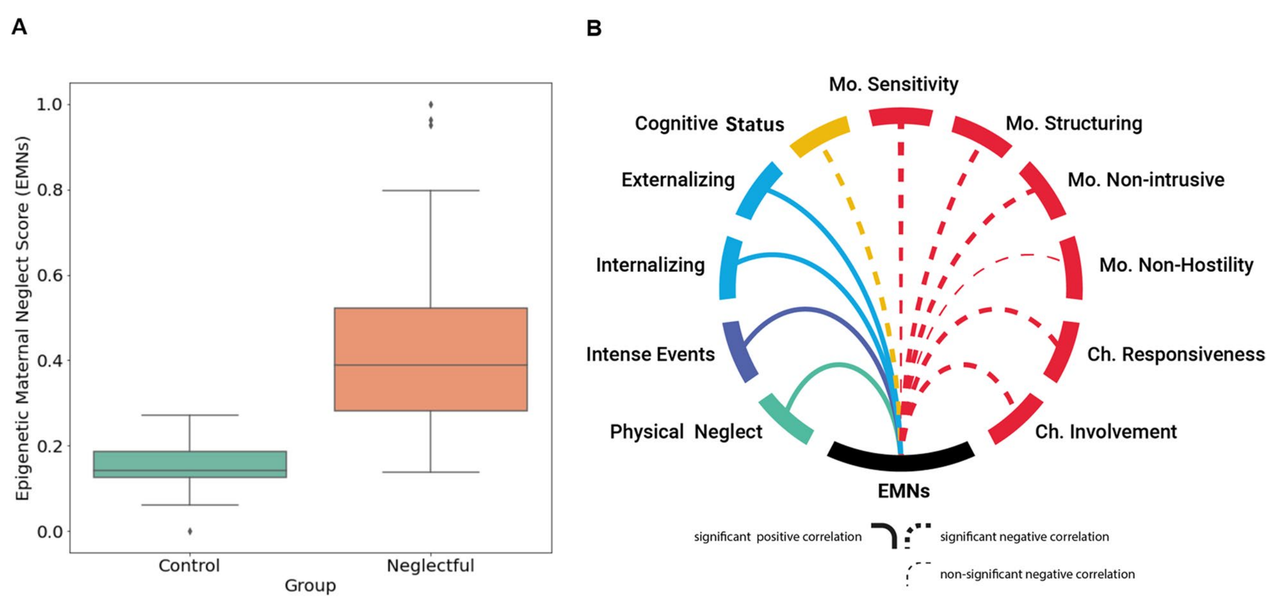

Fig. 2 Descriptive analyses with the set of psychological and behavioral variables: **A** Significant mean difference in epigenetic maternal neglect (EMN) between neglect and control mothers showing outliers; **B** Significant/nonsignificant correlations with EMNs are represented in bold/light font showing positive/negative values represented in solid/dotted connection lines. Green, dark blue, light blue, yellow, and red represented different categories of variables

However, this model failed to converge due to negative variances and lack of fitness: X^2 (49) = 131.8, $p \leq 0.0001$; CFI = 0.859; TLI = 0.813; RMSEA = 0.112; 90% CI = [0.09; 0.136], and SRMR = 0.097. Consequently, a revised model was tested: (a) with a single pathway from the mother's physical neglect as a child to EMN; (b) retaining the positive pathway from EMNs to psychopathology; and (c) maintaining the two negative pathways from EMNs to cognitive status and emotional availability. Model adjustment also recommended omitting child responsiveness from emotional availability as a latent variable because it had a negative variance coefficient (a Heywood case). However, we first ensured that removing it did not substantially affect the internal consistency of the scale, which changed from $\alpha = 0.82$ to $\alpha = 0.75$.

The resulting model in Fig. 3 exhibited a good fit to the data: X^2 (29) = 43.11; p = 0.045; CFI = 0.960; TLI = 0.939; RMSEA = 0.057; 90% CI = [0.009; 0.091], and SRMR = 0.059.

The model shows that EMN relates the mother's childhood experience of physical neglect with significant psychopathological symptoms, lower cognitive status, and lower emotional availability, Table 3 shows significant

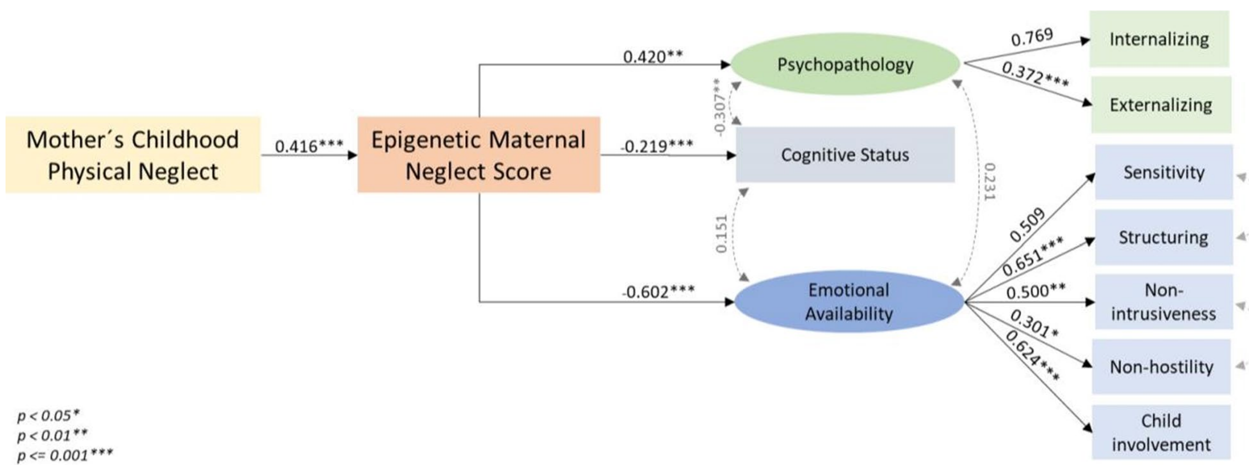

Fig. 3 The final structural equation model shows the standardized path coefficients and significance, and the role of the epigenetic maternal neglect scores in linking the antecedent and consequent variables

Table 3 Significant unstandardized and standardized path coefficients for measurement and structural models

	Estimate	*z* value	*p*	Standardized estimation
Measurement model				
Psychopathological symptoms				
Internalizing	1.000			0.769
Externalizing	0.483	3.336	0.001	0.372
Emotional availability				
Mother sensitivity	1.000			0.509
Mother structuring	0.895	7.685	0.000	0.651
Mother non-intrusiveness	0.606	3.135	0.002	0.500
Mother non-hostility	0.155	1.986	0.047	0.301
Child involvement	0.764	3.930	0.000	0.624
Structural model				
Epig. Maternal Neglect score				
Mother's childhood Physical Neglect	0.026	4.187	0.000	0.416
Psychopathology	1.737	2.764	0.006	0.420
Cognitive Status	−2.680	−3.288	0.001	−0.219
Emotional availability	−2.339	−4.438	0.000	−0.602

unstandardized and standardized path coefficients for measurement and structural models.

Subsequent SEM models, considering the intensity of adverse events or other childhood maltreatment experienced by the mother, apart from physical neglect, failed to produce fitting results ("see Supplement 1 online" for additional SEMs non-fitting results).

Gene enrichment analysis

To assess the biological annotations represented in our data, we performed a gene enrichment analysis. Specifically, to identify gene-disease associations, we used the DisGeNET database with the EnrichR tool online (45, 49–51). The analysis of 1022 genes annotated to the first 1500 CpG sites (ordered by statistical significance) identified 234 genes associated with cognitive impairment, neurodegenerative, and psychopathological disorders. These included several categories related to cognitive functions, depression, and neurobiological diseases such as schizophrenia and autism (Fig. 4A, 4B). Given that 1022 genes account for about 30% of all the functionally defined genes annotated to the differentially methylated CpGs included in the study, we estimate that this is a reasonable subset of genes and thus the results may be considered representative of the data. Interestingly, the 13 genes shared between the 234 cognitive/neurodegenerative/psychopathology-related genes and the EMN genes appear to be associated almost exclusively with cognitive categories, including performance and intelligence impairments, suggesting a predominant association between these categories and the outcome of our model (Fig. 4C). Supporting these results, we found that 71 out of 320 genes annotated to the 385 EMN CpGs are associated with cognitive impairment and neurodegenerative, and psychopathological disorders in the DisGeNET database (Fig. 4D); whereas a group of 34 genes is associated with a distinctive cluster of cognitive impairment categories, the rest are associate to other neurodegenerative and psychopathological disorders such as Alzheimer's diseases, senile dementia, schizophrenia, autism, hyperactive behavior, self-harm behavior, mental depression, anxiety, and bipolar diseases. Overall, cognitive categories show a higher contribution to the EMN, measured as the median of the contribution of all the genes included within each category. In summary, the composition of gene-disease associations in our differentially methylated data, suggests that cognitive dysfunction plays a crucial role in the epigenetic makeup, along with other neurodegenerative disorders and psychopathologies.

Discussion

This study unveils an epigenetic index based on DNAm data (EMN) applied to the dysfunctional neglect condition, which supports the use of epigenetic data to study clinical and socially neglectful behavior. The EMN was obtained submitting the individual epigenome to the trajectories inference machine learning algorithm, which attributed to each mother a given state in the progression from healthy controls to the extreme neglect condition. Our findings also show that EMN links maternal negligence as a daughter to similar practices with her child. EMN also predicts psychopathological symptoms, lower cognitive status, and diminished emotional availability in mother–child interaction. Interestingly, when comparing EMNs with biological age acceleration as measured by the PhenoAge clock (14), a significant correlation was observed ($p < 0.005$). Both metrics showed greater hypermethylation in the neglectful group than in the control group, using the same sample of mothers. However, EMNs appear to provide additional differential information over the epigenetic load of neglectful caregiving. This finding also aligns with the results of accelerated epigenetic aging in adults exposed to childhood maltreatment (52) or lifetime stress (53). More studies with different samples are needed to further clarify the similarities and differences between these complementary concepts applied to dysfunctional parenting and to establish that hypermethylation is associated with the neglect condition.

Our first analyses confirmed the behavioral profile of maternal neglect as compared to controls (1, 2) once corrected for multiple comparisons, independence of

(See figure on next page.)

Fig. 4 Ontology analysis of genes annotated to differentially methylated CpG sites. **A**- The list of 1022 genes annotated to the first 1500 differentially methylated CpGs (ordered by statistical significance) was analyzed using the EnrichR tool online (45). The first 20 cognitive impairment, neurodegenerative and psychopathological disorders categories were selected for the lollipop graph showing gene count (size of the circle) and -Log_{10} (p value) (color scale) from the DisGeNET category (within "Disease and Drugs" categories of EnrichR; **B**- Heatmap depicting the genes in each category presented in the lollipop graphs (red squares indicate the gene belongs within the category; blue squares indicate genes annotated to EMN CpG sites). **C**- Network representation of the EMN genes in the list (see B); blue and green circles indicate DisGeNET categories and genes, respectively (prepared with EnrichR-KG tool). **D**- EMN genes associated with cognitive and neuro-psychopathology categories according to the DisGeNET database. Dark blue squares indicate genes within each category. The upper boxplot shows the contribution of each category to the EMNs calculated as the median of the contribution of all the genes associated with each category. The white-blue-red column indicates EMN contribution of the listed genes

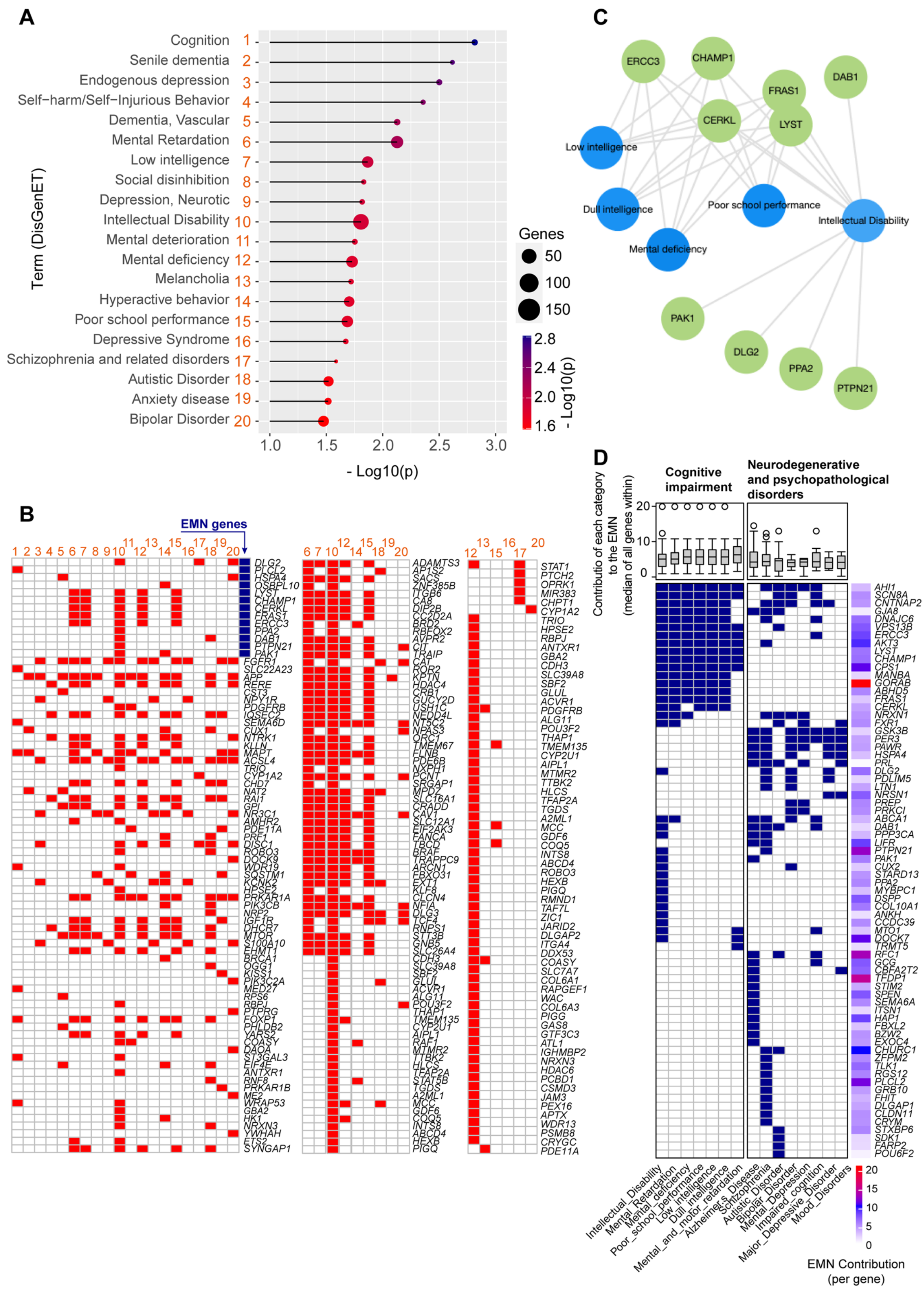

Fig. 4 (See legend on previous page.)

the variables and for unequal group sizes, using robust statistical methods. As anticipated, the neglectful group exhibited a higher epigenetic load compared to the control group. The overall findings regarding the impact of psychological factors on EMNs further validate the significance of our index profiling life adversities and the associated negative consequences prevalent in maternal neglect (6, 8, 9). Importantly, EMN estimation did not include any behavioral information about the target group, highlighting the ability of the method to automatically discover biologically relevant patterns in an unsupervised way without being trained on the control neglect distinction.

Our findings contribute to existing evidence linking DNAm profiles to various life adversities in adults (11, 16). There is behavioral evidence for the intergenerational associations of adverse childhood experiences (4) and abusive and neglect behavior (5), similar to our results. To our knowledge, this is the first human study demonstrating that the mothers' exposure to childhood physical neglect and their later neglectful behavior is associated with their epigenetic load measured by a compound index. In this context, our results support the value of EMNs as a useful index linking the antecedent of having suffered physical neglect to a higher epigenetic load associated with a more extreme neglect position in the control neglect trajectory.

In our SEM model, EMN was positively related to internalizing and externalizing symptoms and negatively to cognitive status, aligning with the neglect vulnerability profile. DNAm has demonstrated susceptibility to psychiatric phenotypes in adults (10) and a single episode of psychosis (54). This connection between EMNs, psychopathological symptoms, and poor cognitive status also aligns with longitudinal behavioral evidence. This evidence indicates the impact of childhood neglect on cognitive function, educational outcomes in adolescence, and mental health problems, including anxiety, depression, PTSD, and psychosis in young adulthood (23).

Finally, EMNs negatively correlated with sensitive-responsive interaction in mother–child bonding (32). The substantial epigenetic burden of adverse experiences appears to redirect the focus of attention away from the child, diminishing the quality of mother–child emotional availability (EA), a characteristic of maternal negligence (6, 55). Higher EA encompasses positive emotional exchanges and effective cognitive organization of coordinated mother–child play actions, crucial for achieving joint goals and influencing the infant's attachment quality, with high relevance for healthy development (22).

Research on the epigenetic component of infant attachment is limited by candidate-gene approaches (56). Our EWAS-based model demonstrates that a higher epigenetic load poses a biological risk for adequate mother–child bonding, potentially impacting the child physical and mental wellbeing negatively. Direct evidence indicating the sharing of life adversity and psychopathology-related genes in differentially methylated regions between mothers and children was in line with a potential epigenetic transmission to the offspring in neglect contexts (57). This possible transmission has been proposed to occur through the fetal germ cells of the offspring when mothers have been exposed to risk factors during pregnancy (58). Moreover, a history of higher adversity experienced by mothers with neglectful behavior was associated with lower mother–child methylation similarity. In turn, longer co-residence time (indicated by the child's age) correlated with higher mother–child similarity. This highlights the importance of both environmental and hereditary factors in the intergenerational methylation process observed in biological dyads (59).

In line with the neglectful psychological profile, the genes annotated to the differentially methylated CpG sites in our data are associated with cognitive impairment, neurodegenerative disorders, and psychopathological conditions. Notably, these conditions seem to contribute more significantly to the EMN index, aligning with behavioral evidence of poor developmental and learning outcomes (23). Overall, our gene enrichment data validates findings from our multivariate behavioral model and highlights genes and pathways as potential links between being neglected as a child and later neglectful behavior (5).

The study's primary strength lies in its utilization of a unique sample comprising mothers and children exposed solely to physical neglect. Additionally, the use of multivariate modeling thanks to the use of a compound epigenetic measure reveals sequential relationships linked by epigenetics between having experienced neglect and engaging in neglectful behavior, along with other negative outcomes. One limitation arising from this study design is the relatively small sample size, which, nonetheless, falls within the median range among DNA studies in maltreated populations (11). Another limitation stemming precisely from being the first study to adopt this approach is the lack of replicability of the results, a challenge that should be addressed in the future studies. Thirdly, the cross-sectional design constrains causal assessment among variables. Fourth, we did not assess the impact that genetic variation, such as common single nucleotide polymorphisms (SNPs), may have on the methylation data. Future studies should incorporate SNP genotyping into their analyses. Lastly, due to the predictive nature of the epigenetic enrichment analysis, our study cannot establish direct biological relationships between genes and the phenotype of interest.

In conclusion, the higher individualized aggregated epigenetic score in the neglectful group effectively models the unique impact of experiencing physical neglect during mothers' childhood on their psychopathological vulnerability, lower cognitive status, and poor mother–child bonding. The use of the EMNs in this study facilitates the detection of enriched DNAm patterns and candidate genes associated with cognitive impairment, neurodegenerative disorders, and psychopathological conditions in neglectful motherhood. These findings support the notion of the biological basis for the behavioral transmission of physical neglect and its associated effects. Furthermore, they could enhance early diagnosis of the neglect condition in both the mother, who has experienced trauma, and her newborn child being performed at primary care screenings. For those cases with early signs of neglect risk, training in mother–child empathic care should be incorporated into targeted psychopathological interventions. This would help to break the cycle of transmission of neglect and prevent subsequent negative outcomes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01839-7.

Additional file1 (XLSX 15375 KB)

Additional file2 (XLSX 50 KB)

Additional file3 (DOCX 197 KB)

Additional file4 (DOCX 20 KB)

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Author contributions
IL, MJR, and YIM developed the idea for the study. SHR collected the data. DGL, JF, CM, and MLR did the analyses. IL, MJR, SHR, and MLR wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Availability of data and materials
The Data Set files analyzed during the current study are available in the supplementary material: Data Set file 1: Excel sheet 1 includes the value of the beta in each CpG for both groups that were used to calculate the EMN index; Excel sheet 2 includes the values of the covariates that were used for the EMN index; Excel sheet 3 includes the values of the variables that were used to test the SEM models. Data Set file 2: Excel sheet 1 includes the genes used in the enrichment; Excel sheets 2 and 3 include the partial and full list of annotated genes, respectively; and Excel sheets 4 and 5 present data of functional categories of the corresponding genes. The full disclosure of the raw sequencing data of mothers and their minors is subject to confidentiality restrictions of the Canary Islands Child Protection Services.

Declarations

Ethical Approval
The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the protocol of the Ethical Committee of Investigation of the Canary Islands University Hospital Complex (code: CHUC_2018_63; date of approval: 14 December 2018).

Competing Interests
The authors declare no competing interests.

Author details
[1]Instituto Universitario de Neurociencia, Universidad de La Laguna, Campus de Guajara, 38201 San Cristóbal de La Laguna, Spain. [2]Facultad de Psicología, Universidad de La Laguna, San Cristóbal de La Laguna, Spain. [3]Department of Microeconomics and Public Economics, Maastricht University School of Business and Economics, Maastricht University - Center of Neuroeconomics, Maastricht, The Netherlands. [4]Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, The Netherlands. [5]Facultad de Ciencias Sociales Aplicadas y de La Comunicación, UNIE Universidad, Madrid, Spain. [6]Department of Pathology and Laboratory Medicine at the David Geffen School of Medicine, UCLA, Los Angeles, USA. [7]Institute for Social Research, University of Michigan, Ann Arbor, MI, USA. [8]Neurology and Neurosurgery Department, Montreal Neurological Institute, Montreal, Canada. [9]McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal, Canada. [10]Ludmer Centre for Neuroinformatics and Mental Health, Montreal, Canada.

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