

Epigenetic signatures linking environmental exposure, cortical structure and psychiatric traits

Sylvane Desrivères

`sylvane.desrivieres@kcl.ac.uk`

Institute of Psychiatry, Psychology & Neuroscience, King's College London, United Kingdom

<https://orcid.org/0000-0002-9120-7060>

Xinyang Yu

Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK <https://orcid.org/0000-0001-8026-4839>

Friederike David

Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany <https://orcid.org/0000-0001-9521-5669>

Antoine Weihs

University Medicine Greifswald

Isabel Schuurmans

Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands

Tianye Jia

Institute of Science and Technology for Brain-Inspired Intelligence, Fudan University, Shanghai, China

Sarina Abrishamcar

Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA

María Alemany-Navarro

Translational Psychiatry Group, Instituto de Biomedicina de Sevilla (IBiS)-CSIC <https://orcid.org/0000-0002-8491-0137>

Nicola J. Armstrong

Curtin University <https://orcid.org/0000-0002-4477-293X>

Jiayu Chen

Tri-institutional Center for Translational Research in Neuroimaging and Data Science (TReNDS) [Georgia State University, Georgia Institute of Technology, Emory University] <https://orcid.org/0000-0001-5059-372X>

Eleanor Conole

The University of Oxford

Benedicto Crespo-Facorro

Hospital Universitario Virgen del Rocio, Department of psychiatry, IBI/CSIC/CIBERSAM/ISCIII, University of Seville, Seville, Spain

Darina Czamara

Max Planck Institute of Psychiatry <https://orcid.org/0000-0001-7381-904X>

Sonia Hesam-Shariati

Neuroscience Research Australia, Sydney, NSW, Australia & School of Psychology, University of New South Wales, Sydney, Australia

Kyle Jensen

Tri-institutional Center for Translational Research in Neuroimaging and Data Science (TReNDS), Georgia State, Georgia Tech, Emory, Atlanta, GA, USA <https://orcid.org/0000-0001-7896-2232>

Jiyang Jiang

Centre for Healthy Brain Ageing <https://orcid.org/0000-0002-2147-6302>

Annchen Knodt

Duke University

Thomas Kremer

Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Shiwei Liu

Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, Indiana, USA <https://orcid.org/0000-0003-4711-5959>

Jingyu Liu

Georgia State University <https://orcid.org/0000-0002-1724-7523>

Kwangsik Nho

for the Alzheimer's Disease Neuroimaging Initiative

Bronwyn Overs

Neuroscience Research Australia, Sydney, NSW, Australia & School of Biomedical Sciences, Faculty of Medicine & Health, University of New South Wales, Sydney, NSW, Australia

Shannon Risacher

Gerontology and Geriatrics, Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA

Gloria Roberts

Discipline of Psychiatry & Mental Health, School of Clinical Medicine, Faculty of Medicine & Health, University of New South Wales, Sydney, NSW, Australia

Rafael Romero-Garcia

Department of Medical Physiology and Biophysics

Jean Shin

Centre hospitalier universitaire Sainte-Justine

Karen Sugden

Duke University

Dennis van 't Ent

VU university <https://orcid.org/0000-0003-2001-1375>

Jenny van Dongen

Vrije Universiteit Amsterdam <https://orcid.org/0000-0003-2063-8741>

Terry Zhou

Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Nina Alexander

University of Marburg

Eric Artiges

INSERM/Université Paris-Saclay <https://orcid.org/0000-0003-4461-7646>

Christian Baeuchi

Department of Psychiatry and Psychotherapy, Technische Universität Dresden, Dresden, Germany;

Tobias Banaschewski

Central Institute of Mental Health, Mannheim <https://orcid.org/0000-0003-4595-1144>

Gareth Barker

King's College London <https://orcid.org/0000-0002-5214-7421>

Eva Beins

Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany

Arun Bokde

Discipline of Psychiatry, School of Medicine and Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland <https://orcid.org/0000-0003-0114-4914>

Tiana Borgers

Institute for Translational Psychiatry

Uli Bromberg

<https://orcid.org/0000-0003-4974-971X>

Rüdiger Brühl

Physikalisch-Technische Bundesanstalt <https://orcid.org/0000-0003-0111-5996>

Robin Bülow

University Medicine Greifswald <https://orcid.org/0000-0003-1884-5784>

Di Chen

Shanghai Key Laboratory of Mental Health and Psychological Crisis Intervention, School of Psychology and Cognitive Science, East China Normal University, Shanghai, China

Isabelle Claus

Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany

Janine Felix

Erasmus MC <https://orcid.org/0000-0002-9801-5774>

Kira Flinkenflügel

University of Münster <https://orcid.org/0000-0001-7738-5429>

Herta Flor

Central Institute of Mental Health Medical Faculty Mannheim Heidelberg University
<https://orcid.org/0000-0003-4809-5398>

Andreas Forstner

Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn
<https://orcid.org/0000-0002-1876-6368>

Vincent Frouin

NeuroSpin, Commissariat à l'Energie Atomique, CEA-Saclay Center <https://orcid.org/0000-0001-9360-6623>

Hugh Garavan

Departments of Psychiatry and Psychology, University of Vermont, 05405 Burlington, Vermont, USA
<https://orcid.org/0000-0002-8939-1014>

Penny Gowland

University of Nottingham <https://orcid.org/0000-0002-4900-4817>

Hans Grabe

University Medicine Greifswald

Antoine Grigis

Dominik Grotegerd

Department of Psychiatry, University of Münster, Germany

Tim Hahn

Muenster University <https://orcid.org/0000-0001-6541-3795>

Stefanie Heilmann-Heimbach

Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany

Andreas Heinz

Department of Psychiatry and Psychotherapy, Universität Tübingen

Per Hoffmann

University of Bonn <https://orcid.org/0000-0002-6573-983X>

Sarah Hohmann

Heidelberg University <https://orcid.org/0000-0002-5175-6698>

Nathalie Holz

Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg
<https://orcid.org/0000-0002-6225-9267>

Anke Huels

Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA

Hervé Lemaître

Groupe d'Imagerie Neurofonctionnelle <https://orcid.org/0000-0002-5952-076X>

Jean-Luc Martinot

Institut National de la Santé et de la Recherche Médicale <https://orcid.org/0000-0002-0136-0388>

Marie-Laure Paillère Martinot

APHP.Sorbonne Université, Institut IDEAL, Pitié-Salpêtrière Hospital <https://orcid.org/0000-0002-0452-2450>

Carina Mathey

Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany

Susanne Meinert

University of Münster <https://orcid.org/0000-0003-2177-7161>

Abigail Miller

Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany

Ryan Muetzel

Erasmus University Medical Center <https://orcid.org/0000-0003-3215-1287>

Frauke Nees

Institute of Medical Psychology, Ludwig-Maximilians-Universität (LMU) in Munich, Munich, Germany

Markus Nöthen

University of Bonn <https://orcid.org/0000-0002-8770-2464>

Dimitri Papadopoulos Orfanos

<https://orcid.org/0000-0002-1242-8990>

Tomas Paus

University of Montreal <https://orcid.org/0000-0003-1495-9338>

Jean-Baptiste Poline

McGill University, Faculty of Medicine, Montreal Neurological Institute and Hospital, McConnell Brain Imaging Center, Ludmer Centre for Neuroinformatics and Mental Health <https://orcid.org/0000-0002-9794-749X>

Richie Poulton

University of Otago <https://orcid.org/0000-0002-1052-4583>

Luise Poustka

Department of Child and Adolescent Psychiatry and Psychotherapy, University Medical Centre Gottingen

Michael Smolka

Technische Universität Dresden <https://orcid.org/0000-0001-5398-5569>

Frederike Stein

Department of Psychiatry and Psychotherapy, University of Marburg, Germany

David Steins

Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany

Lea Teutenberg

Department of Psychiatry and Psychotherapy, University of Marburg, Germany

Reremoana Theodore

Dunedin Multidisciplinary Health & Development Research Unit, Department of Psychology, University of Otago, Dunedin, New Zealand

Paula Usemann

Department of Psychiatry and Psychotherapy, University of Marburg, Germany

Nilakshi Vaidya

Charité - Universitätsmedizin Berlin <https://orcid.org/0000-0002-4600-7158>

Henry Völzke

University Medicine Greifswald

Henrik Walter

University Medical Centre Charité <https://orcid.org/0000-0002-9403-6121>

Joanna Wardlaw

The University of Edinburgh <https://orcid.org/0000-0002-9812-6642>

Robert Whelan

Trinity College <https://orcid.org/0000-0002-2790-7281>

Paul Wirsching

Department of Psychiatry and Psychotherapy CCM, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, <https://orcid.org/0000-0003-1228-6846>

Katharina Wittfeld

University Medicine Greifswald <https://orcid.org/0000-0003-4383-5043>

Dorret Boomsma

Vrije Universiteit Amsterdam, The Netherlands <https://orcid.org/0000-0002-7099-7972>

Vince Calhoun

Georgia State University <https://orcid.org/0000-0001-9058-0747>

Avshalom Caspi

Department of Psychology and Neuroscience, Duke University, Durham, North Carolina, <https://orcid.org/0000-0003-0082-4600>

Udo Dannlowski

Institute for Translational Psychiatry, University of Münster <https://orcid.org/0000-0002-0623-3759>

Ahmad Hariri

Duke University <https://orcid.org/0000-0003-3052-9880>

Jacqueline Hoare

Department of Psychiatry and Mental Health, Neuroscience Institute, University of Cape Town, Cape Town, South Africa

Tilo Kircher

Philipps-University Marburg <https://orcid.org/0000-0002-2514-2625>

Andreas Meyer-Lindenberg

Central Institute of Mental Health <https://orcid.org/0000-0001-5619-1123>

Philip Mitchell

Discipline of Psychiatry & Mental Health, School of Clinical Medicine, Faculty of Medicine & Health, University of New South Wales, Sydney, NSW, Australia

Terrie Moffitt

Duke University

Igor Nenadić

Department of Psychiatry and Psychotherapy, Philipps Universität Marburg, Rudolf-Bultmann-Str. 8, 35039 Marburg, Germany <https://orcid.org/0000-0002-0749-7473>

Zdenka Pausova

The Hospital for Sick Children <https://orcid.org/0000-0002-7784-1380>

Perminder Sachdev

Centre for Healthy Brain Ageing, School of Clinical Medicine, University of New South Wales, Sydney, NSW, Australia <https://orcid.org/0000-0002-9595-3220>

Andrew Saykin

Indiana University School of Medicine <https://orcid.org/0000-0002-1376-8532>

Peter Schofield

Discipline of Psychiatry & Mental Health, School of Clinical Medicine, Faculty of Medicine & Health, University of New South Wales, Sydney, NSW, Australia

Gunter Schumann

Centre for Population Neuroscience and Stratified Medicine (PONS), Institute for Science and Technology of Brain Inspired Intelligence <https://orcid.org/0000-0002-7740-6469>

Dan Stein

University of Cape Town and Neuroscience Institute <https://orcid.org/0000-0001-7218-7810>

Heather Zar

SAMRC Unit on Child and Adolescent Health, Department of Paediatrics and Child Health, University of Cape Town <https://orcid.org/0000-0002-9046-759X>

Sophia Thomopoulos

University of Southern California <https://orcid.org/0000-0002-0046-4070>

Karen Mather

Centre for Healthy Brain Ageing, Psychiatry, University of New South Wales (UNSW)

<https://orcid.org/0000-0003-4143-8941>

Simon R. Cox

Lothian Birth Cohorts, Department of Psychology, University of Edinburgh <https://orcid.org/0000-0003-4036-3642>

Charlotte Cecil

, Erasmus Medical Centre, University Medical Center Rotterdam <https://orcid.org/0000-0002-2389-5922>

Janice Fullerton

University of New South Wales

Philipp Sämann

Max Plank Institute of Psychiatry <https://orcid.org/0000-0001-8523-3628>

Alexander Teumer

University Medicine Greifswald <https://orcid.org/0000-0002-8309-094X>

Paul Thompson

Imaging Genetics Center, Mark and Mary Stevens Neuroimaging & Informatics Institute, Keck School of Medicine of the University of Southern California, Marina del Rey, California, USA

Article

Keywords:

Posted Date: April 6th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9151930/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: **Yes** there is potential Competing Interest. T.B. served in an advisory or consultancy role for AGB Pharma, eye level, Infectopharm, Medice, Neurim Pharmaceuticals, Oberberg GmbH and Takeda. He received conference support or speaker's fee by Janssen-Cilag, Medice and Takeda. He received royalties from Hogrefe, Kohlhammer, CIP Medien and Oxford University Press; the present work is unrelated to these relationships. G.J.B. has received honoraria from General Electric Healthcare for teaching on scanner programming courses. L.P. served in an advisory or consultancy role for Roche and Vifor Pharma and received speaker's fee by Shire. She received royalties from Hogrefe, Kohlhammer and Schattauer. The present work is unrelated to the above grants and relationships. The other authors declare no competing interests. H.J.G. has received travel grants and speakers honoraria from Neuraxpharm, Servier, Indorsia and Janssen Cilag.

Abstract

Environmental exposures influence the risk of psychiatric disorders, yet the biological mechanisms by which such experiences become embedded in brain structure remain poorly understood. The human cerebral cortex is crucial for cognition and emotional regulation, and variation in cortical thickness (CT) and surface area (SA) is linked to various behavioural and psychiatric traits. Here, we present a large-scale epigenome-wide association study that combines peripheral blood DNA methylation (DNAm) with MRI-derived cortical measures in over 7,400 individuals across 20 cohorts within the ENIGMA consortium. We identify mostly non-overlapping DNAm signatures associated with CT and SA, consistent with their distinct developmental and regulatory architectures. CT-associated CpGs are replicated across independent cohorts and are enriched for environmentally responsive regulatory elements and pathways associated with stress, metabolism, and immune signalling. In contrast, SA-associated CpGs cluster within chromatin-regulatory regions involved in early cortical development. Phenome-wide and Mendelian randomisation analyses reveal pleiotropic associations between DNAm, cortical structure, and psychiatric and cognitive traits. These findings suggest that peripheral DNAm captures environmentally sensitive biological processes that link exposure, cortical organisation, and behavioural vulnerability.

Introduction

Environmental experiences, from psychosocial stress and substance use to air pollution and socioeconomic adversity, play a central role in shaping brain and cognitive development and mental-health risk^{1–4}. Yet the biological mechanisms through which such exposures become embedded in brain structure and influence behaviour remain incompletely understood. Structural variation in the human cerebral cortex is a key intermediate phenotype linking environmental influences to cognition, emotional regulation and psychiatric vulnerability^{5–7}.

Macroscopic cortical morphology is typically described along two principal dimensions: cortical thickness (CT) and surface area (SA), both of which can be reliably quantified using structural MRI. These traits reflect distinct neurobiological processes: CT relates to neuronal density, dendritic arborisation, synaptic pruning, and intracortical myelination^{8–11}, whereas SA largely reflects radial glial progenitor proliferation and the tangential expansion of the cortical sheet during early neurodevelopment^{12–14}. Consistent with these differences, CT and SA exhibit dissociable developmental trajectories^{15–18}.

Family and twin studies estimate substantial heritability for both traits, with heritability estimated slightly lower for CT, suggesting that it may be more susceptible to environmental influences or plastic changes over the lifespan^{19,20}. Despite this strong genetic contribution, a substantial proportion of inter-individual variation in cortical structure remains unexplained and may reflect unique environmental influences and gene-environment interplay²⁰. Large-scale genome-wide association studies further support the

biological distinction between CT and SA, identifying largely non-overlapping genetic loci and regulatory architectures associated with each trait²¹.

DNA methylation (DNAm), the addition of a methyl group to CpG dinucleotides, represents a dynamic mechanism through which environmental exposures can influence gene regulation. DNAm patterns are partially heritable but also responsive to developmental and environmental inputs, making them a promising molecular interface linking exposure to biological processes relevant for behaviour and disease^{22–24}. Indeed, epigenome-wide association studies (EWASs) have identified DNAm signatures associated with a wide range of neuropsychiatric and neurodegenerative conditions^{25–31}. In schizophrenia, for example, differential blood DNAm has been observed near synaptic and immune-related genes^{28,32}. Similarly, blood DNAm markers associated with major depressive disorder implicate pathways related to axon guidance, calcium signalling and synaptic plasticity^{29,30}.

Despite increasing interest in DNAm as a biomarker of brain-related phenotypes^{33–36}, relatively little is known about the epigenetic correlates of human cortical morphology. Existing studies linking DNAm to brain structure have been limited by modest sample sizes and minimal replication capacity, leaving open the question of whether epigenetic variation contributes to variation in cortical organisation and its behavioural correlates.

Here, we address this gap through the first large-scale EWAS meta-analysis of cortical morphology. Using data from 7,488 individuals across 20 cohorts participating in the ENIGMA consortium, we tested associations between peripheral blood DNAm and global measures of CT and SA. We complemented single-CpG analyses with differentially methylated region (DMR) analyses and integrative functional annotation, including chromatin-state enrichment, histone marks and gene-expression datasets. To explore potential links with behaviourally relevant outcomes, we further conducted phenome-wide association analyses and Mendelian randomisation analyses examining relationships between DNAm, cortical morphology and major psychiatric disorders, neurodegenerative diseases and educational attainment. Finally, we evaluated the predictive utility of DNAm by deriving methylation profile scores in an independent cohort (workflow in **Fig. S1**). Together, these analyses provide new insights into how environmentally responsive epigenetic processes may contribute to variation in human cortical organisation and its relevance for behaviour and psychiatric vulnerability (see **Fig. 1** for a conceptual framework).

Results

Study participants

The discovery EWAS meta-analyses included 4,925 participants of European ancestry (47.3% male; 29.3% with psychiatric diagnoses), drawn from 11 cohorts spanning ages 17.3–94.2 years (**Tables S1–2**), with DNAm profiled using Illumina EPIC arrays. The replication sample included seven independent European ancestry cohorts of ($N = 2,152$; age range: 8.7–88.5 years; 48.7% male; 20.4% with psychiatric

diagnoses) profiled on the 450K array. Two additional non-European cohorts were examined for replication, including a mixed-ancestry cohort ($N = 195$, 450K) and an African ancestry cohort ($N = 216$, EPIC).

Epigenome-wide associations with cortical morphology

In the discovery meta-analysis, we identified six CpGs associated with average cortical thickness (CT) and five CpGs associated with total surface area (SA) at genome-wide Bonferroni significance ($P < 8.41 \times 10^{-8}$; **Fig. 2**), with consistent effect directions across cohorts (**Fig. S3**). A further 30 CpGs (25 for CT; 5 for SA) surpassed 5% false discovery rate (FDR; **Table S4**). For CT, the strongest signals included cg13518625 (intergenic between *DUSP4* and *SARAF*; $Z = 5.831$, $P = 5.51 \times 10^{-9}$), cg03636183 (*F2RL3*), cg12303084 (*ZMYND8*), cg00024404 (*SERINC5*), and cg21566642 (within the *ALPG/ALPI* cluster), where higher methylation was associated with higher CT, whereas cg10002435 (*NPLOC4*) was negatively associated with CT. For SA, the strongest associations included cg21050046 (*TBC1D5/SATB1*; $Z = -6.54$, $P = 6.17 \times 10^{-11}$), cg23346269 (*KRTAP20-4*), cg01305291 (*MTERFD2/SNED1*), cg03546806 (*LHX5/RBM19*), and cg26845363 (*FAM159A*). All SA-associated CpGs showed negative associations, indicating that higher methylation at these loci was consistently associated with lower total SA. CT- and SA-associated CpGs were non-overlapping, consistent with distinct epigenetic architectures for these cortical metrics.

Regional associations patterns

To assess whether global associations were spatially coherent, we examined regional effects across six major cortical areas for FDR-significant CpGs (**Fig. 3a**; **Table S5**). Most CT-associated CpGs showed consistent effects across regions. For example, cg13518625 (*DUSP4/SARAF*) and cg03636183 (*F2RL3*) were positively associated with CT in all regions, with the strongest effects in the insula, frontal, and parietal cortices; cg10002435 (*NPLOC4*) showed consistently negative associations, strongest in the insula. SA-associated CpGs showed uniformly negative regional associations, with the top site cg21050046 (*TBC1D5*) displaying robust effects in the cingulate, parietal, and frontal cortices. These indicate regionally consistent DNAm correlates of global CT and SA.

Robustness across covariates and subgroups

We tested the robustness of FDR-significant CpGs to key covariates and subgroup stratification (intracranial volume (ICV) adjustment, sex, diagnosis, and age strata; **Fig. 3b**; **Table S6**). For CT, adjustment for intracranial volume (ICV) had negligible effects. Associations were generally attenuated or absent in clinical cases, and several CpGs showed sex-dependent effect sizes (e.g., stronger male effects for cg03636183 (*F2RL3*) and cg00024404 (*SERINC5*)). For SA, effect directions remained consistent, but effect sizes were attenuated after ICV adjustment.

Age-stratified analyses revealed developmentally dynamic DNAm-brain associations (**Fig. 3c**; **Table S6**). For CT, some loci peaked in late adolescence (e.g., cg13518625 (*DUSP4/SARAF*) and cg01015663 (*TCEA3*); $Z = 4.57$ – 4.31) and attenuated in late adulthood ($Z = 0.39$ – 0.62), whereas others peaked in

mid-adulthood (e.g., cg03636183 (*F2RL3*), cg12303084 (*ZMYND8*) and cg21566642 (*ALPG* cluster); $Z = 5.74\text{--}4.48$). SA-associated CpGs also tended to show the strongest effects in late adolescence or mid-adulthood. Reduced effect estimates in late adulthood may partly reflect lower power (late adulthood, $N = 573$).

Replication of EWAS findings

Among discovery CpGs at $FDR < 0.05$, 11 CT-CpGs and 4 SA-CpGs were available on the 450K array for replication. We defined replication a priori as $P < 0.05$ in the independent replication meta-analysis and a stronger association in the combined meta-analysis ($P < 8.41 \times 10^{-8}$). Under this criterion, five CT-associated CpGs replicated (**Table S4**): cg13518625 (upstream of *DUSP4*, linked to MAPK signalling and depression³⁷), cg03636183 (*F2RL3*) (a robust tobacco exposure biomarker³⁸ also linked to lower brain volume and accelerated cognitive decline³⁹), cg12303084 (*ZMYND8*) (a chromatin reader involved in transcriptional responses to genotoxic stress and neurocognitive function^{40–42}), cg21566642 (within the *ALP* cluster on chr2q34-q37), and cg01015663 (*TCEA3*), (a transcription elongation factor). All replicated CpGs showed consistent effect directions, low between-cohort heterogeneity, stronger significance in the combined analysis ($P < 1.8 \times 10^{-10}$ to 1.7×10^{-8}), and fair-to-excellent probe reliability⁴³.

Additionally, cg14284211 (*FKBP5*) showed nominal replication ($P = 0.047$), consistent direction across discovery and replication, and a robust association in the combined analysis ($P < 3.3 \times 10^{-7}$). *FKBP5* methylation has been linked to childhood adversity, stress-related psychiatric outcomes, and structural alterations in fronto-limbic circuitry⁴⁴, supporting its potential relevance to cortical morphology under stress exposure.

In contrast, no SA-associated CpG replicated at nominal significance, although directions were generally consistent. Non-replication likely reflects limited power ($N = 2,152$), platform coverage differences, and/or greater environmental and technical noise in SA phenotypes.

Differentially methylated regions associated with CT and SA

To identify spatially correlated DNAm patterns, we performed DMR analyses, identifying 53 CT-DMRs and 86 SA-DMRs surviving Šidák correction ($P_{\text{Šidák}} < 0.05$; **Fig. S4**; **Table S7**). For CT, the most significant DMRs mapped to *CEP104/DFFB*, *ZMYND8*, *UBE2T* and *MPIG6B* ($P < 10^{-9}$) and overlapped with loci highlighted in single-CpG analyses (e.g., *ZMYND8*), supporting convergence between regional and site-specific signals. In contrast, 95% of SA-DMRs were negative, mirroring the uniform SA CpG directionality. The strongest SA-DMRs mapped to *GAS2L2*, *LPAR5*, and *MPIG6B* ($P < 5 \times 10^{-9}$), and included three DMRs at 17q21.31, spanning *ARHGAP27* and *KANSL1*, a chromatin modifier disrupted in Koolen-de Vries syndrome⁴⁵ and previously linked to cortical morphology and schizophrenia⁴⁶. Only two DMRs (*MPIG6B* and *LY9*) were shared between CT and SA, suggesting limited overlap and a potential/platelet-related component⁴⁷.

Functional characterisation and enrichment analyses

At FDR significance, CpGs associated with cortical morphology were primarily located within gene bodies and intergenic regions, frequently in OpenSea contexts (**Fig. 4a-b**), consistent with non-promoter regulatory mechanisms. Chromatin state analyses in brain tissues (for CpGs with $P < 1 \times 10^{-5}$; **Fig. 4c**; **Table S8-S9**) showed that CT-associated CpGs were depleted in active promoter states, enriched in transcription start site (TSS)-flanking and enhancer-linked states, and enriched for H3K4me1, a canonical enhancer-associated modification, whereas SA-associated CpGs showed enrichment for the repressive mark H3K27me3 in the brain germinal matrix ($P_{FDR} = 0.044$), implicating polycomb-mediated repression in early neurodevelopment. Tissue expression analyses (FUMA⁴⁸) and DNAm-expression correlations across tissues further supported functional relevance, including robust coupling for replicated CT loci (e.g., *ZMYND8*, *TCEA3*, *F2RL3*) (**Tables S10** and **Fig. S4**). Pathway enrichment (KEGG; **Table S11**; **Fig. 4d**) indicated broader enrichment for CT-linked genes spanning environmental sensing/detoxification (e.g. olfactory transduction; xenobiotics and metabolism), cellular adhesion, axon guidance and neuroplasticity-related signalling, whereas SA-linked genes showed a more restricted enrichment profile. Trait enrichment using EWAS Atlas and overlap with prior EWAS signals (**Tables S12–S13**; **Fig. 5a**) indicated strong overrepresentation of CT-associated CpGs linked to smoking, air pollution, IgG glycosylation, cardiometabolic traits, mortality, and cognition, with more restricted enrichments for SA. See **Supplementary Information** for a detailed description of these results.

Phenome-wide associations in youth

In IMAGEN, the largest discovery cohort ($N = 1,191$; mean age = 19.0 ± 0.7 years), two of five replicated CT-CpGs showed Bonferroni-significant PheWAS associations (**Table S14**; **Fig. 5b**). cg03636183 (*F2RL3*) was associated with multiple substance-use phenotypes (lifetime cannabis, cigarettes, total substance use, stimulant use), with higher methylation associated with lower trait levels; cg21566642 (*ALP* cluster) was similarly associated with lifetime cigarette and cannabis use and total lifetime substance use. Beyond substance use, cg03636183 (*F2RL3*) was also negatively associated with negative life events and psychotic-like experiences, consistent with broader psychosocial burden and emerging mental-health vulnerability.

Importantly, cg21566642 remained associated with CT after adjusting for self-reported smoking ($b = 0.203$, $P = 0.034$; $b = 0.207$, $P = 0.036$ before and after adjustment, respectively), and showed evidence of smoking-related effect modification, with DNAm-CT associations present in non-smokers but absent in smokers (**Fig. 5c**). Other replicated CpGs showed nominal associations with smoking and cognitive/mental health traits, but none survived Bonferroni correction.

Causal inference via Mendelian randomisation and cross-tissue concordance

Bi-directional Mendelian randomisation (MR) tested putative causal relationships between DNAm (Bonferroni EWAS CpGs or Sidák DMRs) and cortical morphology, psychiatric and neurodegenerative disorders, and educational attainment (EA). Forward MR using cis-meQTL instruments identified multiple

CpGs with putative causal effects on CT or SA (**Table S15; Fig. 6a**), supported by Steiger filtering and generally little evidence of horizontal pleiotropy across sensitivity analyses (**Table S16**). The strongest CT signal was for cg12568669 (*FDFT1*), where increased methylation had a robust negative causal effect on CT ($\beta = -0.036$, $P_{FDR} = 6.4 \times 10^{-9}$) and showed pleiotropic associations across outcomes (reduced schizophrenia (SCZ)/and bipolar disorder (BD) risk; increased Alzheimer's disease (AD) risk; higher EA). Reverse MR further indicated that SCZ, BD, and EA exerted putative causal influences on DNAm at cg12568669 (**Table S17; Fig. 6b**), consistent with bi-directional relationships.

For SA, the strongest signals mapped to 17q21.31 (*KANSL1/ARHGAP27*), where increased methylation at cg08113562 (*ARHGAP27*), cg26855231 (*KANSL1*) and cg14374015 (*KANSL1*) was causally associated with reduced SA, whereas a nearby *KANSL1* site (cg02301815) showed the opposite direction, highlighting site-specific heterogeneity. DNAm at these loci showed divergent associations with outcomes, and reverse MR suggested feedback from SA, AD risk and for select sites. Additional SA-associated CpGs at *PPP2R3A* and *MPIG6B* (*C6orf25*) showed positive causal effects on SA and pleiotropic associations with MDD, BD, SCZ and EA, with evidence of reverse effects for SCZ and EA, respectively.

A substantial proportion of CpGs reaching FDR significance in forward MR analyses demonstrated moderate blood-brain DNAm concordance ($r \approx 0.25-0.60+$) across cortical regions (**Fig. 6c; Table S18**), supporting the biological plausibility of peripheral methylation at these sites as proxies of regulatory variation relevant to cortical structure and neuropsychiatric traits.

Methylation profile scores

To evaluate whether aggregate DNAm variation contributes to interindividual differences in cortical morphology, we constructed methylation profile scores (MPS) in an independent sample (ALSPAC; $N = 571$) using discovery EWAS weights. Because EWAS summary statistics preclude data-driven P -value threshold optimisation and more polyepigenetic traits may benefit from broader inclusion of ranked CpGs⁴⁹, we generated sequential MPS using cumulative CpG sets (top 20 to 10,000 CpGs).

CT-based MPS showed no predictive utility for average CT (all $R^2 < 0.001$, $P > 0.4$). In contrast, the SA-based MPS (top 50 CpGs) explained 0.76% of the variance in SA ($R^2 = 0.76\%$; $P = 2.8 \times 10^{-3}$; **Table S19**), with attenuated performance at broader thresholds. Although modest, this is notable given summary-statistic training and is comparable in scale to early-stage polygenic score performance for cortical morphology from a much larger sample²¹.

Discussion

Understanding how environmental exposures become biologically embedded to shape brain structure and mental-health vulnerability is a central challenge in behavioural science and psychiatry. In this large-scale epigenome-wide meta-analysis of >7,400 individuals across 20 ENIGMA cohorts, we identify

peripheral DNA methylation (DNAm) signatures associated with global CT and SA. The largely non-overlapping DNAm architectures observed for CT and SA mirror their well-established developmental and genetic dissociation, suggesting that distinct epigenetic regulatory programmes may link environmental exposure to variation in cortical morphology.

Our findings further indicate that these epigenetic programmes differ in their environmental sensitivity and developmental timing. CT-associated CpGs were enriched in regulatory elements and pathways implicated in stress-responsive signalling, immune processes and metabolic regulation, and overlapped with DNAm markers previously linked to smoking, air pollution and psychosocial stress. By contrast, SA-associated loci clustered primarily within chromatin-regulatory regions implicated in early cortical development, particularly within the 17q21.31 locus spanning *KANSL1* and *ARHGAP27*. Together, these results suggest that DNAm variation may reflect two complementary axes of cortical regulation: an environmentally responsive pathway influencing cortical thickness and a developmentally constrained pathway influencing cortical SA. By integrating epigenomic, neuroimaging and phenome-wide analyses across international population cohorts, this study provides evidence that peripheral DNAm captures exposure-sensitive biological processes linked to human cortical organisation and psychiatric vulnerability. These findings support a framework in which DNAm acts as a scalable molecular interface between environmental exposure, brain structure and behavioural outcomes.

Environmentally sensitive regulation of cortical thickness

Five CT-associated CpGs replicated across independent cohorts: cg13518625 near *DUSP4/SARAF*, cg03636183 (*F2RL3*), cg12303084 (*ZMYND8*), cg01015663 (*TCEA3*), and cg21566642 within the *ALP* gene cluster. These loci implicate DNAm variation in stress-responsive MAPK signalling⁵⁰, calcium homeostasis^{51,52}, chromatin remodelling⁵³, and transcriptional elongation – pathways plausibly relevant to cortical plasticity.

Two replicated CpGs, cg03636183 (*F2RL3*) and cg21566642 (*ALPG*), are among the most robust epigenetic correlates of smoking, pollution, and substance exposure^{54–61}. In our study, both were associated with lifetime smoking, cannabis use and total substance use in adolescence, and cg03636183 was additionally linked to negative life events and psychotic-like experiences. cg21566642-CT associations persisted after smoking adjustment, and cg21566642 showed evidence of DNAm × smoking interaction, consistent with exposure-contingent modulation of DNAm-brain associations. These findings suggest that CT-linked DNAm captures cumulative environmental and psychosocial burden relevant to mental-health vulnerability.

Other replicated and DMR loci point to transcriptional regulation and stress systems. *ZMYND8* (*RACK7*) encodes a chromatin reader that constrains enhancer activation by regulating H3K4me1/H3K4me3 dynamics⁶², whose pathogenic mutations result in neurocognitive deficits⁷⁴ and anxiety-related behaviours⁶⁴. *FKBP5*, a glucocorticoid receptor co-chaperone central to hypothalamic-pituitary-adrenal axis regulation, has genetic variation associated with antidepressant response and recurrence of

depressive episodes⁶⁵. A CT-associated DMR mapped to *COMMD7*, which suppresses TNF-mediated NF- κ B activation⁶⁶ and has been implicated in immune-related regulatory networks⁶⁷. Together, these loci converge on stress-responsive, immune-modulated, and enhancer-linked transcriptional regulation – mechanisms increasingly implicated in psychiatric risk.

Distinct developmental architecture of surface area

A striking finding is the dissociation between CT- and SA-associated DNAm. CT showed predominantly positive and heterogeneous DNAm-CT associations, whereas SA showed uniformly negative DNAm-SA associations. CT-associated CpGs were enriched in enhancer and TSS-flanking regions and for H3K4me1 in adult brain tissues, consistent with context-dependent regulation active during later development or in response to environmental signals⁶⁸. By contrast, SA-associated CpGs clustered into DMRs, most prominently at 17q21.31, spanning *KANSL1* and *ARHGAP27*, and were enriched for the repressive mark H3K27me3 in the germinal matrix. This pattern is consistent with Polycomb-mediated regulation during progenitor proliferation and early cortical expansion, in line with the radial unit hypothesis and the strong GWAS evidence for genetic determination of SA²¹. *KANSL1* encodes a critical chromatin modifier whose pathogenic mutations cause 17q21.31 microdeletion (Koolen–de Vries) syndrome with intellectual disability and structural brain abnormalities^{45,69}. Mouse models of 17q21.31 deletion, duplication, and *Kansl1* haploinsufficiency show alterations in brain volumes and cognitive deficits⁷⁰. Opposing anatomical effects in deletion versus duplication models parallel the site-specific MR effects observed here, supporting dosage-sensitive chromatin regulation as a key mechanism for SA.

These findings delineate two epigenetic axes through which environmental and developmental processes may influence cortical organisation and behavioural vulnerability: an early developmental, chromatin-based pathway influencing SA, and an environmentally sensitive pathway influencing CT.

Environmental, behavioural, and mental-health relevance

Trait enrichment analyses showed that CT-associated CpGs are embedded within DNAm signatures linked to smoking, air pollution, IgG glycosylation, cardiometabolic traits, mortality, and cognitive outcomes. KEGG analyses further highlighted enrichment for pathways related to environmental exposures, xenobiotic metabolism, and notably the olfactory system. This is conceptually important, as olfaction constitutes a primary sensory interface through which atmospheric and ecological conditions influence emotion regulation, stress responsivity, cognitive function, social behaviour, and depressive symptoms, while also modulating inflammatory processes⁷¹. Given the direct projections of olfactory inputs to limbic, insular, and prefrontal regions⁷², enrichment of CT-associated CpGs in olfactory-related pathways provides a plausible mechanism linking environmental exposures to cortical circuits implicated in affect regulation and stress processing⁷³. Notably, these same regions, particularly the prefrontal cortex and insula, showed the strongest regional methylation-CT associations in our analyses, reinforcing the biological coherence of this exposure-sensitive pathway. Moreover, longitudinal neuroimaging studies indicate that prefrontal and insula volume reductions mediate associations

between air pollution exposure and depressive symptom trajectories⁷⁴. Together, these patterns suggest that CT-linked DNAm signatures may reflect biologically embedded responses to the sensory and toxicological environment with downstream implications for affective and cognitive health.

Pleiotropy and cross-tissue regulatory architecture

Cis-meQTL-based Mendelian randomisation supported putative causal and bidirectional associations between DNAm, cortical morphology, and neuropsychiatric outcomes, particularly for CpGs embedded within developmentally and metabolically relevant loci. Rather than indicating disorder-specific causal pathways, the convergence of MR signals across schizophrenia, bipolar disorder, Alzheimer's disease, educational attainment, and cortical measures suggests pleiotropic upstream regulatory processes that couple brain structure with psychiatric and cognitive phenotypes. Given the environmental responsiveness of several loci (including to smoking), we interpret MR as supporting shared pleiotropic regulatory pathways rather than disorder-specific effects.

To address tissue specificity, cross-tissue analyses (**Fig. 6c**) showed that many MR-prioritised CpGs exhibit moderate concordance between blood and cortical tissues, including prefrontal, entorhinal, and superior temporal cortices ($R = 0.25\text{--}0.60$). Loci such as *FDFT1*, *KANSL1*, *PPP2R3A*, *HKR1*, *ARTN*, and *VAR2* demonstrated consistent cross-tissue coupling. Because these CpGs were instrumented using cis-meQTLs, this concordance is consistent with partially shared genetic regulation of methylation across tissues, supporting a cross-tissue cis-regulatory architecture rather than purely blood-specific signals. Although concordance varied in magnitude and direction, particularly at 17q21.31, this heterogeneity likely reflects tissue-dependent chromatin context and cellular composition.

For CT, cg12568669 (*FDFT1*), showed a negative putative causal effect on CT alongside pleiotropic associations with SCZ, BD, AD, and educational attainment. *FDFT1* encodes squalene synthase in cholesterol biosynthesis; pathogenic variants cause a neurodevelopmental disorder with structural brain malformations and global developmental delay⁷⁵, and the gene has been implicated in AD^{76,77}. *FDFT1* transcripts are also susceptible to air pollution-induced oxidative modification⁷⁸, linking exposure-sensitive metabolic regulation to brain structure and ageing-related vulnerability. Together, these observations suggest that CT-associated DNAm may index upstream exposure-linked metabolic and inflammatory regulation embedded within partially conserved regulatory networks spanning blood and cortex, with downstream relevance for psychiatric and neurodegenerative risk.

Translational implications and limitations

Methylation profile scores explained ~0.8% of variance in SA in an independent cohort, paralleling early-stage polygenic scores for cortical morphology⁷⁹ and supporting the feasibility of DNAm-based markers for brain-structural phenotypes. Although modest, such markers could complement genetic risk scores in exposure-informed stratification.

Limitations include reliance on whole-blood DNAm, predominantly European-ancestry discovery samples, and platform differences between EPIC and 450K arrays. Although MR reduces confounding, tissue specificity and residual pleiotropy cannot be excluded, particularly for exposure-sensitive loci.

Conclusion

This large-scale ENIGMA meta-analysis identifies peripheral DNAm signatures associated with CT and SA that map onto dissociable regulatory programmes. The results are consistent with a dual-axis framework of cortical organisation: an early developmental, chromatin-linked axis influencing SA, exemplified by loci at 17q21.31 including *KANSL1*; and a later, environmentally responsive axis influencing CT, enriched for enhancer-linked and stress-related regulatory elements.

MR and cross-tissue analyses suggest that prioritised CpGs reflect partially shared regulatory architecture across blood and cortex and are associated with transdiagnostic psychiatric outcomes, including schizophrenia, bipolar disorder and major depressive disorder. These findings are consistent with pleiotropic regulatory processes through which environmental exposures may become biologically embedded and relate to cortical morphology and mental-health risk.

Together, this work positions peripheral DNAm as an accessible marker of regulatory variation linking environmental context, cortical structure and psychiatric vulnerability.

Methods

Cohorts

All cohorts from the ENIGMA-Epigenetics Working Group obtained ethical approval from local research ethics committees or institutional review boards. Written informed consent was obtained from all participants or their legal guardians in accordance with the Declaration of Helsinki.

The discovery dataset comprised 11 cohorts of European ancestry, including 4 population-based cohorts and 7 psychiatric case-control cohorts. DNAm data were assayed in whole blood using the Illumina Infinium MethylationEPIC array (v1.0 or v2.0; Illumina, Inc., San Diego, CA, USA). An independent replication sample of European ancestry ($N = 2,152$), profiled using the Illumina HumanMethylation450 BeadChip (Illumina, Inc., San Diego, CA, USA), was used to replicate EWAS findings. Contributing studies and sample characteristics are provided in **Table S1**.

Neuroimaging measures

Structural MRI data were acquired locally and processed using a harmonised ENIGMA protocol to ensure cross-cohort comparability²¹ (**Table S2; Supplementary Information**). CT and SA were derived with FreeSurfer using the Desikan-Killiany atlas (34 regions per hemisphere) and aggregated to derive global measures of average CT and total SA.

DNAm preprocessing and quality control

DNAm was measured in whole blood using the Illumina EPIC array (v1.0 or v2.0) in discovery cohorts and the Illumina 450K array in replication cohorts. Raw intensity data were preprocessed using the *preprocessQuantile* function from the *minfi* R package⁸⁰. Methylation was quantified as β -values (range 0-1).

The EPIC v1.0 and v2.0 arrays profile >850,000 and >900,000 CpGs, respectively, with substantial probe overlap ($\sim 77.6\%$)⁸¹ and expanded coverage of regulatory regions^{82–84}. The 450K array interrogates >480,000 CpGs⁸⁵. High reproducibility across platforms has been demonstrated previously^{86–88}.

Epigenome-wide association studies (EWASs)

EWASs were conducted independently within each cohort for global and regional CT and SA, including lobe-wise measures (frontal, temporal, occipital, parietal, cingulate, and insula). Linear regression models were fitted at each CpG with DNAm β -value as the outcome and cortical measures as the predictor. Covariates included age, age², sex, diagnostic status (where applicable), scanner site (where applicable), batch variables (where applicable), the first four DNAm principal components, and the first two principal components of estimated blood cell composition (CD4⁺, CD8⁺, NK, B cell, monocytes, neutrophils). Generalised estimating equations were used in twin cohorts to account for familial relatedness.

EWAS quality control (QC)

Cohort-level EWAS summary statistics were harmonised centrally using the *QCEWAS* R package⁸⁹ and reported in **Fig. S2**. Probes overlapping known SNPs, non-CpG probes, and cross-reactive probes were excluded^{90,91}. Inflation and bias were corrected using the *Bacon* R package⁹² and reported in **Table S3**. Full QC procedures are detailed in the **Supplementary Information**.

Meta-analysis of average CT and total SA

Meta-analyses were performed across all discovery cohorts using CpGs present in all datasets (594,724 probes). EWAS summary statistics were combined using a fixed-effects, sample size-weighted model in METAL⁹³. Between-study heterogeneity was assessed using Cochran's Q statistic. Statistical significance was defined using a Bonferroni-corrected threshold ($P < 8.41 \times 10^{-8}$), with false discovery rate correction applied in secondary analyses. Sensitivity analyses stratified by sex and diagnostic status were conducted (**Supplementary Information**).

Region-specific and stratified analyses

Lobe-wise EWAS were conducted for frontal, parietal, temporal, occipital, cingulate, and insular CT and SA, using the same modelling framework. Stratified analyses were additionally performed by sex, case-

control status, and with or without adjustment for intracranial volume (ICV). Results were meta-analysed across cohorts, and consistency of effect direction and significance was assessed to evaluate robustness.

Replication analysis

Replication of EWAS findings in the independent European sample ($N = 2,152$; 450k array) was assessed for CpGs that reached Bonferroni significance ($P < 8.41 \times 10^{-8}$) in the discovery meta-analysis and were also present on the 450k array (#CpGs: $N_{CT} = 11$; $N_{SA} = 4$). Replication was considered successful if (i) the replication p -value reached nominal significance ($P < 0.05$) in the independent meta-analysis, and (ii) stronger effect sizes, smaller p -values, and $P < 8.41 \times 10^{-8}$ were observed in the meta-analysis combining discovery and replication cohorts.

Differentially methylated regions (DMRs)

We complemented the single-CpG EWAS analyses by investigating the associations of aggregated methylation signals across contiguous loci with CT and SA. DMRs were identified using *comb-p*^{94,95}, accounting for spatial correlation between adjacent CpG sites. Parameters used were a 500 bp autocorrelation window and a seed P -value < 0.01 . DMRs containing at least two CpGs and significant regional P -values after correction for multiple testing (Šidák correction, $P_{\text{Šidák}} < 0.05$) were considered significant and reported. For these analyses, genomic annotations were obtained using the Genomic Regions Enrichment of Annotations Tool (GREAT version 4.0.4)⁹⁶. Genes were linked to DMRs using the “basal plus extension” method in GREAT, which defines proximal regulatory regions as 5 kb upstream to 1 kb downstream of the TSS and distal regions as up to 1,000 kb from the TSS. All annotations were based on the hg19 reference genome.

Given the different density and CpG composition of the EPIC and 450k arrays, which influence the identification of spatially correlated CpGs, we did not seek to replicate DMR findings.

Functional characterisation and enrichment analyses

Genomic annotations: FDR-significant CpGs associated with CT and SA were annotated using Illumina EPIC array annotation files. Gene-centric location was defined according to UCSC RefGene group annotations and classified as promoter (TSS200, TSS1500, or 5'UTR), gene body (Body or 3'UTR), promoter-and-body (mapped to both), or intergenic (mapped to neither promoter nor body). CpGs were additionally categorised by CpG island context (island, north/south shore, north/south shelf, or open sea) using the *Relation_to_Island* field, and by genic status (genic vs intergenic) based on whether a RefGene gene annotation. Annotation frequencies were summarised separately for CT- and SA-associated CpGs and visualised as bar plots.

For downstream enrichment analyses, CpGs showing suggestive evidence of association ($P < 1 \times 10^{-5}$; CT: $N = 68$; SA: $N = 52$) were included. The $P < 1 \times 10^{-5}$ threshold was selected to enhance sensitivity for

detecting coordinated biological patterns across loci while maintaining stringency beyond nominal significance. Enrichment analyses are designed to evaluate convergence across multiple modest-effect CpGs within shared regulatory and transcriptional contexts, rather than to validate individual associations. Restricting analyses to only Bonferroni-significant CpGs would substantially reduce power and limit detection of distributed epigenetic architecture. Accordingly, these enrichment findings should be interpreted as systems-level, hypothesis-generating evidence of regulatory organisation rather than definitive confirmation of specific causal mechanisms.

Chromatin state and histone mark enrichment: To characterise functional genomic context, enrichment of chromatin states and histone marks was assessed using epigenomic annotations from the NIH Roadmap Epigenomics Mapping Consortium. Chromatin states were defined using the core 15-state ChromHMM model

(https://egg2.wustl.edu/roadmap/web_portal/chr_state_learning.html#core_15state), which integrates multiple histone modifications to classify genomic regions into active promoters, enhancers, transcribed regions, Polycomb-repressed regions, and quiescent states. Enrichment analyses were conducted separately for CT- and SA-associated CpGs across brain tissues (including hippocampus, anterior caudate, and cingulate gyrus). Overlap between CpGs and chromatin annotations was assessed relative to the background set of all CpGs tested in the EWAS. Enrichment was quantified as the observed-to-expected overlap ratio, and statistical significance was evaluated using Fisher's exact tests with FDR correction across tissues and chromatin states. Results were visualised as heatmaps of enrichment ratios.

Tissue-specific gene expression: Genes mapped to CT- associated ($N = 114$) and SA-associated ($N = 84$) CpGs were analysed using the GENE2FUNC module of FUMA (Functional Mapping and Annotation)⁴⁸, using default parameters. Enrichment was tested against differentially expressed gene (DEG) sets across 54 tissues in the GTEx v8 dataset⁹⁷. Analyses were conducted separately for all DEGs (both sides), as well as for downregulated-only and upregulated-only gene sets (**Fig. S5**). Differential expression was defined based on mean expression ($\log_2$ TPM), and enrichment was evaluated using hypergeometric tests with FDR correction across tissues.

DNAm-gene expression correlations: Associations between CpG methylation and gene expression were examined using the EWAS Toolkit⁹⁸ across six tissues (brain, colon, kidney, liver, stomach, and testis). For each CpG, correlations between methylation levels and the expression of nearby genes were assessed to evaluate potential transcriptional coupling.

KEGG pathway enrichment: KEGG pathway enrichment analyses were performed separately for CT- and SA-linked using the MetaBrain platform (<https://www.metabrain.nl/>)⁹⁹. Enrichment was tested using hypergeometric tests, and pathways with FDR-adjusted $P < 0.05$ were considered significant.

Trait enrichment analyses: We used the EWAS Toolkit⁹⁸ to assess whether CpGs associated with cortical CT or SA were enriched for previously reported associations with complex traits and diseases. CpG sets

were submitted to the EWAS Toolkit open platform (<https://ngdc.cncb.ac.cn/ewas/toolkit>), which integrates results from published epigenome-wide association studies curated in the EWAS Atlas¹⁰⁰. Enrichment was evaluated by quantifying the overlap between CT- or SA-associated CpGs and CpGs previously linked to specific traits. Statistical significance was assessed using a weighted Fisher's exact test, in which CpG-specific weights reflected the number of independent studies reporting each CpG-trait association (with a default weight of 1 for CpGs without prior reports).

EWAS Catalog look-up: To identify overlap with previously reported EWAS signals, CpGs associated with cortical morphology at $P < 1 \times 10^{-5}$ were queried against the EWAS Catalog database (<https://www.ewascatalog.org/>)¹⁰¹. Word clouds were generated for CT- and SA-associated CpGs to summarise prior trait associations, with term size reflecting the frequency of reported associations in the EWAS Catalog (**Fig. S6**).

Phenome-wide association studies (PheWAS)

PheWAS were conducted to assess the broader phenotypic relevance of the five replicated CT-associated CpGs in the IMAGEN cohort ($N = 1,191$; mean age = 19.02 ± 0.73 years). IMAGEN was selected because it provides a large, well-characterised sample with harmonised neuroimaging, DNA methylation, and extensive behavioural, clinical, and environmental phenotyping collected at the same developmental stage.

Associations were tested across 152 phenotypes spanning anthropometric, cognitive, behavioural, psychological, and environmental variables. Full variable descriptions are included in the **Supplementary methods**. For each CpG, linear regression models were fitted with methylation β -values as the predictor and individual phenotypes as the outcomes. Covariates included age, age², sex, scanner site, the first four principal components of DNAm, and the first two principal components of estimated blood cell composition (CD4⁺, CD8⁺, NK cells, B cells, monocytes, neutrophils). The number of principal components included was chosen to capture the major sources of technical and biological variation in methylation and cell composition while minimising collinearity and overfitting, consistent with standard practice in large-scale EWAS³⁴. Multiple testing was controlled using Bonferroni correction across all CpGs and phenotypes, with a significance threshold of $P < 0.05/(152 \times 5)$.

Mendelian randomisation (MR)

Bi-directional two-sample MR analyses were conducted to investigate putative causal relationships between DNAm at CT- or SA-associated CpG sites and brain-related phenotypes. In forward MR analyses, CpGs were included if they reached Bonferroni significance in the discovery EWAS or were part of Sidák-corrected DMRs, yielding 195 CT-associated and 269 SA-associated CpGs. These CpGs were tested for putative causal effects on : (i) global cortical measures (average CT and total SA); (ii) major psychiatric disorders, including schizophrenia (SCZ), bipolar disorder (BD), and major depressive disorder (MDD); (iii) neurodegenerative diseases, specifically Alzheimer's disease (AD) and Parkinson's disease (PD); and

(iv) educational attainment (EA). FDR correction was applied separately for each outcome. Reverse MR analyses were subsequently performed to assess whether these phenotypes exerted putative causal effects on DNAm at the same CpG sites.

Instrumental variables (IVs) for DNAm were defined as genome-wide significant ($P < 5.0 \times 10^{-8}$) *cis*-methylation quantitative trait loci (*cis*-meQTLs) identified from publicly available whole-blood datasets comprising 3,799 individuals of European ancestry⁶⁷. SNPs were clumped using European-ancestry linkage disequilibrium (LD) reference data from the 1000 Genomes Project Phase 3 ($r^2 < 0.01$ within a 250 kb window). Consistent with prior evidence demonstrating substantial cross-tissue concordance of *cis*-meQTL effects and relative stability across cell types⁶⁷, these blood-based *cis*-meQTLs were assumed to capture regulatory variation that is at least partly shared across tissues, including the brain.

GWAS summary statistics for cortical measures (CT and SA)²¹, SCZ¹⁰², BD¹⁰³, MDD¹⁰⁴, AD¹⁰⁵, PD¹⁰⁶, and EA¹⁰⁷ were obtained from the largest available consortia-based studies. For cortical measures, these GWASs include partial sample overlap with the EWAS cohorts contributing to the present study. While such overlap can bias MR estimates towards the observational association and inflate precision, especially for traits with strong genetic instruments, it does not induce spurious causal directionality. Accordingly, causal estimates involving CT and SA were interpreted cautiously as supporting putative, rather than definitive, causal relationships, with greater emphasis placed on consistency of effect direction, robustness across sensitivity analyses, and concordance between forward and reverse MR.

The primary MR analyses were conducted using the inverse variance weighted (IVW) method¹⁰⁸. Robustness was evaluated using MR-Egger regression, weighted median, simple mode, and weighted mode estimators, applied when at least three independent IVs were available. Sensitivity analyses were restricted to CpG-outcome pairs reaching FDR significance in IVW analyses (**Table S16**). Heterogeneity across instruments was assessed using Cochran's Q statistic, and directional horizontal pleiotropy was evaluated using the MR-Egger intercept¹⁰⁹. For CpGs instrumented by a single SNP, causal estimates were derived using the Wald ratio method.

To ensure adequate instrument strength and mitigate bias due to weak instruments, *F*-statistics were calculated for all IVs, and only instruments with $F > 10$ were retained for analysis¹¹⁰. To further assess the directionality of effects and reduce the risk of reverse causation, Steiger filtering was applied¹¹¹, testing whether each genetic instrument explained a greater proportion of variance in the exposure than in the outcome.

Blood-brain DNA methylation concordance

To evaluate the cross-tissue relevance of CpGs prioritised by MR, we assessed concordance of DNA methylation levels between peripheral blood and brain tissue using the publicly available Blood-Brain DNA Methylation Comparison Tool (<http://epigenetics.essex.ac.uk/bloodbrain/>). This resource provides correlation estimates between matched blood and postmortem brain samples across several cortical

regions, including prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG), and cerebellum (CER).

Analyses were restricted a priori to CpGs showing FDR-significant associations in forward MR analyses with CT, SA, or neuropsychiatric and cognitive outcomes. For each CpG, Pearson correlation coefficients (r) between blood and brain DNAm levels and their P -values were extracted and used to provide locus-specific evidence of cross-tissue concordance and to support the biological plausibility of peripheral DNAm as a proxy for regulatory variation relevant to cortical structure. CpGs with at least nominal significance ($P < 0.05$) in one or more brain tissues in the original dataset are reported; blood-brain correlations are reported descriptively to inform interpretation of MR findings.

Methylation profile scores

To examine whether EWAS signals aggregate into predictive epigenetic signatures for cortical morphology, we constructed methylation profile scores (MPS) using effect-size weights from the discovery EWAS meta-analysis. Standard approaches for constructing risk scores typically rely on applying fixed, optimised P -value thresholds to identify sets of CpGs to include in the score. Because our EWAS only provided summary statistics, the use of cross-validation or permutation-based optimisation is not feasible. In addition, for traits influenced by many small-effect features, scores often benefit from incorporating a broader set of top-ranked associations, particularly when the aim is to maximise explained variance rather than build a minimal predictive panel⁴⁹. Therefore, we applied sequential ranked inclusion of CpGs. CpGs were ordered by EWAS significance, and cumulative MPS were constructed at increasingly inclusive cutoffs (top 20, 50, 100, 500, 1,000, 5,000, and 10,000 CpGs). This strategy allows the score to balance signal inclusion against noise, while avoiding arbitrary significance thresholds that may not align with the underlying epigenetic architecture. For each cutoff, MPS were calculated in the validation cohort (ALSPAC; $N = 571$) as the sum of individual CpG methylation beta values multiplied by the corresponding EWAS effect-size estimate.

Associations between the MPS and cortical traits (average CT or total SA) were tested using linear regression models adjusting for age, age², estimated cell composition, batch, and technical covariates. Predictive performance was quantified as the incremental variance explained (ΔR^2) above covariates.

Declarations

Data availability

The summary statistics will be made available at the ENIGMA consortium webpage: <https://enigma.ini.usc.edu/research/download-enigma-gwas-results/> upon the publication of the study. Cohort-level data can be shared upon reasonable request, after permission from the cohort principal investigators.

Code availability

The code for processing individual cohorts' MRI preprocessing can be found on:

<https://enigma.ini.usc.edu/protocols/imaging-protocols/>. The code for processing individual cohort preprocessing and quality control protocol for DNA methylation data is available at:

<https://github.com/XinyangYu918/Meta-analysis-of-cortical-EWAS>. Publicly available software tools were used to perform analyses and are referenced throughout the paper.

Author contribution statement

X.Y. and S.D. conceptualised the study. X.Y. and A.W. conducted the core analyses. X.Y. and S.D. wrote the first draft of the paper. D.I.B., V.D.C., A.C., C.A.M.C., S.R.C., U.D., J.M.F., A.R.H., J.H., T.K., K.A.M., A.M.-L., P.B.M., T.E.M., I.N., Z.P., P.S.S., P.G.S., A.J.S., P.R.S., G.S., H.V., D.J.S., and H.J.Z. were the principal investigators of participating cohorts. N.A., E.A., C.B., T.B., G.J.B., E.B., A.L.W.B., T.B., U.B., R.B., R.B., D.C., I.C., J.F.F., K.F., H.F., A.J.F., V.F., H.G., P.G., H.J.G., A.G., D.G., T.H., S.H.-H., A.H., P.H., S.H., N.H., A.H., T.J., H.L., J.-L.M., M.-L.P.M., C.M.M., S.L.M., A.M., R.L.M., F.N., M.M.N., D.P.O., T.P., J.-B.P., R.P., L.P., M.N.S., F.S., D.S.S., L.T., R.T., S.I.T., P.U., N.V., H.V., H.W., J.M.W., R.W., P.W., and K.W. collected the imaging and methylation data. A.W., I.K.S., F.S.D., S.A., M.A.-N., N.J.A., J.C., E.L.S.C., B.C.-F., D.C., S.H.-S., K.M.J., J.J., A.R.K., T.L.K., S.L., J.L., K.N., B.J.O., S.L.R., G.R., R.R.-G., J.S., K.S., D.v't.E., J.v.D., and T.Z. processed individual cohort data. S.D. finalised the manuscript with intellectual input from A.W., I.K.S., F.S.D., S.L., J.v.D., V.D.C., K.A.M., S.R.C., J.M.F., C.A.M.C., P.G.S., A.T., S.I.T., and P.M.T. All authors approved the final version of the manuscript.

Competing interests

T.B. served in an advisory or consultancy role for AGB Pharma, eye level, Infectopharm, Medice, Neurim Pharmaceuticals, Oberberg GmbH and Takeda. He received conference support or speaker's fee by Janssen-Cilag, Medice and Takeda. He received royalties from Hogrefe, Kohlhammer, CIP Medien and Oxford University Press; the present work is unrelated to these relationships. G.J.B. has received honoraria from General Electric Healthcare for teaching on scanner programming courses. L.P. served in an advisory or consultancy role for Roche and Vifor Pharma and received speaker's fee by Shire. She received royalties from Hogrefe, Kohlhammer and Schattauer. The present work is unrelated to the above grants and relationships. The other authors declare no competing interests. H.J.G. has received travel grants and speakers honoraria from Neuraxpharm, Servier, Indorsia and Janssen Cilag.

References

1. Faherty, T., Ellis-Bradford, L.-J. A., Onyeaka, H., Harrison, R. M. & Pope, F. D. Reframing air pollution as a cognitive and socioeconomic risk. *Npj Clean Air* **2**, 18 (2026).

2. Luby, J. *et al.* The effects of poverty on childhood brain development: the mediating effect of caregiving and stressful life events. *JAMA Pediatr.* **167**, 1135–1142 (2013).
3. Tost, H., Champagne, F. A. & Meyer-Lindenberg, A. Environmental influence in the brain, human welfare and mental health. *Nat. Neurosci.* **18**, 1421–1431 (2015).
4. EEA Briefing. Pollution and mental health: current scientific evidence. TH-01-26-006-EN-Q - ISBN: 978-92-9480-757-1 - ISSN: 2467-3196 (2026) doi:10.2800/0818218.
5. Polemiti, E. *et al.* How does the macroenvironment influence brain and behaviour-a review of current status and future perspectives. *Mol. Psychiatry* **29**, 3268–3286 (2024).
6. Tooley, U. A., Bassett, D. S. & Mackey, A. P. Environmental influences on the pace of brain development. *Nat. Rev. Neurosci.* **22**, 372–384 (2021).
7. Ferschmann, L., Bos, M. G. N., Herting, M. M., Mills, K. L. & Tamnes, C. K. Contextualizing adolescent structural brain development: Environmental determinants and mental health outcomes. *Curr. Opin. Psychol.* **44**, 170–176 (2022).
8. Natu, V. S. *et al.* Apparent thinning of human visual cortex during childhood is associated with myelination. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 20750–20759 (2019).
9. Norbom, L. B. *et al.* New insights into the dynamic development of the cerebral cortex in childhood and adolescence: Integrating macro- and microstructural MRI findings. *Prog. Neurobiol.* **204**, 102109 (2021).
10. Paolicelli, R. C. *et al.* Synaptic pruning by microglia is necessary for normal brain development. *Science* **333**, 1456–1458 (2011).
11. Walhovd, K. B., Fjell, A. M., Giedd, J., Dale, A. M. & Brown, T. T. Through Thick and Thin: a Need to Reconcile Contradictory Results on Trajectories in Human Cortical Development. *Cereb. Cortex N. Y. N 1991* **27**, 1472–1481 (2017).
12. Hill, J. *et al.* A Surface-Based Analysis of Hemispheric Asymmetries and Folding of Cerebral Cortex in Term-Born Human Infants. *J. Neurosci.* **30**, 2268–2276 (2010).
13. Rakic, P. Evolution of the neocortex: Perspective from developmental biology. *Nat. Rev. Neurosci.* **10**, 724–735 (2009).
14. Reillo, I., de Juan Romero, C., García-Cabezas, M. Á. & Borrell, V. A Role for Intermediate Radial Glia in the Tangential Expansion of the Mammalian Cerebral Cortex. *Cereb. Cortex* **21**, 1674–1694 (2011).
15. Bethlehem, R. a. I. *et al.* Brain charts for the human lifespan. *Nature* **604**, 525–533 (2022).
16. Ge, R. *et al.* Normative modelling of brain morphometry across the lifespan with CentileBrain: algorithm benchmarking and model optimisation. *Lancet Digit. Health* **6**, e211–e221 (2024).
17. Lyall, A. E. *et al.* Dynamic Development of Regional Cortical Thickness and Surface Area in Early Childhood. *Cereb. Cortex N. Y. N 1991* **25**, 2204–2212 (2015).
18. Wierenga, L. M., Langen, M., Oranje, B. & Durston, S. Unique developmental trajectories of cortical thickness and surface area. *NeuroImage* **87**, 120–126 (2014).

19. Panizzon, M. S. *et al.* Distinct genetic influences on cortical surface area and cortical thickness. *Cereb. Cortex N. Y. N* 1991 **19**, 2728–2735 (2009).
20. Strike, L. T. *et al.* Genetic Complexity of Cortical Structure: Differences in Genetic and Environmental Factors Influencing Cortical Surface Area and Thickness. *Cereb. Cortex N. Y. NY* **29**, 952–962 (2019).
21. Grasby, K. L. *et al.* The genetic architecture of the human cerebral cortex. *Science* **367**, eaay6690 (2020).
22. Brainstorm Consortium *et al.* Analysis of shared heritability in common disorders of the brain. *Science* **360**, eaap8757 (2018).
23. Moore, L. D., Le, T. & Fan, G. DNA Methylation and Its Basic Function. *Neuropsychopharmacology* **38**, 23–38 (2013).
24. Zeng, Y. *et al.* Parent of origin genetic effects on methylation in humans are common and influence complex trait variation. *Nat. Commun.* **10**, 1383 (2019).
25. Nativio, R. *et al.* An integrated multi-omics approach identifies epigenetic alterations associated with Alzheimer's disease. *Nat. Genet.* **52**, 1024–1035 (2020).
26. Smith, R. G. *et al.* A meta-analysis of epigenome-wide association studies in Alzheimer's disease highlights novel differentially methylated loci across cortex. *Nat. Commun.* **12**, 3517 (2021).
27. Zhang, L. *et al.* Epigenome-wide meta-analysis of DNA methylation differences in prefrontal cortex implicates the immune processes in Alzheimer's disease. *Nat. Commun.* **11**, 6114 (2020).
28. Hannon, E. *et al.* An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-localization of genetic associations and differential DNA methylation. *Genome Biol.* **17**, 176 (2016).
29. Story Jovanova, O. *et al.* DNA Methylation Signatures of Depressive Symptoms in Middle-aged and Elderly Persons: Meta-analysis of Multiethnic Epigenome-wide Studies. *JAMA Psychiatry* **75**, 949–959 (2018).
30. Li, Q. S., Morrison, R. L., Turecki, G. & Drevets, W. C. Meta-analysis of epigenome-wide association studies of major depressive disorder. *Sci. Rep.* **12**, 18361 (2022).
31. Aref-Eshghi, E. *et al.* Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. *Am. J. Hum. Genet.* **106**, 356–370 (2020).
32. Kiltchewskij, D. J., Reay, W. R. & Cairns, M. J. Schizophrenia is associated with altered DNA methylation variance. *Mol. Psychiatry* **30**, 1383–1395 (2025).
33. Sun, Y. *et al.* Associations of DNA Methylation With Behavioral Problems, Gray Matter Volumes, and Negative Life Events Across Adolescence: Evidence From the Longitudinal IMAGEN Study. *Biol. Psychiatry* **93**, 342–351 (2023).
34. Jia, T. *et al.* Epigenome-wide meta-analysis of blood DNA methylation and its association with subcortical volumes: findings from the ENIGMA Epigenetics Working Group. *Mol. Psychiatry* **26**, 3884–3895 (2021).

35. Walton, E. *et al.* A systematic review of neuroimaging epigenetic research: calling for an increased focus on development. *Mol. Psychiatry* **28**, 2839–2847 (2023).
36. Wheeler, E. N. W. *et al.* DNA methylation and brain structure and function across the life course: A systematic review. *Neurosci. Biobehav. Rev.* **113**, 133–156 (2020).
37. Duric, V. *et al.* A negative regulator of MAP kinase causes depressive behavior. *Nat. Med.* **16**, 1328–1332 (2010).
38. Zeilinger, S. *et al.* Tobacco smoking leads to extensive genome-wide changes in DNA methylation. *PloS One* **8**, e63812 (2013).
39. Linli, Z. *et al.* Smoking is associated with lower brain volume and cognitive differences: A large population analysis based on the UK Biobank. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **123**, 110698 (2023).
40. Dias, M. de F. M., Carvalho, P., Castelo-Branco, M. & Valente Duarte, J. Cortical thickness in brain imaging studies using FreeSurfer and CAT12: A matter of reproducibility. *Neuroimage Rep.* **2**, 100137 (2022).
41. Xia, L. *et al.* CHD4 Has Oncogenic Functions in Initiating and Maintaining Epigenetic Suppression of Multiple Tumor Suppressor Genes. *Cancer Cell* **31**, 653–668.e7 (2017).
42. Gong, F. *et al.* Screen identifies bromodomain protein ZMYND8 in chromatin recognition of transcription-associated DNA damage that promotes homologous recombination. *Genes Dev.* **29**, 197–211 (2015).
43. Sugden, K. *et al.* Patterns of Reliability: Assessing the Reproducibility and Integrity of DNA Methylation Measurement. *Patterns* **1**, 100014 (2020).
44. Klengel, T. *et al.* Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat. Neurosci.* **16**, 33–41 (2013).
45. Koolen, D. A. *et al.* Mutations in the chromatin modifier gene KANSL1 cause the 17q21.31 microdeletion syndrome. *Nat. Genet.* **44**, 639–641 (2012).
46. Stauffer, E.-M. *et al.* The genetic relationships between brain structure and schizophrenia. *Nat. Commun.* **14**, 7820 (2023).
47. Burnouf, T. & Walker, T. L. The multifaceted role of platelets in mediating brain function. *Blood* **140**, 815–827 (2022).
48. Watanabe, K., Taskesen, E., van Bochoven, A. & Posthuma, D. Functional mapping and annotation of genetic associations with FUMA. *Nat. Commun.* **8**, 1826 (2017).
49. Hüls, A. & Czamara, D. Methodological challenges in constructing DNA methylation risk scores. *Epigenetics* **15**, 1–11 (2020).
50. Pan, A. L. *et al.* Dual-Specificity Protein Phosphatase 4 (DUSP4) Overexpression Improves Learning Behavior Selectively in Female 5xFAD Mice, and Reduces β -Amyloid Load in Males and Females. *Cells* **11**, 3880 (2022).
51. Dagan, I. & Palty, R. Regulation of Store-Operated Ca^{2+} Entry by SARAF. *Cells* **10**, 1887 (2021).

52. Jardín, I. et al. Fine-tuning of store-operated calcium entry by fast and slow Ca²⁺-dependent inactivation: Involvement of SARAF. *Biochim. Biophys. Acta BBA - Mol. Cell Res.* **1865**, 463–469 (2018).
53. Dias, K.-R. et al. De Novo ZMYND8 variants result in an autosomal dominant neurodevelopmental disorder with cardiac malformations. *Genet. Med.* **24**, 1952–1966 (2022).
54. Meier, M. H. et al. DNA methylation profiles of long-term cannabis users in midlife: a comprehensive evaluation of published cannabis-associated methylation markers in a representative cohort. *Mol. Psychiatry* **30**, 4576–4590 (2025).
55. Baglietto, L. et al. DNA methylation changes measured in pre-diagnostic peripheral blood samples are associated with smoking and lung cancer risk. *Int. J. Cancer* **140**, 50–61 (2017).
56. Andersen, A. M., Dogan, M. V., Beach, S. R. H. & Philibert, R. A. Current and Future Prospects for Epigenetic Biomarkers of Substance Use Disorders. *Genes* **6**, 991–1022 (2015).
57. Breitling, L. P., Salzmann, K., Rothenbacher, D., Burwinkel, B. & Brenner, H. Smoking, F2RL3 methylation, and prognosis in stable coronary heart disease. *Eur. Heart J.* **33**, 2841–2848 (2012).
58. Fasanelli, F. et al. Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. *Nat. Commun.* **6**, 10192 (2015).
59. Georgiadis, P. et al. Omics for prediction of environmental health effects: Blood leukocyte-based cross-omic profiling reliably predicts diseases associated with tobacco smoking. *Sci. Rep.* **6**, 20544 (2016).
60. Dogan, M. V., Beach, S. R. H. & Philibert, R. A. Genetically contextual effects of smoking on genome wide DNA methylation. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet.* **174**, 595–607 (2017).
61. Sikdar, S. et al. Comparison of smoking-related DNA methylation between newborns from prenatal exposure and adults from personal smoking. *Epigenomics* **11**, 1487–1500 (2019).
62. Shen, H. et al. Suppression of Enhancer Overactivation by a RACK7-Histone Demethylase Complex. *Cell* **165**, 331–342 (2016).
63. Criado-Marrero, M. et al. FKBP5 and early life stress affect the hippocampus by an age-dependent mechanism. *Brain Behav. Immun. - Health* **9**, 100143 (2020).
64. Jiao, F. et al. RACK7 Interacts with PRC2 Complex to Regulate Astrocyte Development. *Adv. Sci.* **12**, 2416350 (2025).
65. Binder, E. B. et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat. Genet.* **36**, 1319–1325 (2004).
66. Burstein, E. et al. COMMD proteins, a novel family of structural and functional homologs of MURR1. *J. Biol. Chem.* **280**, 22222–22232 (2005).
67. Hawe, J. S. et al. Genetic variation influencing DNA methylation provides insights into molecular mechanisms regulating genomic function. *Nat. Genet.* **54**, 18–29 (2022).

68. Giacomani-Lozano, M., Meléndez-Ramírez, C., Martínez-Ledesma, E., Cuevas-Díaz Duran, R. & Velasco, I. Epigenetics of neural differentiation: Spotlight on enhancers. *Front. Cell Dev. Biol.* **10**, 1001701 (2022).
69. Zollino, M. et al. Mutations in KANSL1 cause the 17q21.31 microdeletion syndrome phenotype. *Nat. Genet.* **44**, 636–638 (2012).
70. Arbogast, T. et al. Mouse models of 17q21.31 microdeletion and microduplication syndromes highlight the importance of Kansl1 for cognition. *PLoS Genet.* **13**, e1006886 (2017).
71. Bratman, G. N. et al. Nature and human well-being: The olfactory pathway. *Sci. Adv.* **10**, eadn3028 (2024).
72. Dikeçligil, G. N. & Gottfried, J. A. What Does the Human Olfactory System Do, and How Does It Do It? *Annu. Rev. Psychol.* **75**, 155–181 (2024).
73. Laketić, D. et al. Insular Cortex-Biology and Its Role in Psychiatric Disorders: A Narrative Review. *Brain Sci.* **15**, 793 (2025).
74. Petkus, A. J. et al. Ambient air pollution exposure and increasing depressive symptoms in older women: The mediating role of the prefrontal cortex and insula. *Sci. Total Environ.* **823**, 153642 (2022).
75. Coman, D. et al. Squalene Synthase Deficiency: Clinical, Biochemical, and Molecular Characterization of a Defect in Cholesterol Biosynthesis. *Am. J. Hum. Genet.* **103**, 125–130 (2018).
76. Di Paolo, G. & Kim, T.-W. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat. Rev. Neurosci.* **12**, 284–296 (2011).
77. Puglielli, L., Tanzi, R. E. & Kovacs, D. M. Alzheimer's disease: the cholesterol connection. *Nat. Neurosci.* **6**, 345–351 (2003).
78. Gonzalez-Rivera, J. C. et al. Post-transcriptional air pollution oxidation to the cholesterol biosynthesis pathway promotes pulmonary stress phenotypes. *Commun. Biol.* **3**, 392 (2020).
79. Medland, S. E. et al. Ten years of enhancing neuro-imaging genetics through meta-analysis: An overview from the ENIGMA Genetics Working Group. *Hum. Brain Mapp.* **43**, 292–299 (2022).
80. Aryee, M. J. et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinform. Oxf. Engl.* **30**, 1363–1369 (2014).
81. Noguera-Castells, A., García-Prieto, C. A., Álvarez-Errico, D. & Esteller, M. Validation of the new EPIC DNA methylation microarray (900K EPIC v2) for high-throughput profiling of the human DNA methylome. *Epigenetics* **18**, 2185742 (2023).
82. Moore, J. E. et al. Expanded encyclopaedias of DNA elements in the human and mouse genomes. *Nature* **583**, 699–710 (2020).
83. Lizio, M. et al. Gateways to the FANTOM5 promoter level mammalian expression atlas. *Genome Biol.* **16**, 22 (2015).
84. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012).

85. Touleimat, N. & Tost, J. Complete pipeline for Infinium(®) Human Methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. *Epigenomics* **4**, 325–341 (2012).
86. Fernandez-Jimenez, N. *et al.* Comparison of Illumina 450K and EPIC arrays in placental DNA methylation. *Epigenetics* **14**, 1177–1182 (2019).
87. Solomon, O. *et al.* Comparison of DNA methylation measured by Illumina 450K and EPIC BeadChips in blood of newborns and 14-year-old children. *Epigenetics* **13**, 655–664 (2018).
88. Lussier, A. A. *et al.* Technical variability across the 450K, EPICv1, and EPICv2 DNA methylation arrays: lessons learned for clinical and longitudinal studies. *Clin. Epigenetics* **16**, 166 (2024).
89. Van der Most, P. J., Küpers, L. K., Snieder, H. & Nolte, I. QCEWAS: automated quality control of results of epigenome-wide association studies. *Bioinforma. Oxf. Engl.* **33**, 1243–1245 (2017).
90. Chen, Y. *et al.* Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* **8**, 203–209 (2013).
91. Pidsley, R. *et al.* Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. *Genome Biol.* **17**, 208 (2016).
92. van Iterson, M., van Zwet, E. W., BIOS Consortium & Heijmans, B. T. Controlling bias and inflation in epigenome- and transcriptome-wide association studies using the empirical null distribution. *Genome Biol.* **18**, 19 (2017).
93. Willer, C. J., Li, Y. & Abecasis, G. R. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26**, 2190–2191 (2010).
94. Pedersen, B. S., Schwartz, D. A., Yang, I. V. & Kechris, K. J. Comb-p: software for combining, analyzing, grouping and correcting spatially correlated P-values. *Bioinformatics* **28**, 2986–2988 (2012).
95. Sidak, Z. Rectangular Confidence Regions for the Means of Multivariate Normal Distributions. *J. Am. Stat. Assoc.* **62**, 626–633 (1967).
96. Tanigawa, Y., Dyer, E. S. & Bejerano, G. WhichTF is functionally important in your open chromatin data? *PLoS Comput. Biol.* **18**, (2022).
97. The GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* **369**, 1318–1330 (2020).
98. Xiong, Z. *et al.* EWAS Open Platform: integrated data, knowledge and toolkit for epigenome-wide association study. *Nucleic Acids Res.* **50**, D1004–D1009 (2022).
99. de Klein, N. *et al.* Brain expression quantitative trait locus and network analyses reveal downstream effects and putative drivers for brain-related diseases. *Nat. Genet.* **55**, 377–388 (2023).
100. Li, M. *et al.* EWAS Atlas: a curated knowledgebase of epigenome-wide association studies. *Nucleic Acids Res.* **47**, D983–D988 (2019).
101. Battram, T. *et al.* The EWAS Catalog: a database of epigenome-wide association studies. *Wellcome Open Res.* **7**, 41 (2022).

102. Trubetskoy, V. *et al.* Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature* **604**, 502–508 (2022).
103. O’Connell, K. S. *et al.* Genomics yields biological and phenotypic insights into bipolar disorder. *Nature* **639**, 968–975 (2025).
104. Adams, M. J. *et al.* Trans-ancestry genome-wide study of depression identifies 697 associations implicating cell types and pharmacotherapies. *Cell* **188**, 640-652.e9 (2025).
105. Bellenguez, C. *et al.* New insights into the genetic etiology of Alzheimer’s disease and related dementias. *Nat. Genet.* **54**, 412–436 (2022).
106. Nalls, M. A. *et al.* Identification of novel risk loci, causal insights, and heritable risk for Parkinson’s disease: a meta-analysis of genome-wide association studies. *Lancet Neurol.* **18**, 1091–1102 (2019).
107. Okbay, A. *et al.* Polygenic prediction of educational attainment within and between families from genome-wide association analyses in 3 million individuals. *Nat. Genet.* **54**, 437–449 (2022).
108. Burgess, S., Butterworth, A. & Thompson, S. G. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet. Epidemiol.* **37**, 658–665 (2013).
109. Burgess, S. & Thompson, S. G. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur. J. Epidemiol.* **32**, 377–389 (2017).
110. Burgess, S., Thompson, S. G., & CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. *Int. J. Epidemiol.* **40**, 755–764 (2011).
111. Hemani, G., Tilling, K. & Smith, G. D. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLOS Genet.* **13**, e1007081 (2017).

Figures

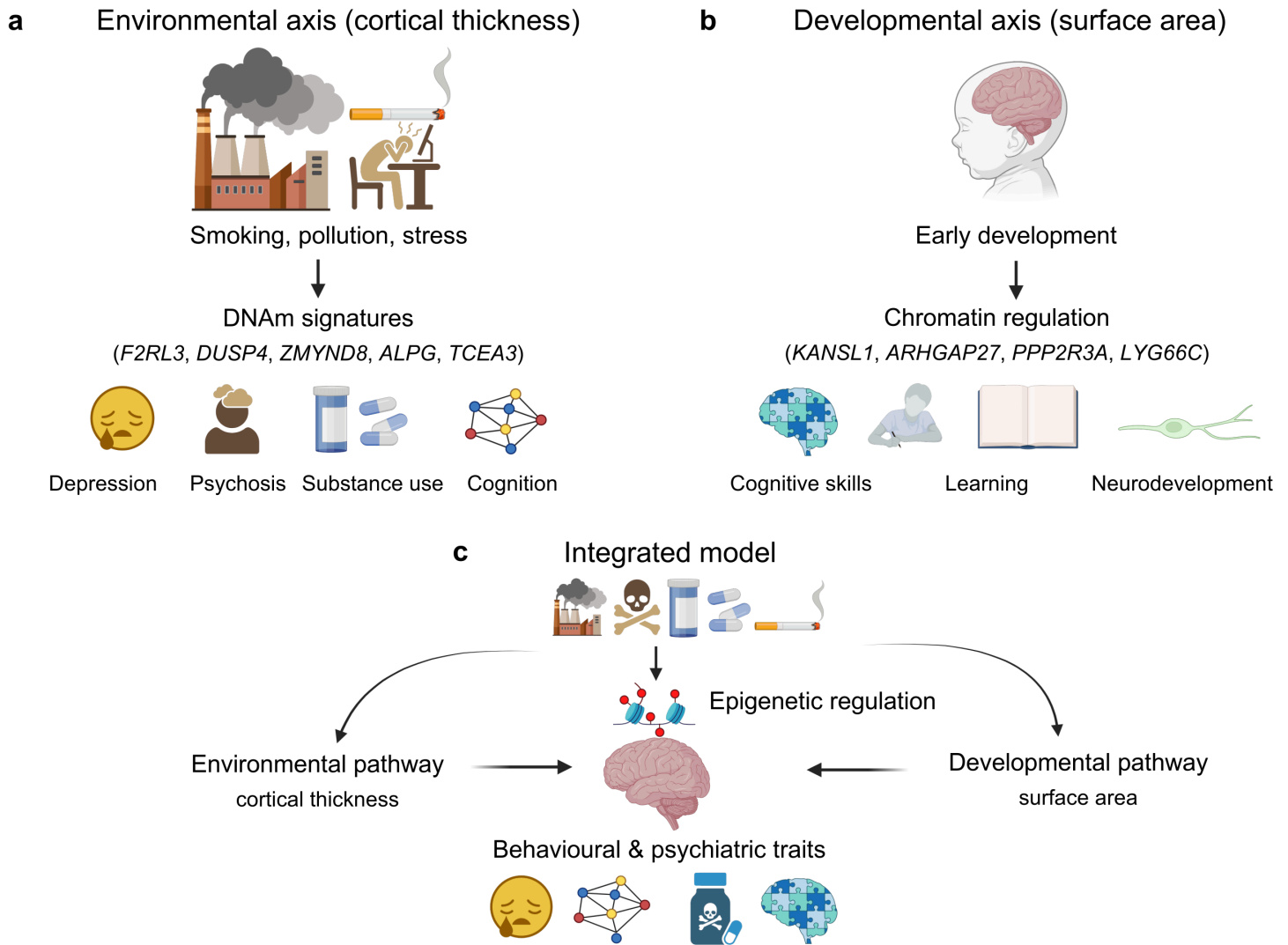


Figure 1

Conceptual representation of the dual epigenetic pathways linking environmental exposure, cortical organisation and behavioural vulnerability.

Environmental exposures may influence human brain structure through epigenetic mechanisms detectable in peripheral DNAm. Our findings suggest two complementary regulatory pathways. The first pathway links environmentally responsive DNAm signatures to variation in CT, with enrichment in stress-responsive, immune and metabolic regulatory processes. Several CT-associated loci overlap with DNAm markers of smoking, pollution and psychosocial stress, suggesting that CT-associated DNAm may capture cumulative environmental exposure relevant to psychiatric vulnerability. The second pathway links DNAm variation to SA, primarily through loci involved in chromatin regulation and early neurodevelopment, including the 17q21.31 region spanning *KANSL1*. Together, these findings support a framework in which DNAm acts as a molecular interface linking environmental exposure, cortical organisation and behavioural outcomes

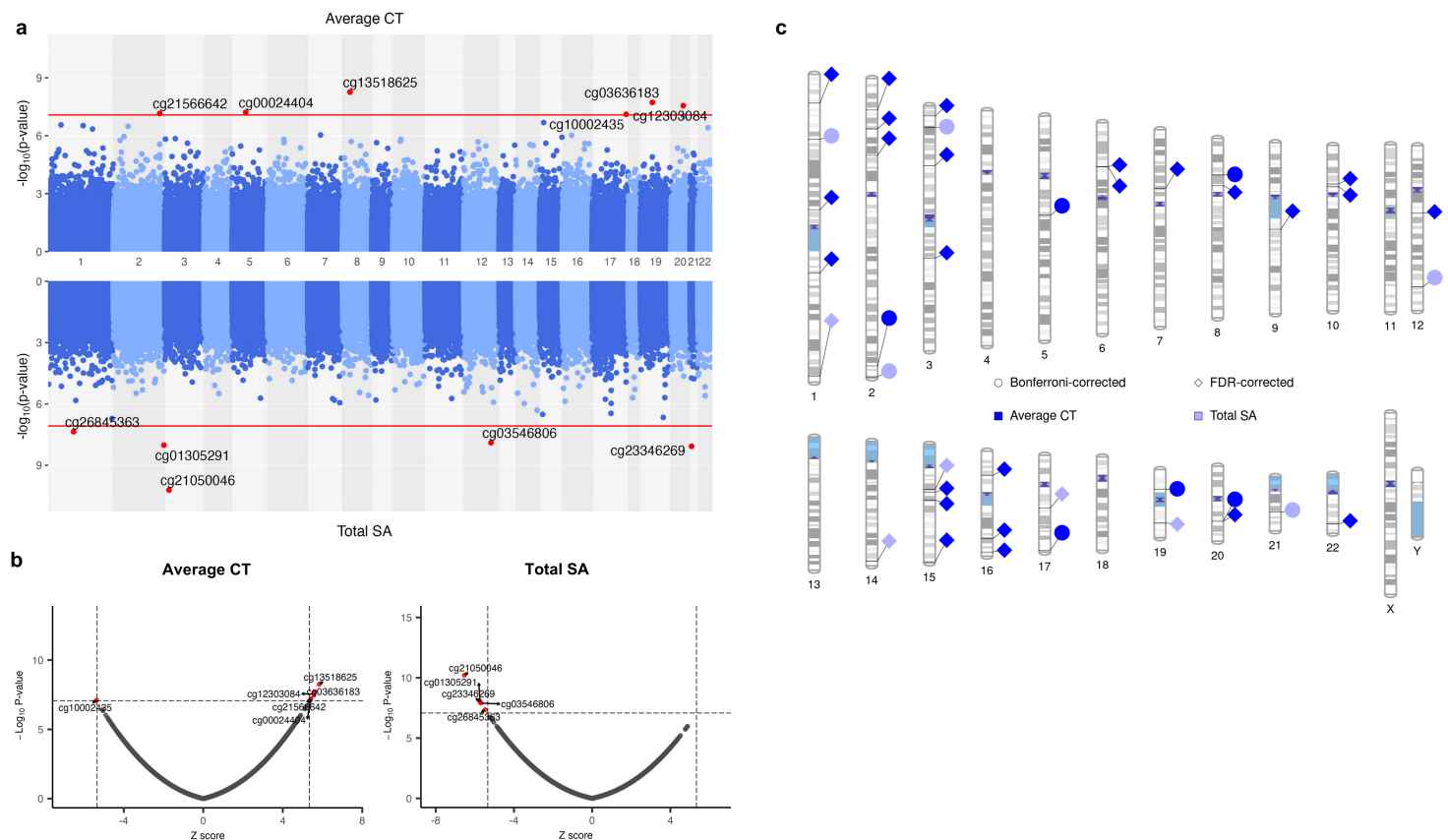


Figure 2

Epigenome-wide associations of DNA methylation with cortical thickness and surface area.

a, Manhattan plots showing $-\log_{10}(P\text{-values})$ for CpG associations with average cortical thickness (CT; top) and total cortical surface area (SA; bottom) across the meta-analysis of discovery cohorts. The red horizontal line denotes the Bonferroni-corrected significance threshold. **b**, Volcano plots displaying Z-scores against $-\log_{10}(P\text{-values})$ for all tested CpGs in the EWAS of CT and SA. **c**, Ideogram illustrating the chromosomal distribution of genome-wide significant CpGs associated with CT and SA. Squares indicate CpGs reaching false discovery rate (FDR) significance, while circles indicate CpGs additionally surpassing the Bonferroni threshold; all Bonferroni-significant CpGs are therefore a subset of FDR-significant CpGs.

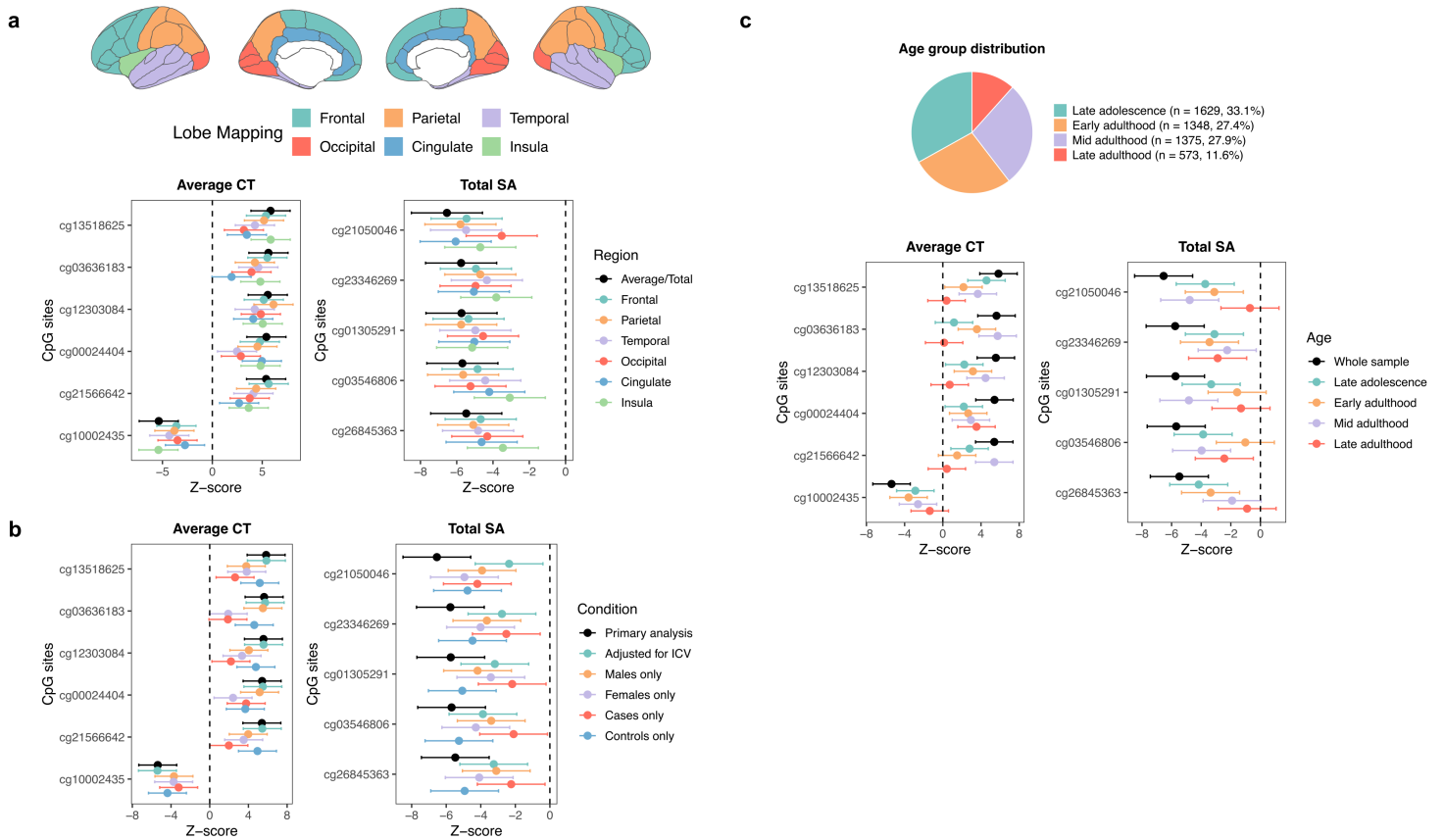


Figure 3

Regional and stratified association patterns of significant CpGs.

a, Z-scores with 95% confidence intervals for significant CpGs across major cortical lobes for average CT and total SA, including frontal, parietal, temporal, occipital, cingulate, and insular regions. **b**, Z-scores with 95% confidence intervals for significant CpGs across stratified analyses, including the primary model, adjustment for intracranial volume (ICV), and subgroup analyses by sex (males only, females only) and case-control status (cases only, controls only). **c**, Z-scores with 95% confidence intervals for significant CpGs in stratified analyses across different age groups: adolescence (18–25 years), early adulthood (25–40 years), mid-adulthood (40–65 years), and late adulthood (> 65 years).

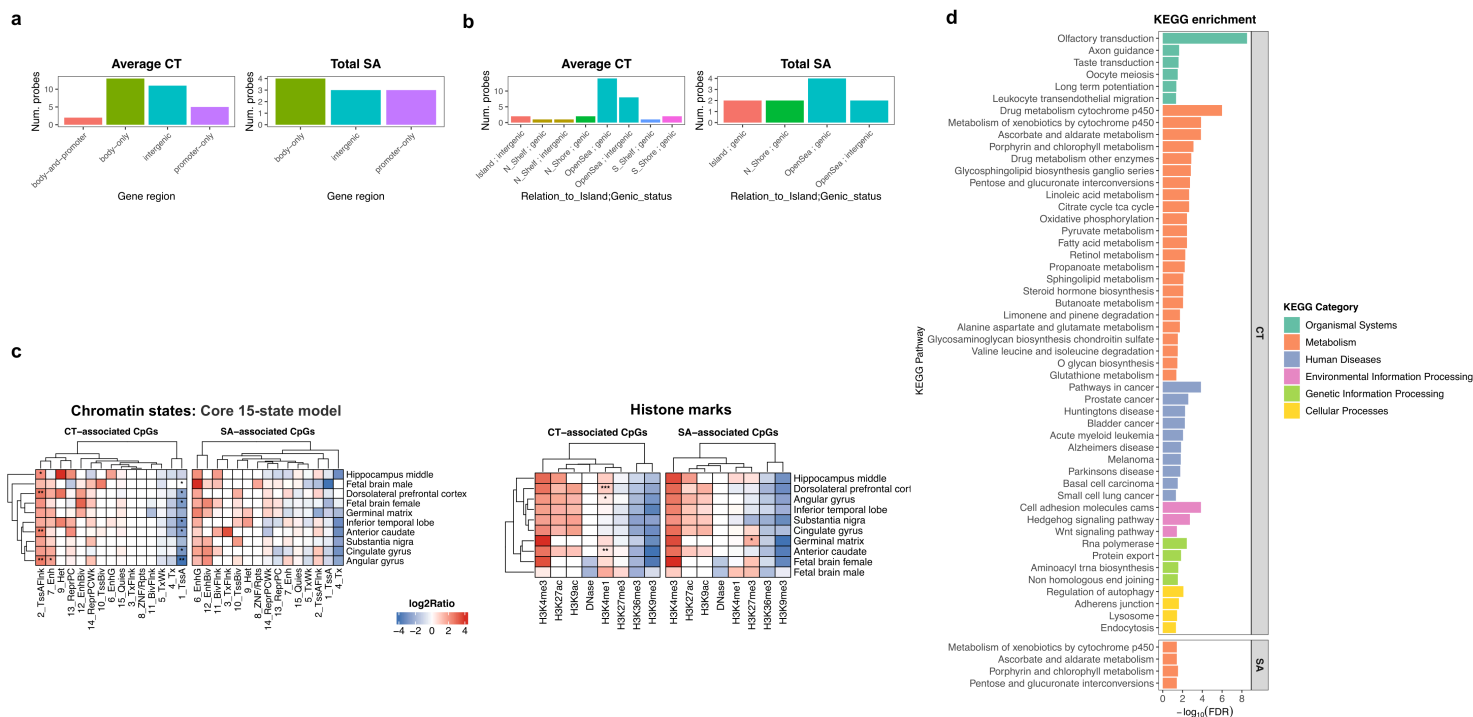


Figure 4

Functional characterisation of CpGs associated with CT and SA.

CpG island context of FDR-significant CpGs, demonstrating enrichment in gene bodies and intergenic regions (**a**) and OpenSea and Shore regions relative to CpG islands (**b**), consistent with non-promoter regulatory functions. **c**, Chromatin state enrichment of CT- and SA-associated CpGs ($P < 1 \times 10^{-5}$; CT: $N = 68$; SA: $N = 52$) across brain tissues (left), based on the 15-state ChromHMM model from the NIH Roadmap Epigenomics Mapping Consortium, and histone mark enrichment (right). Enrichment is shown as $\log_2(\text{observed/expected})$ ratios, with red indicating overrepresentation and blue underrepresentation relative to the background. Statistical significance was assessed using hypergeometric tests with FDR correction across tissues ($*** P_{FDR} < 0.001$, $** P_{FDR} < 0.01$, $* P_{FDR} < 0.05$). **d**, KEGG pathway enrichment analysis of genes mapped to significant CpGs ($P < 1 \times 10^{-5}$) for CT (top) and SA (bottom), performed using the MetaBrain network platform. Pathways are grouped by functional category and plotted by $-\log_{10}(\text{FDR})$.

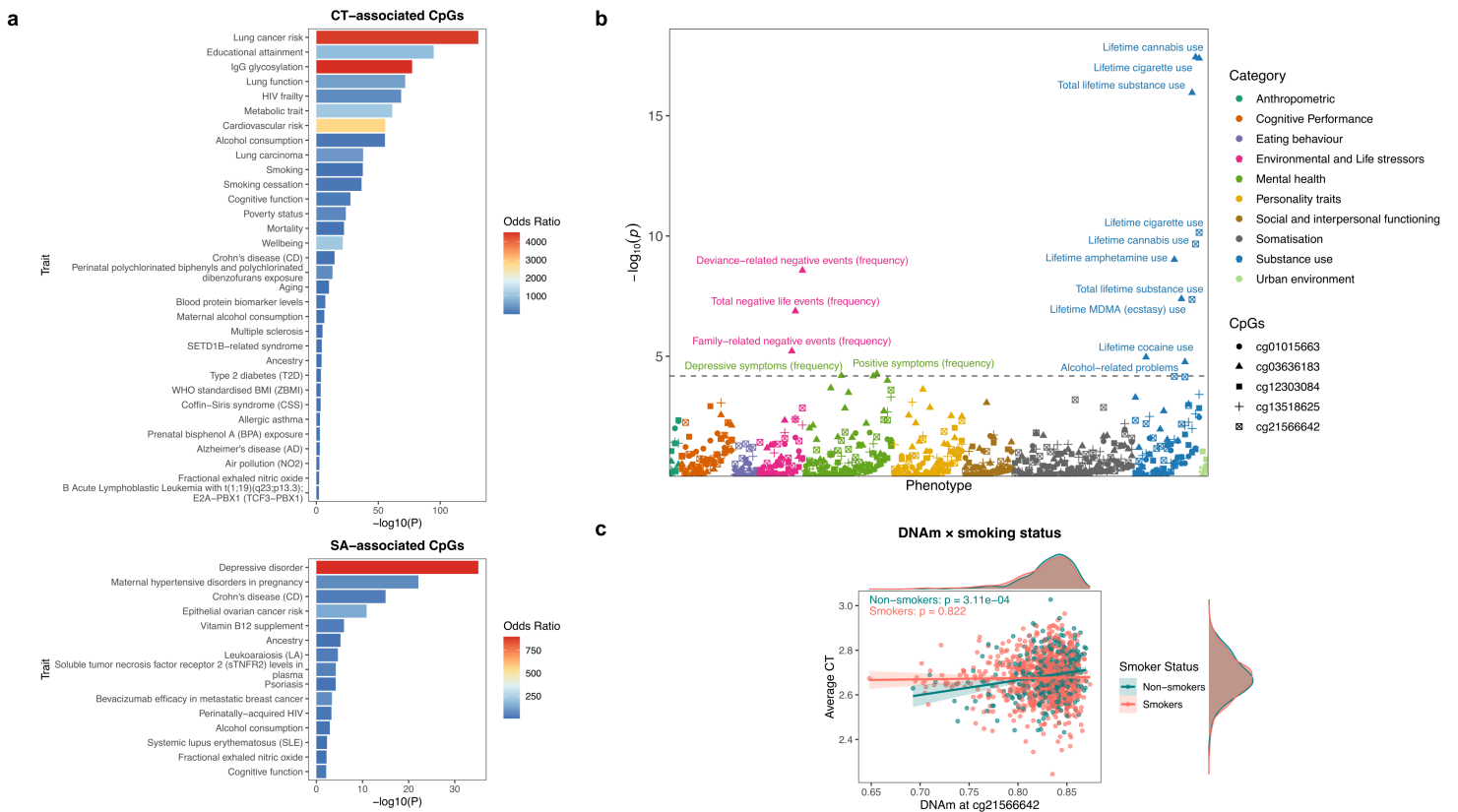


Figure 5

Phenotypic associations and environmental modulation of significant CpGs linked to cortical structure.

a, Trait enrichment analysis for CT-associated (top) and SA-associated (bottom) CpGs ($P < 1 \times 10^{-5}$), based on published EWAS studies curated using the EWAS toolkit. Odds ratios and $-\log_{10}(P)$ -values) are shown for enriched traits. **b**, Phenome-wide association study (PheWAS) results for five replicated CT-associated CpGs associated with average CT across 10 phenotype domains in the IMAGEN cohort, including anthropometric traits, cognition, eating behaviour, environmental exposures and life stressors, mental health, personality, social functioning, somatisation, substance use, and urban environment. Associations are ranked by $-\log_{10}(P)$ -value; the dashed line indicates the Bonferroni significance threshold. **c**, Interaction between DNAm at cg21566642 and smoking status (smokers vs. non-smokers) on average CT. Plots show stratified regression lines and corresponding P -values for the association between DNAm and CT within each smoking group.

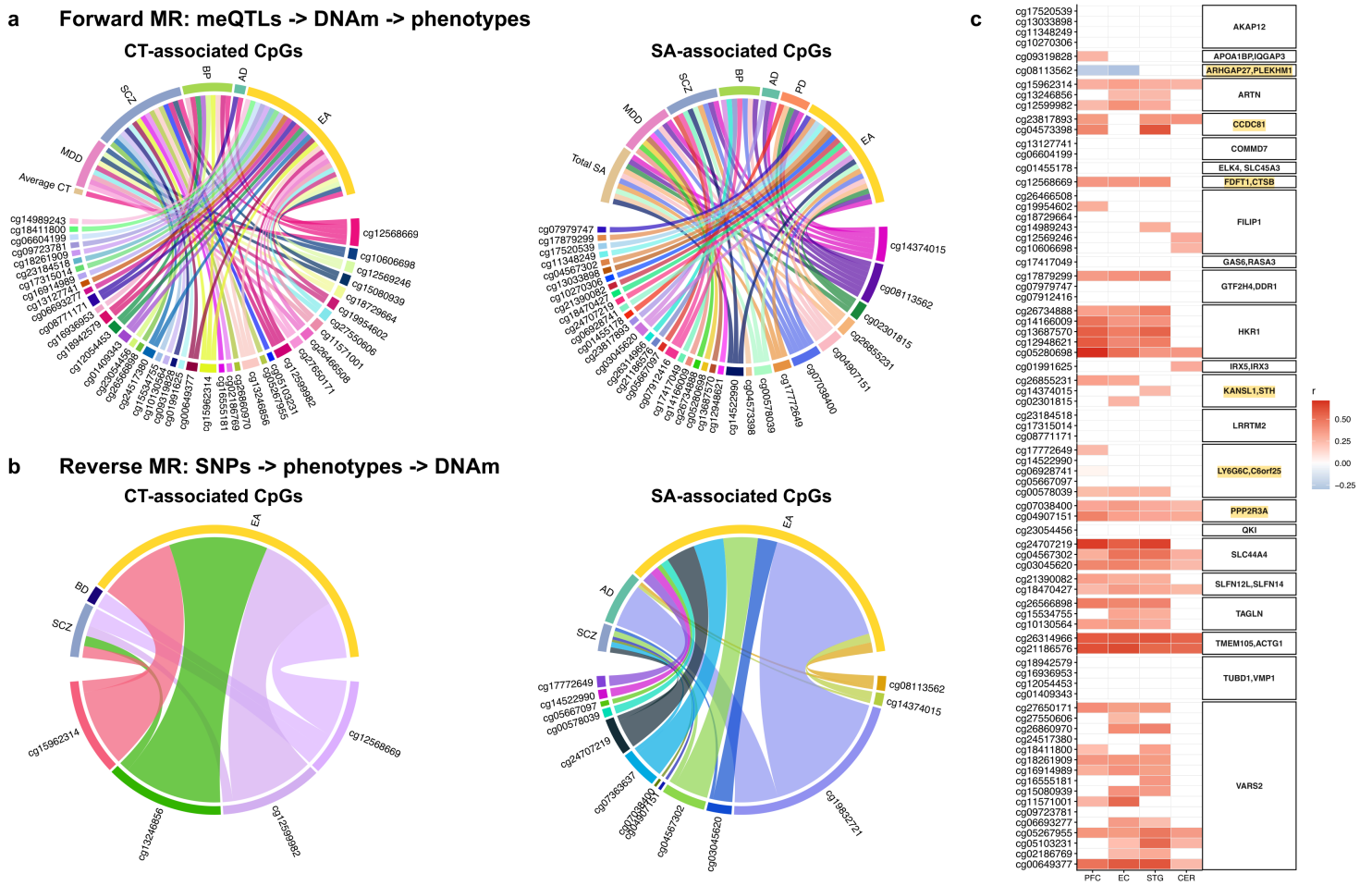


Figure 6

Shared putative causal CpG associations between cortical structure and neuropsychiatric or cognitive traits and trans-tissue concordance.

Circos plots depicting CpGs associated with average CT and total SA. Panel **a** shows putative causal effects of DNAm on cortical morphology and neuropsychiatric or cognitive outcomes, based on Mendelian randomisation analyses. Panel **b** shows the putative causal effects of cortical morphology and neuropsychiatric or cognitive outcomes on DNA methylation. Only CpGs reaching FDR significance within each outcome are displayed. Each connecting link represents a CpG with evidence of a shared putative causal association between cortical structure and at least one of the tested outcomes. Panel **c**, cross-tissue concordance of MR-prioritised CpGs between blood and cortical tissues. Heatmap showing Pearson correlation coefficients (r) between peripheral blood DNA methylation and postmortem brain methylation levels for CpGs reaching FDR significance in forward MR analyses. Correlations were extracted from the Blood-Brain DNA Methylation Comparison Tool across prefrontal cortex (PFC), entorhinal cortex (EC), superior temporal gyrus (STG), and cerebellum (CER). Colour intensity reflects the magnitude and direction of correlation (red, positive; blue, negative). Blank cells indicate no significant correlation. Genes containing at least one CpG potentially causal for CT or SA are highlighted in orange. Most MR-prioritised CpGs demonstrate moderate cross-tissue concordance across one or more cortical

regions, supporting the biological plausibility of peripheral DNAm signals as proxies of regulatory variation relevant to cortical structure and neuropsychiatric traits.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.docx](#)
- [Supplementarytables.xlsx](#)