DNA methylation and brain structure and function across the life course: a systematic review

Citation for published version:
Wheater, ENW, Stoye, DQ, Cox, SR, Wardlaw, JM, Drake, AJ, Bastin, ME & Boardman, JP 2020, 'DNA methylation and brain structure and function across the life course: a systematic review', Neuroscience and Biobehavioral Reviews. https://doi.org/10.1016/j.neubiorev.2020.03.007

Digital Object Identifier (DOI):
10.1016/j.neubiorev.2020.03.007

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Version created as part of publication process; publisher's layout; not normally made publicly available

Published In:
Neuroscience and Biobehavioral Reviews

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
DNA methylation and brain structure and function across the life course: a systematic review

Short running title: DNA methylation and brain imaging

Emily N. W. Wheater¹, David Q. Stoye¹, Simon R. Cox², Joanna M. Wardlaw³, Amanda J. Drake⁴, Mark E. Bastin³, James P. Boardman¹,3*

Affiliations
¹ Medical Research Council Centre for Reproductive Health, University of Edinburgh, United Kingdom.
² Department of Psychology, University of Edinburgh, United Kingdom.
³ Centre for Clinical Brain Sciences, University of Edinburgh, United Kingdom.
⁴ University/British Heart Foundation Centre for Cardiovascular Science, University of Edinburgh, United Kingdom.

*Corresponding author:
Professor James P Boardman (james.boardman@ed.ac.uk)
Queen's Medical Research Institute (W1.26)
47 Little France Crescent,
Edinburgh,
EH16 4TJ,
UK.

Postal addresses of authors
Emily N. W. Wheater, David Q. Stoye, Amanda J. Drake, James P. Boardman: Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, United Kingdom
Simon R. Cox, Mark E. Bastin: Psychology Building, 7 George Square, Edinburgh, EH8 9JZ, United Kingdom
Joanna Wardlaw: Chancellor’s Building, 49 Little France Crescent, Edinburgh, EH16 4SB, United Kingdom
Highlights

- 60 studies of association between DNAm and human brain MRI were identified
- Differential DNAm is associated with brain structure and function throughout life
- There is modest consistency between DNAm and image endophenotypes
- Approaches for reducing heterogeneity in DNAm-MRI analyses are proposed

Abstract

MRI has enhanced our capacity to understand variations in brain structure and function conferred by the genome. We identified 60 studies that report associations between DNA methylation (DNAm) and human brain structure/function. Forty-three studies measured candidate loci DNAm; seventeen measured epigenome-wide DNAm. MRI features included region-of-interest and whole-brain structural, diffusion and functional imaging features. The studies report DNAm-MRI associations for: neurodevelopment and neurodevelopmental disorders; major depression and suicidality; alcohol use disorder; schizophrenia and psychosis; ageing, stroke, ataxia and neurodegeneration; post-traumatic stress disorder; and socio-emotional processing. Consistency between MRI features and differential DNAm is modest. Sources of bias: variable inclusion of comparator groups; different surrogate tissues used; variation in DNAm measurement methods; lack of control for genotype and cell-type composition; and variations in image processing. Knowledge of MRI features associated with differential DNAm may improve understanding of the role of DNAm in brain health and disease, but caution is required because conventions for linking DNAm and MRI data are not established, and clinical and methodological heterogeneity in existing literature is substantial.

Key words
DNA methylation, magnetic resonance imaging, brain, epigenetics.
1. Introduction

A growing number of studies have investigated associations between epigenetic signatures and neuroimaging markers of human disease, behaviour and cognition. This area of research is motivated by the understanding that epigenetic processes contribute to brain development, ageing and disease, and they may mediate interaction between genomic predisposition and environmental pressures that modify brain structure and function.

The term epigenetics refers to a set of molecular mechanisms that modulate the function of the genome in different cell types without altering the genome itself. DNA methylation (DNAm) is one such mechanism whereby a methyl group is covalently added to cytosine residues in a Cytosine-phosphate-Guanine context (CpG). This is thought to alter the accessibility of a locus to transcriptional machinery or modifying proteins, thereby influencing gene expression. DNA methylation may also occur after an initial change in gene expression and function as a form of longer-term control (Jones, 2012).

The pathogenesis of several genetic diseases with neurological phenotypes involve DNAm dysregulation. Rett syndrome is a severe neurological disorder characterised by regression of acquired skills, stereotypic movements, microcephaly, seizures, and intellectual disability. It results from a loss of function mutation in the X-linked methyl-CpG binding protein 2 gene (MECP2), which is a chromatin associated protein required for normal neuronal function throughout life. The loss of function mutation in MECP2 serves to reduce the binding affinity of MECP2 to methylated DNA with effects on regulation of gene expression at transcriptional and post-transcriptional levels (Lyst and Bird, 2015). Angelman syndrome is characterised by cognitive impairment, movement or balance disorder, typical abnormal behaviours, and impaired speech and language. It arises from a mutation or deletion of the maternal ubiquitin protein ligase 3A gene (UBE3A) and the neuronal tissue specific paternal imprinting of the gene that silences the paternal allele (Rangasamy et al., 2013). Fragile X syndrome arises
due to CGG triplet expansion within the *FMR1* gene promoter which becomes hypermethylated and results in a reduction of gene expression (Wijetunge et al., 2013). In addition to these genetic neurological disorders associated with DNAm dysregulation, variation in DNAm has been implicated in the pathogenesis of a number of complex neurodevelopmental, neurological and psychiatric diseases including autism, schizophrenia, and problems associated with trauma and stress (De Rubeis et al., 2014; Grayson and Guidotti, 2013; Klengel et al., 2014; Oberlander et al., 2008; Palumbo et al., 2018).

DNAm may be modifiable by environmental factors including physiological and emotional stress, child abuse, nutritional deprivation, and other lifestyle factors that operate from fetal life to old age (Cao-Lei et al., 2015; Del Blanco and Barco, 2018; Gouin et al., 2017; Heijmans et al., 2008; Joehanes et al., 2016; McGowan et al., 2009; Provenzi et al., 2018; Vidal et al., 2014). As such, it has been proposed that environmentally-induced changes in DNAm may enable short-term survival adaptation, but may also induce adaptations that contribute to impaired development of neural networks and increased risk of pathology (Gluckman et al., 2010; Hoffmann et al., 2017).

Structural, diffusion and functional brain MRI enable the parsing of complex behavioural traits and diseases onto quantitative indicators of brain structure and function. Such imaging endophenotypes have been used to demonstrate the impact of common genetic variants on brain health and disease (Elliott et al., 2018), but the role of DNAm in contributing to brain structure and / or function, as opposed to being a consequence of changes in structure or function, is less certain. To obtain a comprehensive overview of the extent to which differential DNAm associates human behaviour and disease we performed a systematic review of studies that have analysed DNAm with quantitative brain MRI data. By doing so we aimed to provide a comprehensive summary of what is known about DNAm-MRI associative relationships across the life course, and to identify image features associated with differential DNAm.
Finally, we captured sources of heterogeneity in the extant literature to better inform the development of methods and conventions for analysing DNAm with MRI data.

2. Materials and methods

We performed a systematic literature search based on the PRISMA framework, according to a pre-registered protocol on PROSPERO (CRD42018090928).

2.1 Search strategy

Scopus, Web of Science, MEDLINE and EMBASE (via OVID) were searched in March 2018 to identify studies that integrated DNA methylation and human in vivo MRI (for search strategy see supplementary material). The search was composed of three OR clusters that contained terms relating to the key domains, which were combined with AND: MRI neuroimaging AND Brain AND DNA methylation. Bibliographies were searched for additional studies. There were no language restrictions on the search.

2.2 Screening and study selection

Search results were imported to EndNote X8 prior to removal of duplicates and retrieval of full texts prior to import to Covidence software (https://www.covidence.org/home). Screening was carried out by ENWW and DQS independently and studies were included if they met the following criteria: report of primary results; recruited human participants of any age with in vivo neuroimaging and DNAm; investigated the relationship between brain imaging using MRI and epigenome-wide or loci specific DNAm. Exclusion criteria: no original data reported (reviews, abstracts, letters, and grey literature as defined by Grey Literature International Committee Guidance); no test of association between brain imaging findings and DNA methylation; analysis was carried out in animals or in post-mortem human tissue; DNAm-MRI analyses of central nervous system malignancy.
2.3 Data extraction

ENWW extracted data for all included studies and DQS duplicated extraction for a subset of 25%. Extraction included: participant characteristics, study design, method of DNAm ascertainment, MRI modality and image feature(s) of interest, and statistical method, consideration of cell sub-type composition includes cell sorting techniques or statistical methods to estimate and control for cell sub type composition and consideration of genotype or ancestry. The total number of participants per phenotypic category were estimated. Due to participant overlap in some studies, the largest study population reported was used to estimate the total number of participants.

2.4 Risk of bias assessment

In the absence of a validated quality assessment tool for studies linking DNAm with MRI data, we extracted data on study characteristics that might affect risk of bias: design, presence / absence of a comparator group; DNA source; candidate or epigenome-wide approach for evaluating differential DNAm; ascertainment of cell type composition; consideration of genotype; image processing methodology; and region-of-interest versus whole brain MRI analysis, and considered their prevalence in the epigenetic-neuroimaging literature.

2.5 Data synthesis

We provide a narrative synthesis structured around the type of association between DNAm and image feature, target population characteristics, tabulated, and categorised by disease.

3. Results

3.1 Overview of the literature

Figure 1 is the PRISMA flow chart of study identification and inclusion. Our search strategy yielded 3442 in total: 3438 resulting from the search and an additional 4 papers were identified post search and were included. After removal of duplicates and exclusions at screening, 336
articles were eligible for full text evaluation. Of the full text articles that were screened for eligibility for inclusion, 276 were removed due to lack of eligibility. In total, 60 studies were included in this review (estimated 6775 participants): 34 (57%) included a control or comparator group. Table 1 summarises diseases and conditions in phenotypic categories, number of participants per category, MRI modality, and method used to analyse DNAm (EWAS or candidate loci). All studies report populations from resource rich settings: United States (25), United Kingdom (3), Germany (4), Switzerland (4), Germany and Switzerland (1), South Korea (7), Canada (3), Italy (2), Japan (1), China (2), Singapore (2), Republic of Ireland (2), Australia (2), Spain (1), Sweden (1).

3.2 DNA methylation

All studies used surrogate tissues to probe methylation status: blood (n=46); saliva (n=12); one study reported using both blood and saliva, and one reported using blood/saliva/buccal cells (Ismaylova et al., 2017; Nikolova et al., 2014). The majority of studies did not report adjustments for cell type composition of samples used to estimate DNAm (n=48).

Forty-three studies used a candidate gene analysis approach and 17 performed an epigenome-wide association study (EWAS). The majority of included studies that used a candidate gene approach measured methylation using bisulfite pyrosequencing (n=36). Three studies measured methylation at a candidate gene using the EpiTYPER® system (Haas et al., 2016; Shelton et al., 2017, 2016). One study carried out a restriction enzyme digest and sequencing to measure DNAm at a candidate site (McMillan et al., 2015). Three studies measured DNAm using Illumina arrays, and used the output to inform candidate gene DNAm-MRI analyses (Sadeh et al., 2016; Walton et al., 2014; Wiemerslage et al., 2017). Overall nineteen candidate loci were studied. SLC6A4 (Na+/Cl- Dependent Serotonin Transporter) was the gene of interest in 11 studies; BDNF (member of nerve growth factor family), FKBP5 (member of the immunophilin family) and OXTR (oxytocin receptor) were selected as candidate genes in four studies; NR3C1 (glucocorticoid receptor) in three studies; FMR1
(FMRP Translational Regulator 1), **COMT** (enzyme involved in catecholamine degradation) and **DAT/SLC6A3** (Na⁺/K⁺ dependent dopamine transporter) in two studies. The following genes were selected candidates in one study: **OXT** (encodes oxytocin and neurophysin 1 precursor protein), **C9orf72** (thought to be a transcriptional regulator in the brain), **CACNA1C** (voltage gated calcium channel), **DRD2** (dopamine receptor), **KLF13** (transcription regulator), **NCAPH2/LMF2** (encoding both a non-SMC subunit of the condensin II complex and a lipase maturation factor protein from the same locus), **PPM1G** (member of the PP2C family of Ser/Thr protein phosphatases), **HTR3A** (member of the ligand-gated ion channel receptor superfamily), **SKA2** (Spindle And Kinetochore Associated Complex Subunit 2), **SOD** (superoxide dismutase) and **TESC** (tescalcin – regulates cell pH by controlling H+/Na+ exchange across the plasma membrane).

All of the EWAS studies used Illumina Infinium arrays to measure DNAm: Illumina HumanMethylation27 (n=6), Illumina 450 (n=13), and Illumina EPIC (n=1). These arrays measure methylation at 27,578, 485,512 and 866,836 CpG sites, respectively. Five studies investigated DNAm age, a method that uses a subset of CpG sites associated with age to investigate age and age acceleration (Chouliaras et al., 2018; Davis et al., 2017; Hodgson et al., 2017; Raina et al., 2017; Wolf et al., 2016).

Of studies which only report DNAm at candidate genes, two reported that the sample used to measure DNAm was mononuclear blood cells (lymphocytes and monocytes) (Chouliaras et al., 2018; Ursini et al., 2011) whereas none of the studies that measured DNAm by bisulfite pyrosequencing reported correction for cell type composition. 12 of the 20 studies which used Illumina arrays performed a correction for cell type composition. Only one study reported direct counting of cell types included in their samples (Freytag et al., 2017).

### 3.3.1 Magnetic Resonance Imaging
The majority of studies used 3T MRI scanners (n = 42); five studies used 1.5T scanners; five included participants scanned at 1.5T and 3T scanners; and eight studies did not report field strength. Many included studies carried out functional MRI (n=29), of which three studies also carried out structural analysis. Structural MRI, to investigate volumes, cortical morphology, and conventional clinical MRI measures, was used in 26 studies. Diffusion MRI to measure properties of white matter microstructure was used in nine studies, and MR angiography was used in one.

### 3.3.2 MRI features associated with DNA methylation across studies

Table 2 shows 10 MRI features that are associated with differential DNA methylation in two or more studies. The image features are: hippocampal volume (n=11); hippocampal functional connectivity/activity (n=4); amygdala functional connectivity/activity (n=9); PFC functional connectivity/activity (n=8); regional FA measures (n=6); cortical thickness (n=6); regional diffusion metrics (Ax/MD/RD) (n=5); global FA (n=2); and cortical volume (n=2). For most of these, differential DNA methylation occurred at different loci. However, amygdala functional connectivity/activity was associated with differential DNA methylation in SLC6A4 in six studies, and OXTR in two studies. PFC connectivity/activity was associated with DNA methylation in BDNF in two studies. Hippocampal volume was associated with FKBP5 DNA methylation in two studies. Activation within the prefrontal cortex in working memory tasks were associated with COMT DNA methylation in two studies. The studies all employed a candidate gene approach to DNA methylation analysis and directions of association were mixed across the studies.

Six studies tested an association between SLC6A4 methylation and amygdala reactivity. Four out of five studies that reported associations between SLC6A4 methylation and the emotional face processing task assessed by fMRI in response to threat stimuli found positive associations with amygdala reactivity and connectivity (Ismaylova et al., 2018; Nikolova et al., 2014; Schneider et al., 2018; Swartz et al., 2017). One study investigated resting state functional connectivity and reported a positive association between SLC6A4 methylation and
amygdala coupling with the salience network (Muehlhan et al., 2015). However, in a further study that investigated SLC6A4 methylation and brain activity in the context of both resting state connectivity and the emotional face processing task, there were no significant associations reported with regard to the amygdala either with regards to whole brain analysis or amygdala region-of-interest analysis (Ismaylova et al., 2017). The sixth study that reported an association between SLC6A4 and amygdala connectivity was also task-based fMRI, and used a visual emotional attention shifting paradigm (Frodl et al., 2015). These papers assessed DNAm within the promoter region of the SLC6A4 gene, with the exception of Schneider et al. 2018, where a retrotransposon element, AluJb, was studied.

Two studies reported significant associations between OXTR methylation and amygdala connectivity (Puglia et al., 2015; Ziegler et al., 2015). Each study used a different fMRI task paradigm: the first used a task employing words involving social phobia relevant verbal stimuli in a cohort of patients with social anxiety disorder (Ziegler et al., 2015); and the second used an emotional face-matching block-design task in a group of healthy study participants (Puglia et al., 2015). Both studies measured DNAm in blood but each targeted a different region of the OXTR gene in bisulfite sequencing. Ziegler et al. 2015 targeted exon 3 of the OXTR gene due to previous work demonstrating an association between methylation in this region and social cognition (Unternaehrer et al., 2012; Ziegler et al., 2015). Puglia et al. 2015 targeted a single CpG site at position -934 relative to the transcription start site, where methylation had previously been associated with autism spectrum disorder (Gregory et al., 2009; Puglia et al., 2015). The directions of associations reported in these two studies were mixed, and may be explained by the variations in study design and participant characteristics.

Two studies reported significant associations between BDNF methylation and PFC functional connectivity (Moser et al., 2015; Ursini et al., 2016). Moser et al. performed DNAm-MRI analysis in a combined group of analysis of study participants with PTSD, sub-threshold PTSD and healthy control women, and assessed neural response to a Modified Crowell Procedure
(Moser et al., 2015). The second study reported BDNF DNAm associations with PFC activity during a working memory task in a healthy group of study participants (Ursini et al., 2016). Both of these studies measured DNAm at different regions of the BDNF gene. Ursini et al. 2016 measured BDNF DNAm in blood at a region containing the rs6265 SNP, which lies in a coding region of the gene, which may interact with environmental factors to modulate risk for schizophrenia, while Moser et al. 2015 measured BDNF promoter DNAm in saliva, where methylation has been associated with trauma associated psychiatric disease in children (Moser et al., 2015; Ursini et al., 2016). The directions of associations reported in these studies were mixed.

Two studies reported associations between FKBP5 methylation and hippocampal volume (Klengel et al., 2013; Resmini et al., 2016). In study participants with childhood trauma related PTSD there was a negative correlation between FKBP5 methylation in blood and right hippocampal head volume (Klengel et al., 2013). In patients with Cushing’s syndrome there was a positive association between FKBP5 methylation in blood and bilateral hippocampal volume (Resmini et al., 2016). Both of these studies measured DNAm at glucocorticoid responsive regions of the FKBP5 gene, but report significant associations in different regions. Klengel et al. 2013 reported significant associations with intron 7, while Resmini et al. 2016 reports a significant association with a CpG site within intron 2 (Klengel et al., 2013; Resmini et al., 2016).

Two studies reported associations between COMT DNAm and PFC activation during working memory tasks (Ursini et al., 2011; Walton et al., 2014). The two studies assessed brain activity during different working memory tasks: the n-back task (Ursini et al., 2011); and the Sternberg Item Recognition task (Walton et al., 2014). The COMT gene encodes two isoforms of the catechol-O-methyltransferase enzyme: one soluble and one membrane bound, each with their own promoters, with the membrane bound isoform being the form most commonly found in the brain. Positive associations between MB-COMT promoter DNAm in blood and activity in
the left dlPFC and vIPFC (Broca’s area 45) during the Sternberg Item Recognition task were found in a group of participants including healthy controls and schizophrenia patients (Walton et al., 2014). In the second study of healthy participants significant negative associations were reported between methylation at COMT rs4680 SNP in blood and activity in the bilateral PFC (BA 45/14 and BA47) during the n-back task in participants homozygous for the Val/Val allele at the rs4680 COMT SNP (Ursini et al., 2011). The difference in direction of associations reported by these two studies may be explained by the variations in study design and participant characteristics.

3.4 Associations between DNA methylation and MRI features categorised by phenotype

3.4.1 Neurodevelopment and neurodevelopmental disorders

Table 3 summarises eight studies that focused on neurodevelopment: typical development; the impact of early life stress on young adulthood; BDNF polymorphism in early life; ADHD; preterm brain injury; hippocampal sclerosis in mesial temporal lobe epilepsy; birthweight discordance between twins and its impact on cortical anatomy. Four of the eight studies included a comparison group, and four were case series. Two studies report data from the Growing up towards Healthy Outcomes in Singapore (GUSTO) cohort study (Chen et al., 2015; Guillaume et al., 2018).

Two studies used a candidate gene approach and both reported positive associations, but between different MRI features and different genes: the first between DNA methylation at the SLC6A4 promoter and cortical thickness in an uncontrolled case series of 102 children with ADHD (Park et al., 2015); and the second between DNA methylation at FKBP5 introns and dlPFC activation during the go no-go task in a case series of young adults with a history of early life stress exposure (Harms et al., 2017). The remaining six studies used the Illumina 450k array to measure DNA methylation, and all reported significant associations between DNA methylation at multiple loci and features including fractional anisotropy (FA), sub-cortical ROI volumes, cortical thickness,
volume and surface area (Casey et al., 2017; Chen et al., 2015, 2018; Guillaume et al., 2018; Long et al., 2017; Sparrow et al., 2016). Two studies reported associations between DNAm at SMOC2 and MRI brain imaging features (Chen et al., 2018; Guillaume et al., 2018). Guillaume et al. 2018 performed an EWAS in neonates and report an association between DNAm at this gene and FA in the left ventrolateral thalamus (Guillaume et al., 2018). Chen et al. 2018 reported differential methylation and discordance in twin pairs discordant for ADHD diagnosis, and identified six probes associated with the SMOC2 gene which were differentially methylated and were associated with differences in striatum (among 48 probes that were differentially methylated with regard to striatum volume) (Chen et al., 2018). SMOC2 encodes SPARC-related modular calcium binding protein 2.

Two studies reported an association between neonatal water diffusion properties and DNAm (Guillaume et al., 2018; Sparrow et al., 2016). In a case control study of preterm infants and term born controls principal component analysis of saliva DNAm identified a component that was associated with shape of the right corticospinal tract (Sparrow et al., 2016); and in neonates from the GUSTO cohort study there was an association between umbilical cord blood DNAm at the SMOC2 gene and FA in the left ventrolateral thalamus (Guillaume et al., 2018).

### 3.4.2 Major depressive disorder (MDD) and suicidality

Table 4 describes 10 studies of MDD and 1 of suicidality. Seven studies had overlapping participants: five have some overlapping participants from an outpatient psychiatric clinic of Korea University Anam Hospital and community controls (Choi et al., 2015; K. Han et al., 2017; K. M. Han et al., 2017; Na et al., 2016; Won et al., 2016); and two other studies had overlapping participants (Booij et al., 2015; Frodl et al., 2015). Nine studies included a comparator group and 2 were case series of individuals with familial risk for MDD.
Ten of eleven studies used a candidate gene approach based on blood samples and one carried out EWAS on salivary DNA. Five candidate genes were studied in the context of MDD: \( SLC6A4 \) (n=5), \( BDNF \) (n=2), \( TESC \) (n=1), \( FKBP5 \) (n=1) and \( CACNA1C \) (n=1). The single EWAS study reported an association between DNA methylation and hippocampal volume, noting that DNA methylation mediated the effect of cortisol on hippocampal volume (Davis et al., 2017).

All four fMRI studies were task based. Two used the emotional face processing paradigm to investigate amygdala reactivity in relation to the \( SLC6A4 \) AluJb element or its promoter, and both report significant correlations between activation and \( SLC6A4 \) DNA methylation among cases (Schneider et al., 2018; Swartz et al., 2017). Another study reported a correlation between DNA methylation at the \( SLC6A4 \) and limbic system activation during a visual emotional attention shifting task in an analysis which pooled MDD cases and controls (Frodl et al., 2015). Together these studies suggest that \( SLC6A4 \) methylation positively correlates with limbic system reactivity in response to threat or negative emotional stimuli. However, these studies measured and reported \( SLC6A4 \) methylation at different sites, and analysed them differently. For example, Frodl et al used a summary mean measure across 11 CpG sites in the promoter, Swartz et al calculated a residualised change score from 20 CpG sites, and Schneider et al calculated mean methylation at the AluJb element of the promoter from 6 CpG sites (Frodl et al., 2015; Schneider et al., 2018; Swartz et al., 2017). The fourth fMRI study investigated brain responses to images that were associated with suicidal means in relation to DNA methylation in the \( CACNA1C \) transcription factor binding site, and reported differential brain activation in association with DNA methylation at two CpGs (Kim et al., 2017).

### 3.4.3. Alcohol use disorder

Table 5 summarises 4 studies of alcohol use disorder, all of which investigated DNA methylation in candidate genes in relation to task-based fMRI. Two were case-control study designs and two were uncontrolled case series. Three candidate genes were assessed: \( DRD2 \), \( SLC6A3/DAT \) and \( PPM1G \). Three different tasks were used: testing impulse control, reactivity to alcohol
cues and processing of reward/punishment cues. Three of these studies measured DNAm in blood, and one in saliva. Three of the four studies reported positive correlations between functional activation in limbic system structures and DNAm among participants with alcohol use disorder (Bidwell et al., 2018; Ruggeri et al., 2015; Wiers et al., 2015). The remaining study found no significant correlations in the alcohol dependent cases, but did find negative correlations in functional activation in the nucleus accumbens and methylation at the SLC6A3 promoter in the context of a monetary incentive delay task (Muench et al., 2018).

3.4.4. Schizophrenia and psychosis

Table 6 summarises seven studies of schizophrenia and psychosis, all of which included a comparator group. Six of the included studies use data from participants in the Mind Clinical Imaging Consortium (MCIC) all of whom have MRI, DNAm and SNP data available (Deng et al., 2016; Hass et al., 2015; Hu et al., 2018; Liu et al., 2015; Walton et al., 2014; Wang et al., 2017).

The majority of these studies measured blood DNAm on the Illumina HumanMethylation27 array (n=4), and one study carried out a candidate gene analysis on blood MB-COMT DNAm. Five of these used the Illumina HumanMethylation27 array, and two carried out bisulfite pyrosequencing of two genes: OXTR and MB-COMT. fMRI was the most commonly used modality (n=5), followed by sMRI (n=3). Of these, one study used both modalities. fMRI tasks included: a sensorimotor task and the Sternberg Item Recognition Paradigm for working memory ( Hu et al., 2018; Walton et al., 2014). Two studies did not report the fMRI paradigm (Deng et al., 2016; Wang et al., 2017).

3.4.5. Ageing, stroke, ataxia and neurodegeneration

Table 7 summarises eight studies that investigated aspects of ageing, including healthy ageing, Alzheimer’s disease, minor cognitive impairment, stroke, WMH burden and C9orf72 expansion and Fragile X Associated Tremor Ataxia Syndrome (FXTAS).
Imaging modalities used were as follows: dMRI (n=2), sMRI (n=5) d/sMRI (n=1). All studies used blood samples to measure DNAm. Five studies carried out candidate gene analysis and three measured DNAm in an epigenome-wide manner. The candidate genes studied were: *C9orf72* (n=1), *NCAPH2/LMF2* (n=1), *FMR1* (n=2), *SOD* (n=1). Two studies carried out a longitudinal analysis as well as a cross-sectional analysis (McMillan et al., 2015; Swartz et al., 2017). One of these showed, in a group of 11 *C9orf72* expansion patients, that there was more rapid grey matter atrophy in the right hippocampus, right thalamus and left middle frontal cortex where there was decreased methylation in the expansion region (McMillan et al., 2015). The other found in 87 study participants that increases in SLC6A4 promoter methylation corresponded to increases in amygdala reactivity in response to threat related stimuli in task-based fMRI (Swartz et al., 2017). Two studies that investigated associations between *FMR1* methylation and brain imaging in women with FXTAS had overlapping participants (Shelton et al., 2017, 2016). The three EWAS studies investigated DNAm age in relation to its associations with brain imaging features (Chouliaras et al., 2018; Hodgson et al., 2017; Raina et al., 2017). Two of these three studies investigated dMRI: one found a negative association of FA with epigenetic age acceleration, while the other found a negative association between global mean diffusivity (MD) and Hannum age and a positive association between FA and Hannum age (Chouliaras et al., 2018; Hodgson et al., 2017). In an analysis with sMRI a positive association was found between WMH burden category and DNAm age acceleration (Raina et al., 2017).

### 3.4.6. Post-traumatic stress disorder

Table 8 summarises seven studies that were carried out in participants with PTSD (Table 6). Several studies had overlapping participants. Three studies report associations in an overlapping group of participants: PTSD cases and non-PTSD controls, where the case population was mothers who have experienced interpersonal violence (IPV) (Moser et al., 2015; Schechter et al., 2017, 2015). Two further studies had overlapping participants – a cohort of trauma exposed veterans (Sadeh et al., 2016; Wolf et al., 2016). An additional group
of veterans was also studied in relation to PTSD (McNerney et al., 2018). A further study recruited women who had experienced early life trauma to investigate associations between DNAm and sMRI features (Klengel et al., 2013). Five candidate genes were studied: NR3C1 (n=2), SKA2 (n=1), BDNF (n=1), FKBP5 (n=1), 5HT3A (n=1). All three modalities were employed to investigate neuroimaging features: dMRI (n=1), sMRI (n=3), fMRI (n=3). Overlap in study populations lay between two studies which measured DNAm using the Illumina 450k array (Sadeh et al., 2016; Wolf et al., 2016). Wolf et al. (2016) carried out analysis of DNAm age while Sadeh et al. (2016) carried out a candidate gene analysis of SKA2 (Sadeh et al., 2016; Wolf et al., 2016). McNerney et al. (2018) measured DNAm at the NR3C1 gene using bisulfite pyrosequencing (McNerney et al., 2018). All three of the articles investigating a population of mothers who have experienced IPV studied candidate genes: NR3C1, BDNF and 5HT3A (Moser et al., 2015; Schechter et al., 2017, 2015). All three used fMRI, with two using the same task (Modified Crowell procedure), and one using a unique task video stimulus depicting neutral, menacing, and male-female prosocial interactions (Moser et al., 2015). The two studies that reported on IPV PTSD mothers in relation to the modified Crowell procedure showed positive associations between DNAm at NR3C1 and BDNF and cluster activation in vmPFC OFC posterior cingulate cortex (Moser et al., 2015; Schechter et al., 2015).

3.4.7. Miscellaneous: healthy adults, socio-emotional processing, Cushing’s syndrome

Two of the included studies in this section investigated patient populations, and the remainder reported healthy participants. One case-control study investigated DNAm-MRI in Cushing’s Syndrome patients, and another case series study investigated social anxiety disorder (Resmini et al., 2016; Ziegler et al., 2015). Both studies discovered significant correlations between DNAm and neuroimaging findings. Fourteen studies in this section adopt a candidate gene analysis approach, with 1 being epigenome-wide (Table 9). The candidate genes studied were: COMT (n=1), OXTR (n=3), NR3C1 (n=1), SLC6A4 (n=5), OXT (n=1), FKBP5 (n=1), KLF13 (n=1), BDNF (n=1). DNAm was measured in the following tissues: saliva (n=3); blood (n=10); blood/saliva/buccal (n=1); saliva and blood (n=1). Two MRI modalities are represented
in this section: sMRI (n=3), and fMRI (n=10), and two studies used both modalities (s/fMRI (n=2). fMRI tasks included: N back task, social phobia associated verbal stimuli, emotional perspective, emotional attribution, social perception, emotional face processing, responses to high calorie versus low calorie foods.

The three studies that investigated associations with OXTR methylation measured DNAm at two different positions: one in exon 3 and the other two at a CpG site -934 base pairs upstream of the TSS which has consistent methylation across blood and brain tissues (Jack et al., 2012; Puglia et al., 2015; Rubin et al., 2016; Ziegler et al., 2015). While these studies all used fMRI, they also used different tasks: social phobia related verbal stimuli, the emotional face matching task, and the social perception task (where objects move in ways to suggest animacy or random movement). One study investigated OXTR DNAm in exon 3, which had previously been implicated in social cognition, in a patient population with social anxiety disorder (Ziegler et al., 2015). The other two investigated healthy participants; one of these was in the context of social perception and individual variability in social perception (displays of animacy) (Jack et al., 2012; Puglia et al., 2015).

Five papers analysed associations between SLC6A4 methylation and neuroimaging features. They included fMRI (n=4) and sMRI (n=2). fMRI studies used resting state and/or emotional face processing tasks. Ismaylova et al. (2017) estimated DNAm from three different surrogate tissues: blood, saliva, and buccal cells and reported that right lateral parietal area (RLP) resting state connectivity with the lateral occipital cortex and frontal poles has a positive association with SLC6A4 DNAm (Ismaylova et al., 2017). Muehlhan et al. (2015) found positive associations between functional coupling between amygdala and nodes of the salience network with DNAm of the SLC6A4 promoter in blood (Muehlhan et al., 2015). In the emotional face processing task, two studies report positive association between SLC6A4 methylation and amygdala reactivity/connectivity in response to threat/fear related faces. Nikolova et al reported a positive relationship between saliva DNAm at SLC6A4 and left
amygdala reactivity to threat stimuli, while Ismaylova et al reported that twins with higher saliva DNAm at SLC6A4 display greater connectivity between ACC and left amygdala (Ismaylova et al., 2018; Nikolova et al., 2014). The two studies that used sMRI both found positive associations between DNAm at SLC6A4 and grey matter volumes (Dannlowski et al., 2014; Ismaylova et al., 2017). However, they did not replicate each other by identifying the same brain regions as being associated even when using the same tissue to estimate DNAm.

4. Discussion

This systematic review of 60 studies involving approximately 6775 participants suggests that differential DNAm may be associated with MRI features of brain structure and / or function for conditions / diseases within the following categories: neurodevelopment and neurodevelopmental disorders; MDD and suicidality; alcohol use disorder; schizophrenia and psychosis; ageing, stroke, ataxia and neurodegeneration; post-traumatic stress disorder; healthy adults and socio-emotional processing. We found that 10 image features were associated with differential DNAm in two or more of the 60 studies. However, quantitative synthesis of DNAm-MRI associations was not possible due to the lack of consistency in DNAm findings, and / or heterogeneity in image features across studies. While the results presented here could provide new insights into the role of DNAm in health and disease across the life course, caution is required because the clinical and methodological heterogeneity of included studies was large. We identified the following sources of methodological heterogeneity and potential sources of bias: variable inclusion and characterisation of comparator groups in study designs; use of different tissues as a surrogate for brain; variation in methods for estimating DNAm and ascertainment of cell type composition; lack of control for genotype; and variations in image processing methodology and selected MRI features.

4.1 Study populations and comparator group validity
Twenty-six studies (43%) did not include a comparator group, and some others recruited cases and controls but combined the groups in DNAm-MRI analysis or restricted DNAm-MRI analysis to one group only. These issues limit inference about disease association. Several aspects of study design that are fundamental to good epidemiology should be adopted in future DNAm-MRI studies to reduce potential sources of bias and enable maximum inference. These include detailed descriptions of participant selection, the population from which they were selected, and the method used to select. Not only is this important for understanding case definition, but also because research participation is influenced by social class, education, and ethnicity, and some of these factors may influence DNAm (Stafford et al., 2013). The same descriptive standards should be applied to comparator groups to ensure clinical validity, and attention should be given to matching comparator groups for ethnicity and age because both affect DNAm (Fraser et al., 2012; Heyn et al., 2013; Horvath, 2013; Talens et al., 2010).

4.2 Surrogate tissues for brain DNAm and cell composition

Surrogate tissues include blood and saliva buccal cell samples. This is important when interpreting data because of tissue and cell heterogeneity in DNAm patterns. Buccal cells, a major cell type found in saliva, have been proposed to have more validity than blood as a surrogate tissue for brain since they have a common embryological cell lineage, both being derived from the ectoderm germ layer (Smith et al., 2015). One study that ascertained DNAm from three sample types (blood, saliva, buccal - buccal cells and saliva samples are collected differently) found that DNAm obtained from non-blood surrogate tissues most strongly associated with brain processes in living humans in the context of fMRI study (Ismaylova et al., 2017). This is consistent with a previous study in postmortem brain tissue in animal models, which found that saliva and brain are more similar in their DNA methylation patterns than are blood and brain (Lowe et al., 2013). Surrogate tissues have heterogeneous cell compositions which can impact the DNAm signature of the sample: blood comprises lymphocytes, neutrophils, basophils, monocytes and eosinophils; and saliva contains
predominantly buccal cells but can include leukocytes. Crucially, the compositions of both surrogates are influenced by disease status. Hence, adjustment for cell composition should be considered in DNAm analysis.

4.3 Candidate gene versus epigenome-wide approaches

Forty-three included studies in this review used a candidate gene analysis approach, with candidacy most often rooted in knowledge or hypotheses about the biological underpinnings of the target disease. The majority of candidate gene studies focused on promoter regions, which risks neglect of other important regulatory regions, because it limits research to currently understood genomic elements that have a clear role in gene expression regulation.

Although there are inherent sources of bias in the candidate approach, it has been suggested that candidate gene studies can provide a useful starting point for hypothesis construction for example by investigating candidates that have emerged from the GWAS literature (Nikolova and Hariri, 2015). However, we did not find that candidate selection was based on the GWAS literature in the majority of studies. For example, SLC6A4 was the candidate gene in eleven studies. SLC6A4 encodes a serotonin transporter responsible for serotonin re-uptake from the synapse, and is one of the targets for the serotonin reuptake inhibitor class of antidepressants. Despite the importance of this gene product in the pharmacology of depression, recent GWAS studies report no association between candidate polymorphisms at SLC6A4 (or a number of other candidate genes) and major depression (Border et al., 2019). This suggests that candidate genes that encode important pharmacological targets are not necessarily aetiologically significant. This is not to say that the methylation status of these genes is irrelevant: for example, it could be useful for investigating medication response, or as a biomarker. Frodl et al carried out an DNAm-MRI analysis with medication status as a covariate, and but found that this did not modify their findings regarding SLC6A4 methylation and brain function in MDD patients and controls, while Booij et al have reported differential methylation at the SLC6A4 promoter in association SSRI exposure (Booij et al., 2015; Frodl
et al., 2015). In a human population of MDD no difference was found in methylation between medication free subjects and those on medication in a post mortem study (Sabunciyan et al., 2012). These uncertainties suggest that further research into association between medication exposure and DNAm is warranted, and that hypothesis formation around candidate genes should be explicit in terms of theorised mechanism of disease or biomarker development. Further, mechanistic studies are crucial to aid our understanding of the interaction between DNAm and disease or therapy.

The rationales given for the selection of candidate genes for DNAm-MRI analysis were varied. Several studies selected candidates based on prior unbiased analyses in relation to phenotype (Kim et al., 2017; Ruggeri et al., 2015; Sadeh et al., 2016). For example, both PP1MG and SKA2 were identified in EWAS analyses (Ruggeri et al., 2015; Sadeh et al., 2016), and CACNA1C was identified from a hypothesis free Methyl-Seq (Kim et al., 2017). Other candidate genes were chosen because they have a strong genetic link to the patient population being studied, such as C9orf72 expansion in ALS-clinic patients and FMR1 in FXTAS patients (McMillan et al., 2015; Shelton et al., 2017, 2016). For the most part candidate genes were selected because their gene products are considered key components of a pathway of relevance to a condition or phenotype studied. NR3C1 and FKBP5, the genes that encode the glucocorticoid receptor and FK506 binding protein (which regulates glucocorticoid receptor sensitivity), are both involved in cortisol signalling and were selected in studies investigating stress response or stress related neuropsychiatric conditions such as PTSD or MDD, or in Cushing’s Syndrome where cortisol is known to be dysregulated. BDNF, which encodes for brain derived neurotrophic factor, was studied in association to MDD, PTSD and working memory. COMT encodes catechol-O-methyltransferase, an enzyme that degrades catecholamines such as dopamine, epinephrine and norepinephrine, was the candidate gene in studies investigating DNAm-MRI in the context of working memory in schizophrenia and in healthy participants. OXTR encodes the receptor for oxytocin and was selected in studies that investigated an aspect of emotional or social processing. SLC6A4 encodes the serotonin
reuptake transporter and is a target for the serotonin reuptake inhibitor class of anti-depressants. It was the selected candidate gene in studies of MDD and ADHD patients and emotional processing, and resting state amygdala activity.

Seventeen studies used an EWAS approach. There was no overlap between differentially methylated regions identified using this method versus the selected candidate gene approach for any of the phenotypic categories, which highlights the value of epigenome-wide approaches for unbiased investigation of the putative role of DNAm in brain structure and/or function. However, one study which employed an epigenome-wide approach to DNAm analysis identified COMT and SLC6A3 as being negatively associated with right amygdala volume in neonates homozygous for BDNF rs6265 Met/Met allele (Chen et al., 2015). Both of these genes were selected as candidates in other studies but associations were reported in different populations with regards to age and clinical background, and with different image features (Muench et al., 2018; Ursini et al., 2011; Walton et al., 2014; Wiers et al., 2015). Many of the studies that utilised epigenome-wide methods for measuring DNAm did not go on to report on individual loci. In future studies whole genome-based approaches could play an important role in generating novel candidates and pathways for future research that could provide mechanistic insight into pathogenesis.

4.4 Interindividual variability, effect of genotype, and temporal stability

Sources of variability that are crucial for understanding of how changes in DNAm associate with endophenotypes include interindividual variability which may be stochastic, genetic or environmental in origin, and temporal stability (Lancaster et al., 2018). Twin studies are useful for separating genetic and environmental contributions to variability, and several are included in the DNAm-MRI literature to date (Casey et al., 2017; Chen et al., 2018; Ismaylova et al., 2018). Twenty-four studies controlled for genotypic effects, for example by covarying for single nucleotide polymorphisms (SNPs), or for the first PC of the variance in genome wide genetic
data. This is because variation in DNAm may be explained partly by common genetic variation, such as a SNP that removes a methylation site. Therefore, genotyping in DNAm-MRI analyses is necessary for determining whether epigenetic associations with brain structure and function are genotype-specific, or whether associations are independent of genotype.

The stability of some epialleles varies over time with dynamic methylation and demethylation at CpG sites occurring in typical development and ageing, and in response to environmental exposures (Lancaster et al., 2018). Only fifteen studies reported the timing of DNAm sampling in respect to MRI acquisition; we suggest that this information is included in future studies because it is required to compare study populations and interpret phenotypic associations. Longitudinal investigation could be used to establish the temporal stability of methylation at a genomic region or site, the impact of medication, and for assessing potential causal relationships.

All of the included studies carried out cross-sectional analysis and two additionally carried out longitudinal analysis. Three other studies performed mediation analysis to assess causal relationships (Casey et al., 2017; Freytag et al., 2017; Harms et al., 2017). Studies designed to investigate association or causation should report the magnitude of differential DNAm between groups since the described changes in DNAm are often small and the biological relevance of these remains uncertain. Ultimately however, differential DNAm-MRI data will need to be interpreted in the wider context of mechanistic studies of gene expression in order to make inferences about causality. For example, experiments that demonstrate changes in gene expression associated with the differential methylation patterns reported from DNAm-MRI studies would be an important first step in dissecting causality, although it is important to note that changes in DNAm may actually occur as a consequence of changes in gene expression.

4.5 Neuroimaging considerations
Structural and functional quantitative neuroimaging features have been included in this review, assessed using sMRI, fMRI and dMRI. Others have emphasised the importance of selecting image features that represent a deficient or pathological phenotype when investigating associations between DNAm and MRI (Lancaster et al., 2018). Five DNAm-MRI associations recurred in two or more studies (SLC6A4 and amygdala reactivity; OXTR and amygdala reactivity; BDNF and PFC function; FKBP5 and hippocampal volume; COMT and PFC function), and a narrative synthesis of these demonstrates that there were mixed directions of associations, which may be explained by the variation in where in a genomic region DNAm was measured and in which tissue, in tasks employed during fMRI and in participant characteristics. A variety of fMRI paradigms have been used to probe brain function in this field, such as resting state and emotional processing, and dMRI studies have used different diffusion gradient encoding schemes and strengths (b-values) to measure the mobility of water molecules in vivo. A further layer of complexity is added in terms of differences in image processing pipelines such as CIVET (http://www.bic.mni.mcgill.ca/ServicesSoftware/CIVET), FreeSurfer (https://surfer.nmr.mgh.harvard.edu) and SPM (https://www.fil.ion.ucl.ac.uk/spm) for sMRI; and voxel based methods e.g. TBSS (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS) or tractography methods e.g. probabilistic neighbourhood tractography (http://www.tractor-mri.org.uk/tractography) for analysis of dMRI (Ad-Dab’bagh et al., 2006; Anblagan et al., 2015; Bastin et al., 2010; Clayden et al., 2007, 2006; Fischl, 2012; Smith et al., 2006). In the future, it will be necessary to form consensus and harmonisation about the optimal acquisition protocols and analysis pipelines to allow findings from different groups to be combined more readily. Initiatives such as the ENIGMA consortium which aims to understand brain structure, function and disease using brain imaging and genetic data from groups around the world are an important step in this direction.

4.6 Strengths and limitations

This systematic review has several strengths. It was based on a predefined protocol and followed standard guidelines with rigorous screening of >3,400 articles and without language
or publication year restrictions. Our analysis was based on 60 studies examining healthy individuals and a wide range of neurological and psychiatric diseases in more than 6,000 patients across the life course. This provided a detailed overview of a growing body of literature linking DNA methylation (DNAm) and MRI data types, and enabled us to identify the key sources of methodological variation that should be addressed as standards and conventions in DNAm-MRI analyses become established.

Our study also has limitations. First, there was substantial clinical and methodological heterogeneity which prohibited quantitative synthesis for any single phenotype category, or MRI feature. Second, in the absence of validated tools for assessing quality and risk of bias in DNAm-MRI analyses, we were unable to evaluate these issues quantitively.

4.7 Conclusion

There is some evidence of association between differential DNAm and human brain structure and/or function across the life course. However, varied methodologies used to acquire and analyse DNAm-MRI data prevent quantitative synthesis. The development of standards and conventions for studies linking DNAm with MRI data is required, with particular focus on: detailed case and comparator group definition; surrogate tissue type; adjustment for cell composition and common genetic variation; consistent approaches to DNAm estimation using whole genome approaches; and use of image acquisition protocols, analysis pipelines and image feature selection that best support pooled analyses that are likely to be required to achieve statistical power for EWAS-neuroimaging studies. In summary, this review has found that: differential DNAm is associated with image features of brain structure and function in health and disease across the life course (using data from over 6,000 individuals); evidence that differential DNAm is associated with specific image features is modest; and it has identified specific sources of sample and methodological heterogeneity in existing DNAm-MRI analyses. We anticipate that identification of these will expedite rational development of analytic methods in this emerging field so that researchers might more rapidly design studies that support causal inference.
Acknowledgements

ENWW is supported by the Wellcome Trust Translational Neuroscience PhD fellowship programme at the University of Edinburgh (203769/Z/16/A). The work was undertaken in the MRC Centre for Reproductive Health, which is funded by MRC Centre Grant (MRC G1002033). DQS is supported by a fellowship from Theirworld (https://theirworld.org/). The authors declare no conflicts of interest.
Abbreviations used in tables

ACR, anterior corona radiata
AD, alcohol dependence
AxD, axial diffusivity
ADC, apparent diffusion coefficient
aMCI, amnesic mild cognitive impairment
BCC, body of the corpus callosum
CC, corpus callosum
CLASP, Constrained Laplacian-based Automated Segmentation with Proximities
CSF, cerebral spinal fluid
CST, corticospinal tract
DG, dentate gyrus
DMR, differentially methylated region
FA, fractional anisotropy
FXTAS, Fragile X Associated Tremor Ataxia Syndrome
GCC, genu of corpus callosum
GM, grey matter
HC, healthy controls
HS, hippocampal sclerosis
IFG, inferior frontal gyrus
IT, inferior temporal
MD, mean diffusivity
MDD, major depressive disorder
MFG, middle frontal gyrus
mOFC, medial orbitofrontal cortex
MRF, Markov Random Field
MTLE, mesial temporal lobe epilepsy
NAcc, nucleus accumbens
NR, not reported
PCR, posterior corona radiata
PHC, parahippocampal cingulum
PNT, probabilistic neighbourhood tractography
PTR, posterior thalamic radiation
RD, radial diffusivity
RLP, right lateral parietal area
RS, resting state
SFG, superior frontal gyrus
STG, superior temporal gyrus
TBSS, tract-based spatial statistics
vIPFC, ventrolateral prefrontal cortex
vIThalamus, ventrolateral thalamus
WB, whole brain
WMH, white matter hyperintensities

**Gene names**

SKA2, Spindle and Kinetochore Associated Complex Subunit 2

*SLC6A4*, Solute Carrier Family 6 Member 4

*BDNF*, Brain Derived Neurotrophic Factor

*FKBP5*, FK506 Binding Protein 51

*OXTR*, Oxytocin Receptor

*NR3C1*, Nuclear Receptor Subfamily 3 Group member 1

*FMR1*, Fragile X Mental Retardation 1

*COMT*, Catecholamine Transferase

*DAT/SLC6A3*, Dopamine Transporter/Solute Carrier Family 6 Member 3

*OXT*, Oxytocin/Neurophysin 1 Prepropeptide

*C9orf72*, Chromosome 9 Open Reading Frame 72
CACNA1C, Calcium Voltage-Gated Channel Subunit Alpha1 C

DRD2, Dopamine Receptor 2

KLF13, Kruppel Like Factor 13

NCAPH2, Non-SMC Condensin II Complex Subunit H2

LMF2, Lipase Maturation Factor 2

PPM1G, Protein Phosphatase Magnesium Dependent 1 Gamma

HTR3A, 5-Hydroxytryptamine Receptor 3A

SOD, Superoxide Dismutase

TESC, Tescalcin

References

Ad-Dab’bagh, Y., Einarson, D., Lyttelton, O., Muehlboeck, J.-S., Mok, K., Ivanov, O., Vincent, R.D., Lepage, C., Lerch, J., Fombonne, E., Evans, A.C., 2006. The CIVET image-processing environment: a fully automated comprehensive pipeline for anatomical neuroimaging research. Proc. 12th Annu. Meet. Organ. Hum. Brain Mapp.

Anblagan, D., Bastin, M.E., Sparrow, S., Piyasena, C., Pataky, R., Moore, E.J., Serag, A., Wilkinson, A.G., Clayden, J.D., Semple, S.I., Boardman, J.P., 2015. Tract shape modeling detects changes associated with preterm birth and neuroprotective treatment effects. NeuroImage Clin. 8, 51–58. https://doi.org/10.1016/j.nicl.2015.03.021

Bastin, M.E., Munoz Maniega, S., Ferguson, K.J., Brown, L.J., Wardlaw, J.M., MacLullich, A.M., Clayden, J.D., 2010. Quantifying the effects of normal ageing on white matter structure using unsupervised tract shape modelling. Neuroimage 51, 1–10. https://doi.org/10.1016/j.neuroimage.2010.02.036

Bidwell, L.C., Karoly, H.C., Thayer, R.E., Claus, E.D., Bryan, A.D., Weiland, B.J., Yorkwilliams, S., Hutchison, K.E., 2018. DRD2 promoter methylation and measures of alcohol reward: functional activation of reward circuits and clinical severity. Addict. Biol. 24, 539–548. https://doi.org/10.1111/adb.12614

Booij, L., Szyf, M., Carballedo, A., Frey, E., Morris, D., Dymov, S., Vaisheva, F., Ly, V.,
Fahey, C., Meaney, J., Gill, M., Frodl, T., 2015. DNA Methylation of the Serotonin Transporter Gene in Peripheral Cells and Stress-Related Changes in Hippocampal Volume: A Study in Depressed Patients and Healthy Controls. PLoS One 10, 1–14. https://doi.org/10.1371/journal.pone.0119061

Border, R., Johnson, E.C., Ph, D., Evans, L.M., Ph, D., Smolen, A., Ph, D., Berley, N., Sullivan, P.F., Keller, M.C., Ph, D., 2019. No Support for Historical Candidate Gene or Candidate Gene-by-Interaction Hypotheses for Major Depression Across Multiple Large Samples. Am. J. Psychiatry 176, 376–387. https://doi.org/10.1176/appi.ajp.2018.18070881

Cao-Lei, L., Elgbeili, G., Massart, R., Laplante, D.P., Szyf, M., King, S., 2015. Pregnant women’s cognitive appraisal of a natural disaster affects DNA methylation in their children 13 years later: Project Ice Storm. Transl. Psychiatry 5, e515-9. https://doi.org/10.1038/tp.2015.13

Casey, K.F., Levesque, M.L., Szyf, M., Ismaylova, E., Verner, M.-P., Suderman, M., Vitaro, F., Brendgen, M., Dionne, G., Boivin, M., Tremblay, R.E., Booij, L., 2017. Birth Weight Discordance, DNA Methylation, and Cortical Morphology of Adolescent Monozygotic Twins. Hum. Brain Mapp. 38, 2037–2050. https://doi.org/10.1002/hbm.23503

Chen, L.I., Pan, H., Tuan, A.N.H., Teh, L., Maclusaic, J.L., Mah, S.M., McEwen, L.M., Li, Y., Chen, H., Broekman, B.F.P., Buschdorf, J.P., Chong, Y.S., Kwek, K., Saw, S.M., Gluckman, P.D., Fortier, M. V., Rifkin-Graboi, A., Kobor, M.S., Qiu, A., Meaney, M.J., Holbrook, J.D., GUSTO Study Group, 2015. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism influences the association of the methylome with maternal anxiety and neonatal brain volumes. Dev. Psychopathol. 27, 137–150. https://doi.org/10.1017/S0954579414001357

Chen, Y.C., Sudre, G., Sharp, W., Donovan, F., Chandrasekharappa, S.C., Hansen, N., Elnitski, L., Shaw, P., 2018. Neuroanatomic, epigenetic and genetic differences in monozygotic twins discordant for attention deficit hyperactivity disorder. Mol. Psychiatry 23, 683–690. https://doi.org/10.1038/mp.2017.45
Choi, S., Han, K.-M., Won, E., Yoon, B.-J., Lee, M.-S., Ham, B.-J., 2015. Association of brain-derived neurotrophic factor DNA methylation and reduced white matter integrity in the anterior corona radiata in major depression. J. Affect. Disord. 172, 74–80. https://doi.org/10.1016/j.jad.2014.09.042

Chouliaras, L., Pishva, E., Haapakoski, R., Zsoldos, E., Mahmoud, A., Filippini, N., Burraga, J., Mill, J., Kivima, M., Lunnon, K., Ebmeier, K.P., 2018. Peripheral DNA methylation, cognitive decline and brain aging: pilot findings from the Whitehall II imaging study. Epigenomics 10, 585–595. https://doi.org/10.2217/epi-2017-0132

Clayden, J.D., Bastin, M.E., Storkey, A.J., 2006. Improved segmentation reproducibility in group tractography using a quantitative tract similarity measure. Neuroimage 33, 482–492. https://doi.org/10.1016/j.neuroimage.2006.07.016

Clayden, J.D., Storkey, A.J., Bastin, M.E., 2007. A Probabilistic Model-Based Approach to Consistent White Matter Tract Segmentation. IEEE Trans. Med. Imaging 26, 1555–1561.

Dannlowski, U., Kugel, H., Redlich, R., Halik, A., Schneider, I., Opel, N., Grotegerd, D., Schwarte, K., Schettler, C., Ambree, O., Rust, S., Domshcke, K., Arolt, V., Heindel, W., Baune, B.T., Suslow, T., Zhang, W., Hohoff, C., 2014. Serotonin transporter gene methylation is associated with hippocampal gray matter volume. Hum. Brain Mapp. 35, 5356–5367. https://doi.org/10.1002/hbm.22555

Davis, E.G., Humphreys, K.L., McEwen, L.M., Sacchet, M.D., Camacho, M.C., MacIsaac, J.L., Lin, D.T.S., Kobor, M.S., Gotlib, I.H., 2017. Accelerated DNA methylation age in adolescent girls: associations with elevated diurnal cortisol and reduced hippocampal volume. Transl. Psychiatry 7, e1223. https://doi.org/10.1038/tp.2017.188

De Rubeis, S., He, X., Goldberg, A.P., Poultnay, C.S., Samocha, K., Cicek, A.E., Kou, Y., Liu, L., Fromer, M., Walker, S., Singh, T., Klei, L., Kosmicki, J., Shih-Chen, F., Aleksic, B., Biscaldi, M., Bolton, P.F., Brownfeld, J.M., Cai, J., Campbell, N.G., Carracedo, A., Chahrour, M.H., Chiocchetti, A.G., Coon, H., Crawford, E.L., Curran, S.R., Dawson, G., Duketis, E., Fernandez, B.A., Gallagher, L., Geller, E., Guter, S.J., Hill, R.S., Ionita-
Laza, J., Jimenez Gonzalez, P., Kilpinen, H., Klauck, S.M., Kolevzon, A., Lee, I., Lei, I., Lei, J., Lehtimaki, T., Lin, C.F., Ma’ayan, A., Marshall, C.R., McInnes, A.L., Neale, B., Owen, M.J., Ozaki, N., Parellada, M., Parr, J.R., Purcell, S., Puura, K., Rajagopalan, D., Rehnstrom, K., Reichenberg, A., Sabo, A., Sachse, M., Sanders, S.J., Schafer, C., Schulte-Ruther, M., Skuse, D., Stevens, C., Szatmari, P., Tammimies, K., Valladares, O., Voran, A., Li-San, W., Weiss, L.A., Willsey, A.J., Yu, T.W., Yuen, R.K., Study, D.D.D., Homozygosity Mapping Collaborative for, A., Consortium, U.K., Cook, E.H., Freitag, C.M., Gill, M., Hultman, C.M., Lehrer, T., Palotie, A., Schellenberg, G.D., Sklar, P., State, M.W., Sutcliffe, J.S., Walsh, C.A., Scherer, S.W., Zwick, M.E., Baret, J.C., Cutler, D.J., Roeder, K., Devlin, B., Daly, M.J., Buxbaum, J.D., 2014. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515, 209–215. https://doi.org/10.1038/nature13772

Del Blanco, B., Barco, A., 2018. Impact of environmental conditions and chemicals on the neuronal epigenome. Curr. Opin. Behav. Sci. 45, 157–165. https://doi.org/10.1016/j.cobpa.2018.06.003

Deng, S., Lin, D., Calhoun, V.D., Wang, Y., Member, S., 2016. Predicting Schizophrenia by Fusing Networks from SNPs, DNA Methylation and fMRI Data *. Conf. Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. IEEE Eng. Med. Biol. Soc. Annu. Conf. 1447–1450. https://doi.org/10.1109/EMBC.2016.7590981

Elliott, L.T., Sharp, K., Alfaro-almagro, F., Shi, S., Miller, K.L., Douaud, G., Marchini, J., Smith, S.M., 2018. Genome-wide association studies of brain imaging phenotypes in UK Biobank. Nature 562, 210–216. https://doi.org/10.1038/s41586-018-0571-7

Fischl, B., 2012. FreeSurfer. Neuroimage 62, 774–781. https://doi.org/10.1016/j.neuroimage.2012.01.021

Fraser, H.B., Lam, L.L., Neumann, S.M., Kobor, M.S., 2012. Population-specificity of human DNA methylation. Genome Biol. 13. https://doi.org/10.1186/gb-2012-13-2-r8

Freytag, V., Carrillo-roa, T., Milnik, A., Samann, P.G., Vukojevic, V., Coynel, D., Demougine, P., Egli, T., Gschwind, L., Jessen, F., Loos, E., Maier, W., Riedel-heller, S.G., Scherer,
M., Vogler, C., Wagner, M., Binder, E.B., Quervain, D.J. De, Papassotiropoulos, A., 2017. A peripheral epigenetic signature of immune system genes is linked to neocortical thickness and memory. Nat. Commun. 8. https://doi.org/10.1038/ncomms15193

Frodl, T., Szyf, M., Carballedo, A., Ly, V., Dymov, S., Vaisheva, F., Morris, D., Fahey, C., Meaney, J., Gill, M., Booij, L., 2015. DNA methylation of the serotonin transporter gene (SLC6A4) is associated with brain function involved in processing emotional stimuli. J. Psychiatry Neurosci. 40, 296–305. https://doi.org/10.1503/jpn.140180

Gluckman, P.D., Hanson, M.A., Buklijas, T., 2010. A conceptual framework for the developmental origins of health and disease. J. Dev. Orig. Health Dis. 1, 6–18. https://doi.org/10.1017/S2040174409990171

Gouin, J.P., Zhou, Q.Q., Booij, L., Boivin, M., Cote, S.M., Hebert, M., Ouellet-Morin, I., Szyf, M., Tremblay, R.E., Turecki, G., Vitaro, F., 2017. Associations among oxytocin receptor gene (OXTR) DNA methylation in adulthood, exposure to early life adversity, and childhood trajectories of anxiousness. Sci. Rep. 7, 7446. https://doi.org/10.1038/s41598-017-07950-x

Grayson, D.R., Guidotti, A., 2013. The dynamics of DNA methylation in schizophrenia and related psychiatric disorders. Neuropsychopharmacology 38, 138–166. https://doi.org/10.1038/npp.2012.125

Gregory, S.G., Connelly, J.J., Towers, A.J., Johnson, J., Biscocho, D., Markunas, C.A., Lintas, C., Abramson, R.K., Wright, H.H., Ellis, P., Langford, C.F., Worley, G., Delong, R., Murphy, S.K., Cuccaro, M.L., Persico, A., Pericak-Vance, M.A., 2009. Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. BMC Med. 7. https://doi.org/10.1186/1741-7015-7-62

Guillaume, B., Wang, C., Poh, J., Shen, M.J., Ong, M.L., Tan, P.F., Karnani, N., Meaney, M., Qiu, A., 2018. Improving mass-univariate analysis of neuroimaging data by modelling important unknown covariates: Application to Epigenome-Wide Association Studies. Neuroimage 173, 57–71. https://doi.org/10.1016/j.neuroimage.2018.01.073
Haas, B.W., Filkowski, M.M., Cochran, R.N., Denison, L., Ishak, A., Nishitani, S., 2016. Epigenetic modification of OXT and human sociability. Proc. Natl. Acad. Sci. U. S. A. 113. https://doi.org/10.1073/pnas.1602809113

Han, K., Won, E., Sim, Y., Kang, J., Han, C., Kim, Y., Kim, S., Joe, S., Lee, M., Tae, W., Ham, B., 2017. Influence of FKBP5 polymorphism and DNA methylation on structural changes of the brain in major depressive disorder. Sci. Rep. 1–12. https://doi.org/10.1038/srep42621

Han, K.M., Won, E., Kang, J., Choi, S., Kim, A., Lee, M.S., Tae, W.S., Ham, B.J., 2017. TESC gene-regulating genetic variant (rs7294919) affects hippocampal subfield volumes and parahippocampal cingulum white matter integrity in major depressive disorder. J. Psychiatr. Res. 93, 20–29. https://doi.org/10.1016/j.jpsychires.2017.05.010

Harms, M.B., Birn, R., Provencal, N., Wiechmann, T., 2017. Early life stress, FK506 binding protein 5 gene (FKBP5) methylation, and inhibition-related prefrontal function: A prospective longitudinal study. Dev. Psychopathol. 29, 1895–1903. https://doi.org/10.1017/S095457941700147X

Hass, J., Walton, E., Wright, C., Beyer, A., Scholz, M., Turner, J., Liu, J., Smolka, M.N., Roessner, V., Sponheim, S.R., Gollub, R.L., Calhoun, V.D., Ehrlich, S., 2015. Associations between DNA methylation and schizophrenia-related intermediate phenotypes — A gene set enrichment analysis. Prog. Neuropsychopharmacol. Biol. Psychiatry 59, 31–39. https://doi.org/10.1016/j.pnpbp.2015.01.006

Heijmans, B.T., Tobi, E.W., Stein, A.D., Putter, H., Blauw, G.J., Susser, E.S., Slagboom, P.E., Lumey, L.H., 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc. Natl. Acad. Sci. U. S. A. 105, 17046–17049. https://doi.org/10.1073/pnas.0806560105

Heyn, H., Moran, S., Hernando-Herraez, I., Sayols, S., Gomez, A., Sandoval, J., Monk, D., Hata, K., Marques-Bonet, T., Wang, L., Esteller, M., 2013. DNA methylation contributes to natural human variation. Genome Res. 23, 1363–1372. https://doi.org/10.1101/gr.154187.112
Hodgson, K., Carless, M.A., Kulkarni, H., Curran, J.E., Sprooten, E., Knowles, E.E., Mathias, S., Goring, H.H.H., Yao, N., Olvera, R.L., Fox, P.T., Almasy, L., Duggirala, R., Blangero, J., Glahn, D.C., 2017. Epigenetic Age Acceleration Assessed with Human White-Matter Images. J. Neurosci. 37, 4735–4743.
https://doi.org/10.1523/JNEUROSCI.0177-17.2017

Hoffmann, A., Sportelli, V., Ziller, M., Spengler, D., 2017. Epigenomics of major depressive disorders and schizophrenia: Early life decides. Int. J. Mol. Sci. 18, 1–25.
https://doi.org/10.3390/ijms18081711

Horvath, S., 2013. DNA methylation age of human tissues and cell types. Genome Biol. 14.
https://doi.org/10.1186/gb-2013-14-10-r115

Hu, W., Lin, D., Cao, S., Liu, J., Chen, J., Calhoun, V.D., Wang, Y., Member, S., 2018. Adaptive Sparse Multiple Canonical Correlation Analysis With Application to Imaging (Epi)Genomics Study of Schizophrenia. IEEE Trans. Biomed. Eng. 65, 390–399.
https://doi.org/10.1109/TBME.2017.2771483

Ismaylova, E., Di Sante, J., Szyf, M., Nemoda, Z., Yu, W.J., Pomares, F.B., Turecki, G., Gobbi, G., Vitaro, F., Tremblay, R.E., Boojl, L., 2017. Serotonin transporter gene promoter methylation in peripheral cells in healthy adults: Neural correlates and tissue specificity. Eur. Neuropsychopharmacol. 27, 1032–1041.
https://doi.org/10.1016/j.euroneuro.2017.07.005

Ismaylova, E., Lévesque, M.L., Pomares, F.B., Szyf, M., Fahim, C., Vitaro, F., Brendgen, M., Dionne, G., Boivin, M., Tremblay, R.E., Boojl, L., 2018. Serotonin transporter promoter methylation in peripheral cells and neural responses to negative stimuli: A study of adolescent monozygotic twins. Transl. Psychiatry 8. https://doi.org/10.1038/s41398-018-0195-6

Jack, A., Connelly, J.J., James, P., 2012. DNA methylation of the oxytocin receptor gene predicts neural response to ambiguous social stimuli. Front. Hum. Neurosci. 6, 1–7.
https://doi.org/10.3389/fnhum.2012.00280

Joehanes, R., Just, A.C., Marioni, R.E., Pilling, L.C., Reynolds, L.M., Mandaviya, P.R.,
Guan, W., Xu, T., Elks, C.E., Aslibekyan, S., Moreno-Macias, H., Smith, J.A., Brody, J.A., Dhingra, R., Yousefi, P., Pankow, J.S., Kunze, S., Shah, S.H., McRae, A.F., Lohman, K., Sha, J., Absher, D.M., Ferrucci, L., Zhao, W., Demerath, E.W., Bressler, J., Grove, M.L., Huan, T., Liu, C., Mendelson, M.M., Yao, C., Kiel, D.P., Peters, A., Wang-Sattler, R., Visscher, P.M., Wray, N.R., Starr, J.M., Ding, J., Rodriguez, C.J., Wareham, N.J., Irvin, M.R., Zhi, D., Barrdahl, M., Vineis, P., Ambatipudi, S., Uitterlinden, A.G., Hofman, A., Schwartz, J., Colicino, E., Hou, L., Vokonas, P.S., Hernandez, D.G., Singleton, A.B., Bandinelli, S., Turner, S.T., Ware, E.B., Smith, A.K., Klengel, T., Binder, E.B., Psaty, B.M., Taylor, K.D., Gharib, S.A., Swenson, B.R., Liang, L., Demeo, D.L., O’Connor, G.T., Herceg, Z., Ressler, K.J., Conneely, K.N., Sotoodehnia, N., Kardia, S.L.R., Melzer, D., Baccarelli, A.A., Van Meurs, J.B.J., Romieu, I., Arnett, D.K., Ong, K.K., Liu, Y., Waldenberger, M., Deary, I.J., Fornage, M., Levy, D., London, S.J., 2016. Epigenetic Signatures of Cigarette Smoking. Circ. Cardiovasc. Genet. 9, 436–447. https://doi.org/10.1161/CIRCGENETICS.116.001506

Jones, P.A., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat. Rev. Genet. 13, 484–492. https://doi.org/10.1038/nrg3230

Kim, J.S., Singh, V., Lee, J.K., Lerch, J., Ad-Dab’bagh, Y., MacDonald, D., Lee, J.M., Kim, S.I., Evans, A.C., 2005. Automated 3-D extraction and evaluation of the inner and outer cortical surfaces using a Laplacian map and partial volume effect classification. Neuroimage 27, 210–221. https://doi.org/10.1016/j.neuroimage.2005.03.036

Kim, Y.J., Park, H.J., Jahng, G.H., Lee, S.M., Kang, W.S., Kim, S.K., Kim, T., Cho, A.R., Park, J.K., 2017. A pilot study of differential brain activation to suicidal means and DNA methylation of CACNA1C gene in suicidal attempt patients. Psychiatry Res. 255, 42–48. https://doi.org/10.1016/j.psychres.2017.03.058

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J.C., Pariente, C.M., Pace, T.W.W., Mercer, K.B., Mayberg, H.S., Bradley, B., Nemeroff, C.B., Holsboer, F., Heim, C.M., Ressler, K.J., Rein, T., Binder, E.B., 2013. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat. Neurosci. 16, 33–41.
https://doi.org/10.1038/nn.3275

Klengel, T., Pape, J., Binder, E.B., Mehta, D., 2014. The role of DNA methylation in stress-related psychiatric disorders. Neuropharmacology 80, 115–132.
https://doi.org/10.1016/j.neuropharm.2014.01.013

Lancaster, K., Morris, J.P., Connelly, J.J., 2018. Neuroimaging Epigenetics: Challenges and Recommendations for Best Practices. Neuroscience 370, 88–100.
https://doi.org/10.1016/j.neuroscience.2017.08.004

Liu, J., Julnes, P.S., Chen, J., Ehrlich, S., Walton, E., Calhoun, V.D., 2015. The association of DNA methylation and brain volume in healthy individuals and schizophrenia patients. Schizophr. Res. 169, 447–452. https://doi.org/10.1016/j.schres.2015.08.035

Long, H., Feng, L., Kang, J., Luo, Z., Xiao, W., Long, L., Yan, X.-X., Zhou, L., Xiao, B., 2017. Blood DNA methylation pattern is altered in mesial temporal lobe epilepsy. Sci. Rep. 7.
https://doi.org/10.1038/srep43810

Lowe, R., Gemma, C., Beyan, H., Hawa, M.I., Bazeos, A., Leslie, R.D., Montpetit, A., Vardhman, K., Ramagopalan, S. V, Lowe, R., Gemma, C., Beyan, H., Hawa, M.I., Leslie, R.D., Montpetit, A., Rakyant, V.K., Ramagopalan, S. V, Lowe, R., Gemma, C., Beyan, H., Hawa, M.I., Bazeos, A., Leslie, R.D., Rakyant, V.K., Ramagopalan, S. V, 2013. surrogate tissue than blood for epigenome- Buccals are likely to be a more informative surrogate tissue than blood for epigenome-wide association studies. Epigenetics 8. https://doi.org/10.4161/epi.24362

Lyst, M.J., Bird, A., 2015. Rett syndrome: a complex disorder with simple roots. Nat. Rev. Genet. 16. https://doi.org/10.1038/nrg3987

McGowan, P.O., Sasaki, A., D’Alessio, A.C., Dymov, S., Labonte, B., Szyf, M., Turecki, G., Meaney, M.J., 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat. Neurosci. 12, 342–348.
https://doi.org/10.1038/nn.2270

McMillan, C.T., Russ, J., Wood, E.M., Irwin, D.J., Grossman, M., McCluskey, L., Van Deerlin, V., Lee, E.B., 2015. C9orf72 promoter hypermethylation is neuroprotective.
McNerney, M.W., Sheng, T., Nechvatal, J.M., Lee, A.G., Lyons, M., Soman, S., Liao, C., Hara, R.O., Hallmayer, J., Taylor, J., Ashford, J.W., Yesavage, J., Adamson, M.M., 2018. Integration of neural and epigenetic contributions to posttraumatic stress symptoms: The role of hippocampal volume and glucocorticoid receptor gene methylation. PLoS One 13, e0192222. https://doi.org/10.1371/journal.pone.0192222

Moser, D.A., Paoloni-giacobino, A., Stenz, L., Adouan, W., Rusconi-serpa, S., Ansermet, F., Dayer, A.G., 2015. BDNF Methylation and Maternal Brain Activity in a Violence-Related Sample 1–13. https://doi.org/10.1371/journal.pone.0143427

Muehlhan, M., Kirschbaum, C., Wittchen, H., Alexander, N., 2015. Epigenetic Variation in the Serotonin Transporter Gene Predicts Resting State Functional Connectivity Strength Within the Salience-Network. Hum. Brain Mapp. 36, 4361–4371. https://doi.org/10.1002/hbm.22923

Muench, C., Wiers, C.E., Cortes, C.R., Momenan, R., Lohoff, F.W., 2018. Dopamine Transporter Gene Methylation is Associated with Nucleus Accumbens Activation During Reward Processing in Healthy but not Alcohol-Dependent Individuals. Alcohol. Clin. Exp. Res. 42, 21–31. https://doi.org/10.1111/acer.13526

Na, K.S., Won, E., Kang, J., Chang, H.S., Yoon, H.K., Tae, W.S., Kim, Y.K., Lee, M.S., Joe, S.H., Kim, H., Ham, B.J., 2016. Brain-derived neurotrophic factor promoter methylation and cortical thickness in recurrent major depressive disorder. Sci Rep 6, 21089. https://doi.org/10.1038/srep21089

Nikolova, Y.S., Hariri, A.R., 2015. Can we observe epigenetic effects on human brain function? Trends Cogn. Sci. 19, 366–373. https://doi.org/10.1016/j.tics.2015.05.003

Nikolova, Y.S., Koenen, K.C., Galea, S., Wang, C., Seney, M.L., Sibille, E., Williamson, D.E., Hariri, A.R., 2014. Beyond genotype: serotonin transporter epigenetic modification predicts human brain function. Nat. Neurosci. 17, 1153–1155. https://doi.org/10.1038/nn.3778

Oberlander, T.F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., Devlin, A.M., 2008.
Prenatal exposure to maternal depression, neonatal methylation of human

glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics
3, 97–106. https://doi.org/10.4161/epi.3.2.6034

Palumbo, S., Mariotti, V., Iofrida, C., Pellegrini, S., 2018. Genes and Aggressive Behavior:
Epigenetic Mechanisms Underlying Individual Susceptibility to Aversive Environments.
Front. Behav. Neurosci. 12, 117. https://doi.org/10.3389/fnbeh.2018.00117

Park, S., Lee, J., Kim, J., Cho, D., Yun, H.J., Han, D.H., Cheong, J.H., Kim, B., 2015.
Associations between serotonin transporter gene (SLC6A4) methylation and clinical
characteristics and cortical thickness in children with ADHD. Psychol. Med. 45, 3009–
3017. https://doi.org/10.1017/S003329171500094X

Provenzi, L., Guida, E., Montirosso, R., 2018. Preterm behavioral epigenetics: A systematic
review. Neurosci. Biobehav. Rev. 84, 262–271.
https://doi.org/10.1016/j.neubiorev.2017.08.020

Pruessner, J.C., Li, L.M., Serles, W., Pruessner, M., Collins, D.L., Kabani, N., Lupien, S.,
Evans, A.C., 2000. Volumetry of Hippocampus and Amygdala with High-resolution MRI
and Three-dimensional Analysis Software: Minimizing the Discrepancies between
Laboratories. Cereb. Cortex 10, 433–442.

Puglia, M.H., Lillard, T.S., Morris, J.P., Connelly, J.J., 2015. Epigenetic modification of the
oxytocin receptor gene influences the perception of anger and fear in the human brain.
Proc. Natl. Acad. Sci. U. S. A. 112, 3308–3313.
https://doi.org/10.1073/pnas.1422096112

Raina, A., Zhao, X., Grove, M.L., Bressler, J., Gottesman, R.F., Guan, W., Pankow, J.S.,
Boenwinkle, E., Mosley, T.H., Fornage, M., 2017. Cerebral white matter hyperintensities
on MRI and acceleration of epigenetic aging: the atherosclerosis risk in communities
study. Clin. Epigenetics 9, 1–9. https://doi.org/10.1186/s13148-016-0302-6

Rangasamy, S., D’Mello, S.R., Narayanan, V., 2013. Epigenetics, Autism Spectrum, and
Neurodevelopmental Disorders. Neurotherapeutics 10, 742–756.
https://doi.org/10.1007/s13311-013-0227-0
Resmini, E., Santos, A., Aulinas, A., Webb, S.M., Vives-Gilabert, Y., Cox, O., Wand, G., Lee, R.S., 2016. Reduced DNA methylation of FKBP5 in Cushing’s syndrome. Endocrine 54, 768–777. https://doi.org/10.1007/s12020-016-1083-6

Rubin, L.H., Connelly, J.J., Reilly, J.L., Carter, C.S., Drogos, L.L., Pournaja, H., Ruocco, A.C., Keedy, S.K., Matthew, L., Tandon, N., Pearlsen, G.D., Clementz, B.A., Tamminga, C.A., Gershon, E.S., Keshavan, M.S., Bishop, J.R., Sweeney, J.A., 2016. Sex and Diagnosis-Specific Associations Between DNA Methylation of the Oxytocin Receptor Gene With Emotion Processing and Temporal-Limbic and Prefrontal Brain Volumes in Psychotic Disorders. Biol. Psychiatry 1, 141–151. https://doi.org/10.1016/j.biopsych.2015.10.003

Ruggeri, B., Nymberg, C., Vuoksimaa, E., Lourdusamy, A., Wong, C.P., Carvalho, F.M., Jia, T., Cattrell, A., Macare, C., Banaschewski, T., Barker, G.J., Bokde, A.L., Bromberg, U., Buchel, C., Conrod, P.J., Fauth-Buhler, M., Flor, H., Frouin, V., Gallinat, J., Garavan, H., Gowland, P., Heinz, A., Ittermann, B., Martinot, J.L., Nees, F., Pausova, Z., Paus, T., Rietschel, M., Robbins, T., Smolka, M.N., Spanagel, R., Bakalkin, G., Mill, J., Sommer, W.H., Rose, R.J., Yan, J., Aliev, F., Dick, D., Kaprio, J., Desrivières, S., Schumann, G., Consortium, I., 2015. Association of Protein Phosphatase PPM1G With Alcohol Use Disorder and Brain Activity During Behavioral Control in a Genome-Wide Methylation Analysis. Am. J. Psychiatry 172, 543–552. https://doi.org/10.1176/appi.ajp.2014.14030382

Sabunciyan, S., Aryee, M.J., Irizarry, R.A., Rongione, M., Webster, M.J., Kaufman, W.E., Murakami, P., Lessard, A., Yolken, R.H., Feinberg, A.P., Potash, J.B., Consortium, G., 2012. Genome-Wide DNA Methylation Scan in Major Depressive Disorder. PLoS One 7, e34451. https://doi.org/10.1371/journal.pone.0034451

Sadeh, N., Spielberg, J.M., Logue, M.W., Wolf, E.J., Smith, A.K., Lusk, J., Hayes, J.P., Sperbeck, E., Milberg, W.P., Mcglinchey, R.E., 2016. SKA2 methylation is associated with decreased prefrontal cortical thickness and greater PTSD severity among trauma-exposed veterans. Mol. Psychiatry 21, 357–363. https://doi.org/10.1038/mp.2015.134
Schechter, D.S., Moser, D.A., Paoloni-Giacobino, A., Stenz, L., Gex-Fabry, M., Aue, T., Adouan, W., Cordero, M.I., Suardi, F., Manini, A., Sancho Rossignol, A., Merminod, G., Ansermet, F., Dayer, A.G., Rusconi-Serpa, S., 2015. Methylation of NR3C1 is related to maternal PTSD, parenting stress and maternal medial prefrontal cortical activity in response to child separation among mothers with histories of violence exposure. Front. Psychiatry 6, 1–12. https://doi.org/10.3389/fpsyg.2015.00690

Schechter, D.S., Moser, D.A., Pointet, V.C., Aue, T., Stenz, L., Paoloni-giacobino, A., Adouan, W., Manini, A., Suardi, F., Vital, M., Sancho, A., Cordero, M.I., Rothenberg, M., Rusconi, S., Dayer, A.G., 2017. The association of serotonin receptor 3A methylation with maternal violence exposure, neural activity, and child aggression. Behav. Brain Res. 325, 268–277. https://doi.org/10.1016/j.bbr.2016.10.009

Schneider, I., Kugel, H., Redlich, R., Grotegerd, D., Burger, C., Burkner, P.C., Opel, N., Dohm, K., Zaremba, D., Meinert, S., Schroder, N., Strassburg, A.M., Schwarte, K., Schettler, C., Ambree, O., Rust, S., Domschke, K., Arolt, V., Heindel, W., Baune, B.T., Zhang, W., Dannlowski, U., Hohoff, C., 2018. Association of Serotonin Transporter Gene AluJb Methylation with Major Depression, Amygdala Responsiveness, 5-HTTLPR/rs25531 Polymorphism, and Stress. Neuropsychopharmacology 43, 1308–1316. https://doi.org/10.1038/npp.2017.273

Shelton, A.L., Cornish, K.M., Godler, D., Bui, Q.M., Kolbe, S., Fielding, J., 2017. White matter microstructure, cognition, and molecular markers in fragile X premutation females. Neurology 88, 2070–2088.

Shelton, A.L., Cornish, K.M., Kolbe, S., Clough, M., Slater, H.R., Li, X., Kraan, C.M., Bui, Q.M., Godler, D.E., Fielding, J., 2016. Brain structure and intragenic DNA methylation are correlated , and predict executive dysfunction in fragile X premutation females. Transl. Psychiatry 6. https://doi.org/10.1038/tp.2016.250

Shinagawa, S., Kobayashi, N., Nagata, T., Kusaka, A., 2016. Neuroscience Letters DNA methylation in the NCAPH2 / LMF2 promoter region is associated with hippocampal atrophy in Alzheimer ’ s disease and amnesic mild cognitive impairment patients.
Neurosci. Lett. 629, 33–37. https://doi.org/10.1016/j.neulet.2016.06.055

Smith, A.K., Kilaru, V., Klengel, T., Mercer, K.B., Bradley, B., Conneely, K.N., Ressler, K.J., Binder, E.B., 2015. DNA extracted from saliva for methylation studies of psychiatric traits: Evidence tissue specificity and relatedness to brain. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. 168, 36–44. https://doi.org/10.1002/ajmg.b.32278

Smith, S.M., Jenkinson, M., Johansen-berg, H., Rueckert, D., Nichols, T.E., Mackay, C.E., Watkins, K.E., Ciccarelli, O., Cader, M.Z., Matthews, P.M., Behrens, T.E.J., 2006. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. Neuroimage 31, 1487–1505. https://doi.org/10.1016/j.neuroimage.2006.02.024

Sparrow, S., Manning, J.R., Cartier, J., Anblagan, D., Bastin, M.E., Piyasena, C., Pataky, R., Moore, E.J., Semple, S.I., Wilkinson, A.G., Evans, M., Drake, A.J., Boardman, J.P., 2016. Epigenomic profiling of preterm infants reveals DNA methylation differences at sites associated with neural function. Transl. Psychiatry 6, e716. https://doi.org/10.1038/tp.2015.210

Stafford, M., Black, S., Shah, I., Hardy, R., Pierce, M., Richards, M., Wong, A., Kuh, D., 2013. Using a birth cohort to study ageing: representativeness and response rates in the National Survey of Health and Development. Eur. J. Ageing 10, 145–157. https://doi.org/10.1007/s10433-013-0258-8

Swartz, J.R., Hariri, A.R., Williamson, D.E., 2017. An epigenetic mechanism links socioeconomic status to changes in depression-related brain function in high-risk adolescents. Mol. Psychiatry 22, 209–214. https://doi.org/10.1038/mp.2016.82

Talens, R.P., Boomsma, D.I., Tobi, E.W., Kremer, D., Jukema, J.W., Willemsen, G., Putter, H., Slagboom, P.E., Heijmans, B.T., 2010. Variation, patterns, and temporal stability of DNA methylation: considerations for epigenetic epidemiology. FASEB J. Res. Commun. 24, 3135–3144. https://doi.org/10.1096/fj.09-150490

Unternaehrer, E., Luers, P., Mill, J., Dempster, E., Meyer, A.H., Staehli, S., Lieb, R., Hellhammer, D.H., Meinschmidt, G., 2012. Dynamic changes in DNA methylation of stress-associated genes (OXTR, BDNF) after acute psychosocial stress. Transl.
Ursini, A.G., Cavalleri, T., Fazio, L., 2016. BDNF rs6265 methylation and genotype interact on risk for schizophrenia. Epigenetics 11, 11–23. https://doi.org/10.1080/15592294.2015.1117736

Ursini, G., Bollati, V., Fazio, L., Porcelli, A., Iacovelli, L., Catalani, A., Sinibaldi, L., Gelao, B., Romano, R., Rampino, A., Taurisano, P., Mancini, M., Giorgio, A. Di, Popolizio, T., Baccarelli, A., Blasi, A. De, Blasi, G., Bertolino, A., 2011. Stress-Related Methylation of the Catechol-O-Methyltransferase Val 158 Allele Predicts Human Prefrontal Cognition and Activity. J. Neurosci. 31, 6692–6698. https://doi.org/10.1523/JNEUROSCI.6631-10.2011

Vidal, A.C., Neelon, S.E.B., Liu, Y., Tuli, A.M., Fuemmeler, B.F., Hoyo, C., Murtha, A.P., Huang, Z., Schildkraut, J., Overcash, F., Kurtzberg, J., Jirtle, R.L., Iversen, E.S., Murphy, S.K., 2014. Maternal Stress, Preterm Birth, and DNA Methylation at Imprint Regulatory Sequences in Humans. Genet. Epigenetics 6, 37–44. https://doi.org/10.4137/GEG.S18067

Vukojevic, V., Kolassa, I., Fastenrath, M., Gschwind, L., Spalek, K., Milnik, A., Heck, A., Vogler, C., Wilker, X.S., Demougin, P., Peter, F., Atucha, E., Stetak, A., Roozendaal, B., Elbert, X.T., Papassotiropoulos, A., Quervain, D.J. De, 2014. Epigenetic Modification of the Glucocorticoid Receptor Gene Is Linked to Traumatic Memory and Post-Traumatic Stress Disorder Risk in Genocide Survivors. J. Neurosci. 34, 10274–10284. https://doi.org/10.1523/JNEUROSCI.1526-14.2014

Walton, E., Liu, J., Hass, J., White, T., Scholz, M., Roessner, V., Gollub, R., Calhoun, V.D., Ehrlich, S., 2014. MB-COMT promoter DNA methylation is associated with working-memory processing in schizophrenia patients and healthy controls. Epigenetics 9, 1101–1107. https://doi.org/10.4161/epi.29223

Wang, M., Huang, T., Calhoun, V.D., Fang, J., Wang, Y., 2017. Integration of multiple genomic imaging data for the study of Schizophrenia using joint nonnegative matrix factorization. IEEE Trans Biomed Eng 1083–1087.
Wiemerslage, L., Islam, R., Van Der Kamp, C., Cao, H., Olivo, G., Ence-Eriksson, F.,
Castillo, S., Larsen, A.L., Bandstein, M., Dahlberg, L.S., Perland, E., Gustavsson, V.,
Nilsson, J., Vogel, H., Schürmann, A., Larsson, E.M., Rask-Andersen, M., Benedict, C.,
Schöth, H.B., 2017. A DNA methylation site within the KLF13 gene is associated with
orexigenic processes based on neural responses and ghrelin levels. Int. J. Obes. 41,
990–994. https://doi.org/10.1038/ijo.2017.43

Wiers, C.E., Shumay, E., Volkow, N.D., Frieling, H., Kotsiari, A., Lindenmeyer, J., Walter, H.,
Bermpohl, F., 2015. Effects of depressive symptoms and peripheral DAT methylation
on neural reactivity to alcohol cues in alcoholism. Transl. Psychiatry 5, e648-8.
https://doi.org/10.1038/tp.2015.141

Wijetunge, L.S., Chattarji, S., Wyllie, D.J.A., Kind, P.C., 2013. Fragile X syndrome: From
targets to treatments. Neuropharmacology 68, 83–96.
https://doi.org/10.1016/j.neuropharm.2012.11.028

Wolf, E.J., Logue, M.W., Hayes, J.P., Sadeh, N., Schichman, S.A., Stone, A., Salat, D.H.,
Milberg, W., McGlinchey, R., Miller, M.W., 2016. Accelerated DNA methylation age:
Associations with PTSD and neural integrity. Psychoneuroendocrinology 63, 155–162.
https://doi.org/10.1016/j.psyneuen.2015.09.020

Won, E., Choi, S., Kang, J., Kim, A., Han, K.-M., Chang, H.S., Tae, W.S., Son, K.R., Joe, S.-H.,
Lee, M.-S., Ham, B.-J., 2016. Association between reduced white matter integrity in
the corpus callosum and serotonin transporter gene DNA methylation in medication-
naive patients with major depressive disorder. Transl. Psychiatry 6, e866-9.
https://doi.org/10.1038/tp.2016.137

Zhou, X., Xu, Y., Xie, Z., Xu, S., Bi, J., 2016. Association of extracellular superoxide
dismutase gene methylation with cerebral infarction. Chinese J. Med. Genet. 33, 378–382. https://doi.org/10.3760/cma.j.issn.1003-9406.2016.03.023

Ziegler, C., Dannlowski, U., Brauer, D., Stevens, S., Laeger, I., Wittmann, H., Kugel, H.,
Dobel, C., Hurtleman, R., Reif, A., Lesch, K.-P., Heindel, W., Kirschbaum, C., Arolt, V.,
Gwerlach, A.L., Hoyer, J., Deckert, J., Zwanzger, P., Domschke, K., 2015. Oxytocin
Receptor Gene Methylation: Converging Multilevel Evidence for a Role in Social Anxiety. Neuropsychopharmacology 40, 1528–1538. https://doi.org/10.1038/npp.2015.2
Figure legend

Figure 1. PRISMA flow diagram of studies
| Disease or condition, number of studies | Number of participants | MRI modality (structural, functional, diffusion) | Epigenetic method: Epigenome-wide (EW) or candidate (candidate loci) |
|----------------------------------------|------------------------|-----------------------------------------------|----------------------------------------------------------|
| Neurodevelopment and neurodevelopmental disorders† n=8 | 715 | 5 sMRI 1 fMRI 2 dMRI | 6 EW 2 candidate (SLC6A4, FKBP5) |
| Major depressive disorder and suicidality n=11 | 922§ | 4 sMRI 4 fMRI 2 dMRI 1 sMRI/dMRI | 1 EW 10 candidate (BDNF, SLC6A4, FKPB5, CACNA1C, TESC) |
| Alcohol use disorder n=4 | 921 | 4 fMRI | 0 EW 4 candidate (PPM1G, DAT/SLC6A3, DRD2) |
| Schizophrenia and psychosis n=7 | 456§ | 2 sMRI 4 fMRI 1 sMRI/fMRI | 5 EW 3 candidate (OXTR, BDNF, COMT) |
| Ageing, stroke, ataxia and neurodegeneration n=8 | 1456§ | 5 sMRI 2 dMRI 1 sMRI/dMRI | 3 EW 5 candidate (C9orf72, FMR1, SOD, NCAPH2/LMF2) |
| Post-traumatic stress disorder n=7 | 450§ | 3 sMRI 3 fMRI 1 dMRI | 1 EW 6 candidate (SKA2, NR3C1, HTR3A, BDNF, FKBP5) |
| Miscellaneous‡ n=15 | 1855 | 3 sMRI 10 fMRI 2 sMRI/fMRI | 1 EW 14 candidate (COMT, OXTR, NR3C1, SLC6A4, OXT, FKBP5, KLF13, BDNF) |

†Includes studies of typical development, twin birth weight discordance, BDNF Val66Met polymorphism, preterm birth, early life stress, attention deficit hyperactivity disorder, medial temporal lobe epilepsy
‡Healthy adults, emotion processing, social anxiety disorder, Cushing’s syndrome
§Estimate due to possible sample overlap within phenotypic category.
Table 2. Associations between image features and DNAm

| Image Feature (number of studies) | Differential DNAm: gene or feature |
|-----------------------------------|-----------------------------------|
| **Global fractional anisotropy (n=2)** | DNAm age (Horvath)  
DNAm age (Hannum) |
| **Regional fractional anisotropy (n = 6)** |  
  - Corpus callosum  
  - Ventrolateral thalamus  
  - Right anterior corona radiata  
  - Right parahippocampal cingulum |
| **Regional radial, mean or axial diffusivity (n = 5)** |  
  - Inferior and middle cerebellar peduncle  
  - Corpus Callosum  
  - Right anterior corona radiata  
  - Right parahippocampal cingulum |
| **Amygdala functional connectivity (n=9)** |  
  - SLC6A4  
  - SLC6A4  
  - SLC6A4  
  - SLC6A3/DAT  
  - SLC6A4  
  - OXTR  
  - OXTR  
  - SLC6A4  
  - SLC6A4 |
| **Hippocampal functional connectivity (n = 4)** |  
  - SLC6A4  
  - Cluster of genes identified through EWAS (Wang et al 2017)*  
  - Cluster of genes identified through EWAS (Hu et al 2018)*  
  - BDNF |
| **Prefrontal cortex functional connectivity (n = 8)** |  
  - FKBP5  
  - MB-COMT  
  - BDNF |
| Brain Region          | Genes and DNAm Age cited in EWAS                                      | Notes |
|-----------------------|-----------------------------------------------------------------------|-------|
| Hippocampal volume    | BDNF, SLC6A4, TESC, DNA methylation age (Horvath)                    | Cluster of genes identified through EWAS (Hass et al 2015)* |
|                       |                                                                       |       |
|                       | OXTR, DNA methylation age (Hannum)                                    |       |
|                       | FKBP5, NR3C1-1F, SLC6A4, FKBP5                                         |       |
| Cortical volume       | Cluster of genes identified through EWAS (Casey et al 2017)*          |       |
|                       | Cluster of genes identified through EWAS (Liu et al 2015)*            |       |
| Cortical thickness    | SLC6A4, BDNF, FKBP5, FMR1, SKA2                                      |       |
|                       | Independent component of DNA methylation (Freytag et al 2017)         |       |
| PFC (volume/thickness)| OXTR, SKA2                                                            |       |

*See Tables 3-9 for list of genes included in clusters identified by EWAS
Table 3. Neurodevelopment and neurodevelopmental disorders

| Author, Country | Sample characteristics | Epigenome-wide/candidate, platform | MRI field strength | Main Findings |
|-----------------|------------------------|-----------------------------------|-------------------|---------------|
| Chen et al., 2015 Singapore † | **BDNF** Val66Met polymorphism in GUSTO study | Epigenome-wide: Illumina 450 array | 1.5T | 19% of total variable CpGs were significantly associated with the volume of the left hippocampus for Val/Val neonates. 5% of total variable CpGs were associated with left hippocampal volume in the Met/Val genotype group 3% of total variable CpGs were significant in the Met/Met genotype group. |
| Park et al., 2015 South Korea | ADHD | Candidate: **SLC6A4** promoter (185bp region) | 3T | Negative association between DNAm of **SLC6A4** CpGs 5-8 in the promoter region and cortical thickness in the right STG, middle occipital gyrus, lingual gyrus, inferior occipital gyrus, precentral gyrus, MTG, cuneus, and cingulate gyrus. |
| Sparrow et al., 2016 United Kingdom | Preterm birth 36 preterm infants (<32 weeks’ gestation); 36 term controls | Epigenome-wide: Illumina 450 array | 3T | 95% of the variance in the preterm methylome was explained by 23 principal components; the 6th principal component explained 2.9% of the variance of DNAm data in the preterm group and was associated with right CST topology. |

| Author, Country | Country | Sample characteristics | Condition | Cases (male); controls (male) | Age | Epigenome-wide/candidate, platform | Tissue sampled | Correction for cell sub-type distribution | Consideration of genotype | Correction for cell sub-type distribution | MRI field strength | Modality | Image feature(s) | Analysis method |
|-----------------|---------|------------------------|-----------|-----------------------------|-----|-----------------------------------|---------------|------------------------------------------|---------------------|------------------------------------------|-----------------|-----------|----------------|-----------------|
| Chen et al., 2015 Singapore † | Singapore | **BDNF** Val66Met polymorphism in GUSTO study | **BDNF** Val66Met polymorphism in GUSTO study | 237 cases (133M) | Mean gestational age 38.7 weeks | Epigenome-wide: Illumina 450 array | Umbilical cord blood | Cell type composition: NR | Consideration of genotype: yes | | | | |
| Park et al., 2015 South Korea | South Korea | ADHD | ADHD | 102 participants (77M) | Mean age 8.9 years | Candidate: **SLC6A4** promoter (185bp region) | Blood | Cell type composition: NR | Consideration of genotype: yes | | | | |
| Sparrow et al., 2016 United Kingdom | United Kingdom | Preterm birth 36 preterm infants (<32 weeks’ gestation); 36 term controls | Preterm birth 36 preterm infants (<32 weeks’ gestation); 36 term controls | Mean age at assessment: | Epigenome-wide: Illumina 450 array | Saliva | Cell type composition: no | Consideration of genotype: yes | | | | | |
| Study | Country | Design | Participants | Methodology | Findings |
|-------|---------|--------|--------------|-------------|----------|
| Casey et al., 2017 | Canada | Cortical anatomy in monozygotic twin cohort | 52 twin pairs (22M) | Mean age 15.7 years | Epigenome-wide: Illumina 450 array | 23 probes mediate the relationship between birthweight discordance and cortical volume, including annotated probes found in 13 genes: *PRPF3, RELN, EXOC2, LEFTY2, CAST, SNK1G2, TRIM5, ABR, TNF, C6orf10, FREM2, GABPA* and *EFNA5*; 15 probes mediate the relationship between birthweight discordance and cortical surface area, including annotated probes found in 9 genes: *TMCO3, TRIM15, KDM2A, EPDR1, PARP14, PBLD, RELN, ACACA* and *CASZ1*. No probes significantly mediated the relationship between birthweight discordance and cortical thickness discordance. |
| Long et al., 2017 | China | HS in MTLE | Cases n=30 (18M); controls n = 30 (18M) | Cases mean 25.3 years; controls mean 28.2 years | Epigenome-wide: Illumina 450 array | From top 10 differentially methylated probes: DNAm in *SLC12A7, SRC, PLIN5* had a positive association with HS; DNAm in *PCNT, CEACAM21, PCGF3, BAT2* displayed a negative association with HS. Genes from top 10 positively associated with HS: *RASAL3, PFDN6:WDR46, UBN1:GLYR1, TRIM49, ACVR1B*. Genes from the top 10 negatively associated with HS: *SPIRE2, NKD1, TNFRSF11B*. | Subgroup analysis: MTLE patients with (n = 9) or without HS (n = 14). |
| Study                          | Country      | Cohort                  | Participants | Age          | Sample Type | Cell Type Composition | Genotype Consideration | fMRI Task                  | NeuroImage Analysis | Results                                                                 |
|-------------------------------|--------------|-------------------------|--------------|--------------|-------------|-----------------------|------------------------|--------------------------|-----------------------|-------------------------------------------------------------------------|
| Harms et al., 2017 United States |              | Early life stress exposure on adult outcome | 33 participants (15M) | Mean age 20.5 years | *FKBP5*, CpG sites in intron 7, 5, and 2 | Saliva | Cell type composition: NR | Consideration of genotype: NR | 3T | fMRI task: go-no go task | Analysis of Functional NeuroImages | Negative association between DNAm of intron 5 of *FKBP5* and activation in the dlPFC in the go no-go task. CpG8 of intron 5 mediates the relationship between childhood stress and dlPFC activation. |
| Chen et al., 2018 United States |              | ADHD                    | 15 monozygotic twin pairs (12M), discordant for ADHD | Mean 9.7 years | Epigenome-wide: Illumina 450 array | Blood | Cell type composition: yes | Consideration of genotype: NR | 1.5T | Structural, T1 | Cerebral cortex, cerebellum, caudate, putamen, thalamus volumes | FreeSurfer | Subset of 14 MZ twins: Of the 67 genes with intrapair probe DNAm which associated with volume discordance in the cerebellum, 49 were negatively associated. Of the 48 genes associated with the striatum, 38 were negatively associated, while of the 78 associated with the thalamus 44 were negatively associated. |
| Guillaume et al., 2018 Singapore† |              | GUSTO birth cohort      | 114 participants | 7-10 days post birth | Epigenome-wide: Illumina 450 array | Umbilical cord blood | Cell type composition: yes | Consideration of genotype: NR | 1.5T, Diffusion | FA | Voxel based comparison of FA maps | EWAS a significant association between probe cg19641625 in the *SMOC2* gene locus, with FA in the left vlThalamus. |

† Overlapping cohorts (GUSTO study)
| Author, Country | Sample characteristics | Epigenome-wide/candidate, platform | MRI field strength | Modality | Image feature(s) | Analysis method | Main Findings |
|----------------|------------------------|-----------------------------------|-------------------|---------|-----------------|----------------|---------------|
| Choi et al., 2015 South Korea † | MDD | Candidate: BDNF, 4 CpGs in the promoter region | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Cases, n=60 (13M); controls, n=53 (17M) | Control for cell sub-type distribution | Brain MRI | Diffusion | FA/RD/AxD of 7 white matter tracts | Consideration of genotype | No significant positive associations. |
| | Cases, mean 41.9 years; controls, mean 41.2 years | Consideration of genotype: NR | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Candidate: BDNF, 4 CpGs in the promoter region | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | BDNF DNA methylation | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | FA/RD/AxD of 7 white matter tracts | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Tract Based Spatial Statistics (TBSS) | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Cases: negative association between DNA methylation of CpG4 and FA of the right ACR, AxD of right ACR is negatively associated with BDNF DNA methylation. RD was not significantly associated with BDNF DNA methylation. | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Controls: no significant positive associations. | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| Booij et al., 2015 Republic of Ireland ‡ | MDD | Candidate: SLC6A4, CpGs 5-15 found 214–625 bp upstream of the gene promoter | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Cases, n=33 (10M); controls, n=36 (15M) | BDNF DNA methylation | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Cases, mean 40.3 years; controls, mean 35.3 years | BDNF DNA methylation | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | Candidate: SLC6A4, CpGs 5-15 found 214–625 bp upstream of the gene promoter | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings |
| | MRI field strength | Modality | Cell type composition: NR | Analysis method | Main Findings | Whole group: negative predictive value of hippocampal volume and sub-volumes (CA2/3 and DG, and CA1) and SLC6A4 DNA methylation (average and site specific – CpG 5&6, CpG 11&12) |
| Study | Location | Diagnosis | Case Numbers | Control Numbers | Case Age | Control Age | Candidate Gene | Cell Type | Consideration of Genotype | fMRI Task | Analysis Tool | Findings |
|-------|----------|-----------|--------------|-----------------|----------|-------------|----------------|-----------|--------------------------|------------|-------------|---------|
| Frodl et al., 2015 | Republic of Ireland ‡ | MDD | Cases, n=25(7M); controls, n=35(13M) | Cases, mean 41.6 years; controls, mean 35.6 |  |  | SLC6A4, promoter | Blood | NR | Visual emotional attention shifting | SPM8 | Whole group: when shifting from emotional valence to geometric properties there is a negative association between DNAm and activation in the right Rolandic operculum and insula, right superior temporal lobe, and pons; when shifting from negative to neutral emotional valence, there is positive association between DNAm and activation in the left anterior insula and inferior frontal operculum. Controls: When looking at emotionally negative stimuli versus neutral stimuli, there was a positive association between DNAm and more activation in the bilateral hippocampus, left inferior operculum, fusiform gyrus and amygdala regions. |
| Na et al., 2016 | South Korea † | MDD | Cases, n=65 (11M); controls, n=65 (15M) | Cases, mean 42.5 years; controls mean 40.3 years |  |  | BDNF, 4 CpGs in the promoter region | Blood | NR | Cortical thickness | FreeSurfer | Cases: negative association between CpG2/4 and cortical thickness in the right rostral middle frontal, mOFC cortical thicknesses and left lingual, STG and SFG cortices. Negative association between CpG2 and cortical thickness in the right IT and pericalcarine and left rostral middle frontal cortices. Negative association between CpG4 and cortical thickness in the right cuneus, precuneus and post central cortices and left frontal pole cortices. Controls: no significant associations found. |
| Won et al., 2016 | South Korea † | MDD | Cases, n=35 (10M); controls, n=49 (15M) | Cases, mean 40.3 years; controls mean 41.1 years |  |  | SLC6A4, 5 CpGs in the promoter | Blood | NR | Diffusion | TBSS | Cases: negative association between SLC6A4 DNAm and FA/AxD in the BCC (medication naïve). Controls: negative association between SLC6A4 DNAm and GCC FA, and positive association with RD. Associations between BCC FA/AxD and CpG3 DNAm are higher in the cases compared to controls. |
| Study                          | Country   | Sample Size        | Trait                   | Candidate Gene | Cell Type Composition | Genotype Considered | Imaging Technique | Findings                                                                 |
|-------------------------------|-----------|--------------------|-------------------------|----------------|-----------------------|---------------------|-------------------|---------------------------------------------------------------------------|
| Kim et al., 2017             | South Korea | Suicidality Cases (suicide attempters), n=14 (1M), controls, n=22 (9M) | Suicidality | CACNA1C, 11 CpG sites in DMR in a transcription factor binding site | Blood | Cell type composition: NR | 3T | *fMRI task: participants shown pictures of facial emotion and objects relating to suicidal means
Activation in 4 left hemisphere regions
SPM5 |
| K. M. Han et al., 2017        | South Korea | MDD Cases, n=84 (13M); controls, n=61 (17M) | MDD | TESC, rs7294919 locus | Blood | Cell type composition: NR | 3T | *Diffusion Structural
Diffusion parameters of the PHC
Hippocampal subfield volumes
Global PNT |
| K. Han et al., 2017           | South Korea | MDD Cases, n=114 (24M); controls, n=88 (27M) | MDD | FKBP5, intron 7 rs1360780, 2 CpGs investigated | Blood | Cell type composition: NR | 3T | *Structural, T1
GM volumes/cortical thickness of 14 cortical/subcortical regions
FreeSurfer |
| **FKBP5 C homozygote group (patients and controls) (n = 118): positive association between DNAm of CpG1 and cortical thickness in the right transverse frontopolar gyrus.** |
| **Cases: in knife vs neutral landscape contrast positive association between CpG4/6 DNAm and brain activation in the left thalamus, MFG, IFG.** |
| **Controls: positive association between CpG6 DNAm and activation in the left MFG and IFG.** |
| Study                  | Location     | MDD Diagnosis       | Cases/Controls | Wave 1 Mean Age | Wave 2 Mean Age | Candidate sites/SNP | Blood Sample | Cell Type Composition | Genotype Consideration | fMRI Task                | Amygdala Reactivity | Analysis Description                                                                 |
|------------------------|--------------|---------------------|----------------|-----------------|-----------------|---------------------|---------------|-----------------------|------------------------|------------------------|----------------------|--------------------------------------------------------------------------------------|
| Swartz et al., 2017    | United States| Family history of depression | Cases n=94 (51M); controls n=89 (46M) | 13.5 years; wave 2, mean 15.6 years | 15.6 years | SLC6A4 - 20 CpG sites in promoter region | Blood | Cell type composition: NR | Consideration of genotype: yes | 3T | fMRI task: emotional face processing | Amygdala reactivity | SPM8 | Subgroup (n=87), longitudinal analysis: SLC6A4 promoter DNAm increases over time is associated with increases in left centromedial amygdala reactivity when participants viewed faces with fearful expressions in contrast to geometric shapes. |
| Davis et al., 2017     | United States| High/low familial MDD risk among females | High risk, n=24; low risk, n=22 | 12.9 years; low risk, mean 12.1 years | 12.1 years | Illumina EPIC, Horvath DNAm age | Saliva | Cell type composition: yes | Correction of genotype: NR | 1.5T/3T | Structural, T1 | Hippocampal and amygdala volumes | FreeSurfer | Whole group analysis: Negative associations between DNAm age residual and left hippocampal volume. Accelerated DNAm age mediates the effect of cortisol on left hippocampal volume. |
| Schneider et al., 2018 | Germany      | MDD                  | Cases, n=137 (57M); controls, n=189 (83M) | 37.4 years; controls, mean 35.6 years | 35.6 years | SLC6A4 AluJb element | Blood | Cell type composition: NR | Consideration of genotype: yes | 3T | fMRI task: emotional face processing (threat/fearful faces) | Amygdala reactivity | SPM8 | Whole group analysis: Significant interaction of amygdala activity with DNAm and diagnosis. Cases: positive association between AluJb DNAm and activity in the right amygdala. No effect of DNAm on bilateral amygdala reactivity Controls: no significant associations |

† Overlapping cohorts
‡ Overlapping cohorts
| Author, Country | Sample characteristics | Condition | Cases (male); controls (male) | Age | Epigenome-wide/candidate, platform | Tissue sampled | Correction for cell sub-type distribution | Consideration of genotype | MRI field strength | Modality | Image feature(s) | Analysis method | Main Findings |
|----------------|------------------------|-----------|-------------------------------|-----|-----------------------------------|---------------|--------------------------------------------|--------------------------|----------------|----------|----------------|---------------|--------------|
| Ruggeri et al., 2015 United Kingdom | Alcohol exposure (IMAGEN cohort) | n=499(222M) | Mean age 14 years | | Candidate: *PPM1G*, 3' UTR | | | | | | | | | Subset of whole sample (n=393) | Positive association between *PPM1G* DNAm and BOLD response in the right subthalamic nucleus in the stop success versus go success contrast | No association with right IFG activation |
| Wiers et al., 2015 United States | Alcohol dependence | Cases n=38 (M); controls, n=17 (M) | Cases, mean age 44.4 years; controls, mean 42.7 years | | Candidate: *SLC6A3/DAT* - 12 CpG sites in promoter region (-1037 to -829) | | | | | | | | | Cases: positive association between alcohol elicited amygdala reactivity (in contrast to soft drink) and *DAT* DNAm found only in cases with low depression scores (n=29) |
| Bidwell et al., 2018 United States | Alcohol dependence | 383 (238M) | Mean age 30.1 years (range 21-56) | | Candidate: *DRD2*, 6 CpGs in promoter region (-126 to -189) | | | | | | | | | ROI analysis: Positive association between DRD2 DNAm and alcohol elicited activation in striatum: right NAcc, bilateral putamen and bilateral caudate. WB analysis: positive associations between DRD2 DNAm and alcohol elicited response in: left superior temporal, |
| Muench et al., 2018 United States | ▪ Alcohol dependence  
▪ Cases, n=45(35M); controls, n=45 (22M)  
▪ Cases: mean 43.3 years; controls: 36.2 years | ▪ Cell type composition: NR  
▪ Consideration of genotype: NR  
▪ Candidate: *SLC6A3/DAT*, 48 CpGs  
▪ Blood  
▪ Cell type composition: NR  
▪ Consideration of genotype: NR | ▪ **BOLD** contrasts of 10 ROIs and WB analysis  
▪ SPM8 | middle temporal, insula, postcentral, supramarginal, putamen regions. | ▪ Cases: no significant associations  
▪ Controls: negative association between *SLC6A3* promoter DNAm and NAcc activation during the anticipation of both high and low monetary loss. The effects were driven by 2 CpGs located at positions -1,001 and -993 from the TSS in the promoter of *SLC6A3*.  
▪ **3T**  
▪ **fMRI** task: Monetary incentive delay task  
▪ **BOLD** responses in bilateral NAcc  
▪ AFNI |
Table 6. Schizophrenia and psychosis

| Author, Country | Sample characteristics | Epigenome-wide/candidate, platform | MRI field strength | Modality | Image feature(s) | Analysis method | Main Findings |
|-----------------|------------------------|-----------------------------------|-------------------|----------|-----------------|----------------|---------------|
| Walton et al., 2014 United States † | ▪ Schizophrenia or schizophreniform disorder ▪ Cases, n=82(62M); controls, n=102(62M) ▪ Cases, mean 33.8 years; controls 32.7 years | ▪ Candidate: MB-COMT, 864bp upstream from TSS of membrane bound isoform of COMT ▪ Blood ▪ Cell type composition: NR ▪ Consideration of genotype: yes | ▪ 1.5T and 3T ▪ fMRI task: Sternberg item recognition paradigm - working memory task ▪ BOLD response in dIPFC and exploratory whole brain analysis | ▪ fMRIB (FSL) | Whole group: ▪ ROI analysis: positive association between MB-COMT promoter DNAm and left dIPFC activation; ▪ Whole brain analysis: positive association between MB-COMT promoter methylation and left dIPFC and vIPFC (BA45), left premotor and primary sensory cortex. No effect in R dIPFC. No effect of diagnosis. |
| Liu et al., 2015 United States † | ▪ Schizophrenia, schizophreniform or schizoaffective disorder ▪ Cases, n=94; controls, n=106 ▪ Cases, mean 34.7 years; controls, mean 32.5 years | ▪ Epigenome-wide: Illumina Infinium HumanMethylation 27 Array ▪ Blood ▪ Cell type composition: NR ▪ Consideration of genotype: NR | ▪ 1.5T and 3T ▪ Structural, T1 ▪ Volumes of cortical and subcortical regions ▪ FreeSurfer | ▪ fMRIB (FSL) | Negative association between the 7th DNAm component and the 1st brain volume component: Brain volume component - left cerebellar cortex, right cerebellar cortex Methylation component - C1orf65, MCCC1, EPHA3, CDX1, RAET1L, LR8, STMN2, GATA4, NALP6, KCNK10, ADRA1D, NPDC1, MCHR1 |
| Hass et al., 2015 | ▪ Schizophrenia | ▪ Epigenome-wide: Illumina Infinium | ▪ 1.5T and 3T ▪ Structural, T1 | ▪ fMRIB (FSL) | Alterations in the DNAm state of genes within the hsa-miR-219a-5p targets are associated with hippocampal volume |
| United States † | Cases, n=103 (75M); Controls, n=111 (71M) | HumanMethylation 27 Array | fMRI: Sternberg Item Recognition Paradigm - working memory task. | fMRI: Small concordance between fMRI data and DNAm data. Predictition accuracy of schizophrenia cases was improved by incorporating the two data types versus prediction models using one or the other. |
|----------------|------------------------------------------|-----------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------|
|                | Cases: mean 34.6 years; controls, mean 32.1 years | Blood | Hippocampal volume, STG thickness | Schizophrenia: positive association between OXTR DNAm and volumes of the left OFC, and right fusiform gyrus and negative association with the right hippocampus. Bipolar disorder: positive association between DNAm and right parahippocampal gyrus volume. Schizoaffective disorder (men): Negative association between OXTR DNAm and left MFG volume. |
|                |                                          | Cell type composition: yes | Working memory load dependent neural activity (percentage BOLD signal change) in the dlPFC |                                                                                   |
|                |                                          | Consideration of genotype: yes | FreeSurfer |                                                                                   |
|                |                                          | fMRI (FSL) |                                                                                   |                                                                                   |
| Deng et al., 2016 United States † | Schizophrenia | Epigenome-wide: Illumina Infinium HumanMethylation 27 Array | NR |                                                                                   |
|                | Case, n=80 (60M); Controls, n=104 (66M) | Tissue sampled: blood | fMRI |                                                                                   |
|                | Cases, mean 34 years; controls, mean 32 years | Cell type composition: NR | NR |                                                                                   |
|                |                                          | Consideration of genotype: NR | NR |                                                                                   |
| Rubin et al., 2016 United States | Schizophrenia and Bipolar disorder | Candidate: OXTR methylation measured at a site 934bp upstream of TSS | 3T |                                                                                   |
|                | Case, n=167 (75M); controls, n=75 (37M) | Blood | Structural, T1 |                                                                                   |
|                | Age | Cell type composition: NR | Volumes of prefrontal regions and temporal limbic regions |                                                                                   |
|                |                                          | FreeSurfer | FreeSurfer |                                                                                   |
| Disorder                  | Women (n) | Men (n) | Mean Age | Consideration of genotype: | Controls: | 
|---------------------------|-----------|---------|----------|----------------------------|-----------|
| Schizophrenia             | 22        | 35      | 39       | NR                        | negative  |
| Schizoaffective disorder  | 19        | 15      | 37       |                           | association between OXTR DNAm and left amygdala, right MFG and IFG volumes. |
| Bipolar disorder          | 51        | 25      | 36       |                           |           |
| Controls                  | 38        | 37      | 36       |                           |           |

**Wang et al., 2017**

**United States**

- **Schizophrenia**
  - Cases, n=80(60M); Controls, n=104 (66M)
  - Cases: mean 34 years; controls mean 32 years

- **Epigenome-wide:**
  - Illumina Infinium HumanMethylation 27 Array
  - Blood
  - Cell type composition: NR
  - Consideration of genotype: NR

- **fMRI module:** midcingulate area, parahippocampal gyrus, postcentral gyrus, supramarginal gyrus, angular gyrus, precuneus, superior temporal gyrus

- **DNAm module:** ANKRD15, ATP6V0A4, C1orf116, C1orf172, C21orf56, CCNA1, CREB3L3, FLJ11017, HOXA4, HTN3, KIF27, LDHD, PAGE5, PDIA2, RCBTB2, RPL26L1, TMEM100

- **Positive association between fMRI and DNAm modules in cases.**
- **Associations in controls do not reach statistical significance.**

- **DNAm module:**
  - ANKRD15, ATP6V0A4, C1orf116, C1orf172, C21orf56, CCNA1, CREB3L3, FLJ11017, HOXA4, HTN3, KIF27, LDHD, PAGE5, PDIA2, RCBTB2, RPL26L1, TMEM100

- **fMRI module:** midcingulate area, parahippocampal gyrus, postcentral gyrus, supramarginal gyrus, angular gyrus, precuneus, superior temporal gyrus
| Hu et al., 2018 | **United States** † |
|----------------|---------------------|
| Schizophrenia  | Cases, n=79; controls, n=104 |
| Age: NR        | Epigenome-wide: Illumina Infinium HumanMethylation 27 Array |
|                | Blood               |
|                | Cell type composition: NR |
|                | Consideration of genotype: NR |
| Field Strength: | Field Strength: NR |
| fMRI task:     | fMRI task: Sensorimotor task |
|                | NR                  |
|                | NR                  |
| Positive       | Positive association between DNAm and fMRI data |
| association     | DNAm: 31 CpG sites that correspond to 30 genes |
| between DNAm and fMRI data | fMRI (7 ROIs): left inferior frontal gyrus (pars triangularis), left hippocampus, right inferior occipital gyrus, bilateral fusiform gyrus, right middle temporal gyrus, left inferior temporal gyrus |

† Mind Clinical Imaging Consortium
Table 7. Ageing, stroke, ataxia and neurodegeneration

| Author, Country | Sample characteristics | Epigenome-wide/candidate, platform | Tissue sampled | Correction for cell sub-type distribution | Consideration of genotype | MRI field strength | Modality | Image feature(s) | Analysis method | Main Findings |
|-----------------|------------------------|-----------------------------------|---------------|------------------------------------------|---------------------------|-----------------|----------|-----------------|----------------|--------------|
| McMillan et al., 2015 United States | **C9orf72** expansion | Candidate: **C9orf72** | Blood | Cell type composition: NR | Consideration of genotype: NR | **3T** | Structural, T1 | Grey matter density | Randomise (FSL) | • **C9orf72** expansion group: positive association between **C9orf72** DNAm and grey matter density in the right hippocampus and thalamus and left premotor cortex. • Longitudinal analysis (n=11): GM atrophy progresses more rapidly over time with decreased **C9orf72** DNAm in patients with **C9orf72** expansion |
| Shelton et al., 2016 Australia † | **Fragile X associated tremor ataxia syndrome (FXTAS)** | Candidate: **FMR1** - exon 1 CpG1 and CpG2; intron 1 CpG6/7, CpG8/9; CpG10-12 | Blood | Cell type composition: NR | Consideration of genotype: NR | **3T** | Structural, T1 | Regional cortical thickness from bilateral MFG and SFG and inferior parietal gyrus | FreeSurfer | • Premutation group: positive associations between methylation of **FMR1** CpG6/7 or CpG1 or CpG10-12 and of cortical thickness in the left inferior parietal gyrus, middle frontal gyrus, and right middle frontal gyrus, superior frontal gyrus. Negative association between methylation in the intronic region with white matter hyperintensities. • Controls: negative associations between FMR1 DNAm at CpGs 2, 6/7, 10–12 and cortical thickness in frontal lobe regions: left middle frontal gyrus and right superior frontal gyrus |
| Study Reference | Country | Condition | Study Design | Candidate | Field Strength | Consideration of Genotype | Cell Type Composition | Image Analysis | Findings |
|-----------------|---------|-----------|--------------|-----------|---------------|--------------------------|----------------------|----------------|---------|
| Zhou et al., 2016 China | Cerebral infarction | Cases, n=83(45M); controls, n=94(52M) | Cases: mean 61.6 years; controls: mean 61.6 years | Extracellular SOD | NR | NR | NR | MR Angiography, Area of cerebral infarction, cerebral arteriosclerosis | Dependence between large/small area of cerebral infarction and extent of extracellular SOD DNAm. Negative but non-significant association between arteriosclerosis severity and extracellular SOD DNAm. |
| Shinagawa et al., 2016 Japan | Alzheimer’s/aMCI | Cases 58(27M): AD, n=30; aMCI n=28 | Cases, mean 71.9 years | NCAPH2/LMF2 promoter – representative CpG site | 1.5T | NR | NR | VBM Grey matter/ VSRAD Z-score (hippocampal atrophy grade), SPM8, DARTEL/VSRAD | Extent of grey matter atrophy non-significant for associations with NCAPH/LMF2 promoter DNAm. Positive association between DNAm of NCAPH2/LMF2 and severity of hippocampal atrophy. |
| Hodgson et al., 2017 United States | Ageing | Cases N=376 | Mean 45.5 years | Illumina 450 array, Horvath DNAm age | 3T | Yes | Yes | Diffusion, FA, TBSS | Negative association of FA with epigenetic age acceleration. |
| Raina et al., 2017 United States | Cerebral white matter hyperintensities | N=713(257M) | Mean age at MRI was 61.8 years; mean age | Illumina 450k array, Hannum/Horvath DNAm age | 1.5T | Yes | Yes | Structural, T1/T2, White matter hyperintensities, Non-automated counting | Positive association between WMH burden and DNAm age acceleration: high WMH burden category was associated with DNAm age acceleration, whereas low WMH burden was not. No individual probe reached significance in any analysis. |
| Study                          | Year | Country   | Age Information | Genotype Consideration | Candidate Genes | Imaging Methods | Results                                                                 |
|-------------------------------|------|-----------|-----------------|------------------------|-----------------|----------------|--------------------------------------------------------------------------|
| Shelton et al., 2017          | 2017 | Australia | at DNA sampling 59.3 years | Consideration of genotype: yes | Candidate: *FMR1* – exon 1 CpG1 and CpG2; intron 1 CpG6/7, CpG8/9; CpG10-12 | Blood, Cell type composition: NR, Consideration of genotype: NR | Premutation group: negative association between inferior and middle cerebellar peduncle’s MD and CpG1, Control: negative association between BCC FA and CpG6/7 |
| Chouliaras et al., 2018       | 2018 | United Kingdom | N = 47(39M), cognitively intact, mean 72.8 years; cognitively impaired, mean 72.3 years (s.d. 6.02) | Consideration of genotype: NR | Epigenome-wide: Illumina 450k array, Horvath/Hannum DNA methylation age | Blood, Cell type composition: yes, Consideration of genotype: NR | No associations were observed between the Horvath epigenetic clock and any of the imaging variables tested, Candidate gene testing did not find any associations that survived correction for multiple comparisons, Negative association between global MD and Hannum age, and positive association between global FA and Hannum age. |

† Overlapping cohort

Table 8. Post-traumatic stress disorder
| Author, Country | Sample characteristics | Epigenome-wide/candidate, platform | MRI field strength | Main Findings |
|----------------|------------------------|----------------------------------|------------------|--------------|
| **Klengel et al., 2013** United States | Childhood trauma related PTSD | Candidate: *FKBP5* - 4 intronic regions, CpG island of TSS and a region upstream of the TSS | 3T | Negative association between FKBP5 (mean at intron 7 bin 2) DNAm and right hippocampal head volume in a subset of the cohort (n=34). |
|                  | Cases n=56(F) | Blood | Structural, T1 | |
|                  | Cases, mean age 28.5 mean years | Cell type composition: NR | Amygdala/Hippocampal volume | |
|                  | Candidate: *FKBP5* - 4 intronic regions, CpG island of TSS and a region upstream of the TSS | Consideration of genotype: yes | Amygdala and hippocampal segmentation (Pruessner et al., 2000) | |
| **Moser et al., 2015 Switzerland †** | Inter-personal violence related PTSD | Candidate: *BDNF* mean methylation of 4 CpGs found in exon IV | 3T | Whole group analysis: |
|                  | Controls, n= 20F; Subthreshold PTSD, n = 8F; IPV-PTSD diagnosis, n = 18F | Saliva | fMRI task: Modified Crowell Procedure | Positive associations between *BDNF* promoter mean methylation and DNAm and cluster activation in the following clusters: in left vmPFC, OFC, sgACC; right vmPFC, OFC; posterior cingulate cortex. |
|                  | Age - (sample of 68 mothers with saliva): Mean 34.3 years (s.d. 5.8 years) | Cell type composition: NR | Contrast of brain activity when mothers watched their own and unfamiliar children during scenes of separation vs play | Negative associations between *BDNF* promoter mean methylation and cluster activation in the following clusters: right hippocampus, right parahippocampal gyrus, right fusiform gyrus; left precuneus; right precuneus; left cerebellum; right STG. |
| Study                  | Country  | PTSD Type       | Cases/Controls | Cell Type Composition | Consideration of Genotype | fMRI Task                | Number of CpGs | Genomic Region | Mean Methylation | Brain Regions                              | Mediation Effect                                  |
|-----------------------|----------|-----------------|----------------|-----------------------|---------------------------|--------------------------|----------------|-----------------|------------------|---------------------------------------------|--------------------------------------------------|
| Schechter et al., 2015 | Switzerland | Inter-personal violence related PTSD | Cases: n=28; controls n=17 | NR3C1 – mean methylation of 13 CpGs | | | 3T | fMRI task: Modified Crowell Procedure | 13 | vmPFC OFC, right gyrus rectus; left dLPFC; left dLPFC, left precentral gyrus; dmPFC; left precuneus, posterior cingulate cortex | Spearman’s correlations between each of the 13 CpG sites and neural activity in prefrontal cortex regions during separation vs. play did not reach statistical significance |
| Sadeh et al., 2016    | United States | PTSD | Cases: a subset (n=145) of cohort size n=200(182M) | SKA2 – 3' UTR. (Illumina probe cg13989295) | | | 3T | Structural, T1 | | cortical thicknesses in prefrontal regions: left frontal pole, SFG and rostral middle frontal gyrus; right frontal pole, SFG, and right OFC/IFG pars orbitalis and rostral MFG | Mediation was significant for right OFC and rostral MFG cluster, and for left prefrontal cluster. Mediation effect not significant for the right frontal pole/SFG cluster, though direction of association was consistent. |
| Wolf et al., 2016     | United States | PTSD | Cases, n=281(247M) | Epigenome-wide: Illumina 450 array, Horvath/Hannum DNAm age | | | 3T | Diffusion | | FA/RD/MD/AxD in right frontal cortex, left frontal cortex, genu of corpus callosum | No associations found with Horvath DNAm age. |
| Study                  | Location          | Type of PTSD | Sample Size | Candidate | Saliva | Cell Type Composition | Consideration of Genotype | fMRI Task                           | Neural Activity in Maternal Brain Regions Association | DNAm Analysis                                                                 |
|------------------------|-------------------|--------------|-------------|-----------|--------|-----------------------|---------------------------|-------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------|
| Schechter et al., 2017 | Switzerland †    | Inter-personal violence related PTSD | Cases, n=18F; controls, n=17F | Candidate: serotonin receptor 3A methylation (HTR3A) – 7 CpG sites in the promoter region | saliva | Cell type composition: NR | Consideration of genotype: NR | fMRI task: video stimuli depicting neutral, menacing, and male-female prosocial interactions. | Neural activity in maternal brain regions association with emotion regulation | Field strength: NR | Negative association between DNAm at CpG 2 III site and neural activity in response to menacing vs prosocial stimuli: dorsomedial PFC, dACC, bilateral dorsolateral prefrontal gyrus, left prefrontal gyrus, left temporal pole, left STG, left MTG; posterior cingulate cortex; left cuneus, V1; cerebellum; right brainstem. |
| Mcnerney et al., 2018  | United States ‡  | Veterans risk of meeting PTSD diagnostic criteria | Cases, n= 67 (59M) | Candidate: NR3C1-1F – promoter binding sites for transcription factor NGF1A | saliva | Cell type composition: NR | Consideration of genotype: NR | 3T Structural, FSPGR Intracranial volume and hippocampal volume FreeSurfer | Positive associations between hippocampal volume and DNAm of NR3C1 gene in low scorers on the PTSD checklist (Civilian version 4; PCL-C), unlikely to meet PTSD diagnostic criteria. Associations were not significant for those who scored highly on the PCL-C. |

† Overlapping cohort
‡ Overlapping cohort
Table 9. Miscellaneous: healthy adults, emotion processing, social anxiety disorder, Cushing’s syndrome.

| Author, Country | Sample characteristics | Condition | Cases (male); controls (male) | Age | Epigenome-wide/candidate, platform | Tissue sampled | Correction for cell sub-type distribution | Consideration of genotype | MRI field strength | Modality | Image feature(s) | Analysis method | Main Findings |
|-----------------|------------------------|-----------|--------------------------------|-----|-----------------------------------|---------------|-------------------------------------------|--------------------------|----------------|----------|----------------|----------------|---------------|
| Ursini et al., 2011, Italy | Healthy participants | Cases, n=84(32M) | Cases, mean age 25.9 years | Candidate: COMT rs4680 (C2) and gene promoter (3 CpGs in the promoter) | Peripheral blood mononuclear cells | Cell type composition: NR | Consideration of genotype: yes | 3T | fMRI task: N-back task–working memory | BOLD responses in the bilateral prefrontal cortex | SPM5 | Val/Val genotype: Negative association between C2 DNAm and bilateral PFC (BA 45/13 and BA47) activity during task | Increased stress and lower C2 DNAm related to greater activity in bilateral PFC (BA10/47; BA44/45) during working memory task | No significant relationship was found between DNAm of sites in the COMT promoter or in LINE-1 and working memory related PFC activity in any other genotype group or in the whole sample. |
| Jack et al., 2012, United States | Healthy participants | Cases, n=42(23M) | Cases, mean age 21.9 years | Candidate: OXTR-coding strand including site -934 | Blood (mononuclear cells) | Cell type composition: NR | Consideration of genotype: NR | 3T | fMRI task: social perception task (animacy versus random movement) | whole brain: BOLD activity | FSL (FEAT) | OXTR DNAm positively associated with activity in left STG, supramarginal gyrus, and right dACC. | Controlling for sex: OXTR DNAm positively associated with activity in the left STG and right dACC. No difference between men and women. |
| Study                                    | Participants Details                                                                 | Candidate | fMRI Task | Brain Activation | Associations                                                                 |
|------------------------------------------|--------------------------------------------------------------------------------------|-----------|------------|------------------|-----------------------------------------------------------------------------|
| Vukojevic et al., 2014 Switzerland       | Healthy participants, Cases, n=72(25M), Cases, median age 23 years                   | NR3C1 CpG3 | 3T         | SPM8             | Women: no significant associations between NR3C1 CpG3 DNAm and brain activation during fMRI task. Men: positive associations between DNAm at NR3C1 CpG3 and brain activity related to successful recognition of previously seen pictures: right hemisphere pars orbitalis, right hemisphere pars triangularis of the inferior frontal gyrus; near superior temporal cortex; left hemisphere cuneus, left hemisphere pericalcarine; near superior frontal cortex. |
| Nikolova et al., 2014 United States      | Healthy participants, Discovery set n=80(38M); replication set: 96 (48M), Discovery set mean age 19.7 years; replication set 13.6 years | SLC6A4; 20 CpG sites in promoter region | fMRI task: emotional faces processing | BOLD - amygdala reactivity to threat (angry/fearful faces vs shapes). | Positive association between SLC6A4 DNAm and left amygdala reactivity in response to threat stimuli; right amygdala did not reach statistical significance. The CpG with the strongest association across both hemispheres is CpG14. Findings consistent in discovery and replication sets |
| Dannlowski et al., 2014 Germany          | Healthy participants, Two independent samples: sample 1 n=94(42M); sample 2 n=95(46M), Sample 1 mean age 36.9 years; sample 2 mean age 34.2 years | SLC6A4 AluJb element – mean methylation of 8 investigated sites | Field strength: NR | Structural, T1 | Positive association between AluJb DNAm in the SLC6A4 gene and bilateral hippocampal grey matter volume in ROI analysis in both samples. In sample 1 peak coordinates cluster extended into the bilateral amygdala. This was not the case in sample 2, but was the case in the combined sample. Whole brain analysis: positive association clusters where a positive association is also found: bilateral insula, right putamen, amygdala and caudate, and left superior occipital gyrus, bilateral hippocampus. No areas showed a negative association between DNAm rates and grey matter volume. |
| Study | Country | Participants | Cases, n= | Age | Candidate | Cell Type | Cell Type Composition | Consideration of Genotype | Imaging Technique | Associations |
|-------|---------|--------------|-----------|-----|-----------|-----------|-----------------------|------------------------|------------------|-------------|
| Puglia et al., 2015 | United States | Healthy participants | 98(42M) | 22.8 years | OXTR CpG site at position -934 relative to TSS | Peripheral blood mononuclear cells | NR | NR | 3T | ROI analysis, PPI/FEAT (FSL) | Positive association between OXTR DNAm and BOLD in the left amygdala in Faces versus Ovals. No association in right amygdala. Higher OXTR DNAm predicted decreased levels of functional connectivity on tasks involving affect appraisal and emotion regulation between right amygdala and brain regions involved in emotional regulation including insular cortex, cingulate cortex, orbitofrontal cortex as well as regions associated with face perception e.g fusiform gyrus. Left amygdala showed no significant voxels. |
| Ziegler et al., 2015 | Germany | Social anxiety disorder | 25(F) | 28.8 years | OXTR gene exon 3 | Blood | NR | Yes | 3T | ROI analysis, SPM8 | In SAD patients (n=25F): negative association between OXTR DNAm and amygdala responsiveness in social phobia vs negative words or neutral words. |
| Muehlhan et al., 2015 | Germany | Healthy participants | 74(45M) | 23.6 years; cases (female), mean age 23.2 years | SLC6A4 mean methylation score at the promoter | Blood | NR | Yes | 3T | ROI analysis, SPM12 | Positive associations between SLC6A4 DNAm and amygdala RS functional coupling with nodes of the salience network: Right amygdala: right SMA and left dACC, left putamen and left insula, right IFG, right putamen, right insula. Left amygdala: right dACC, left ACC, left SMA. |
| Ursini et al., 2016 | Italy | Healthy participants | 141 | NR | BDNF/rs6265 methylation status | Blood | NR | Yes | 3T | ROI analysis, SPM8 | Analysis carried out in 141 healthy subjects: Positive association between BDNF rs6265 DNAm and left prefrontal activity. Val/Val genotype: greater DNAm is associated with reduced activity in the PFC (BA46) subjects with hypoxia related obstetric complications (assessed using the McNeil-Sjöström Scale) compared to those without. |
| Study          | Country       | Participants                                                                 | Candidate | Cell type composition | Consideration of genotype | Task/Analysis                                                                 |
|---------------|---------------|------------------------------------------------------------------------------|-----------|-----------------------|---------------------------|--------------------------------------------------------------------------------|
| Haas et al., 2016 | United States | Healthy participants: n=121(52M)
Cases, mean age 21.3 years | OXT - average OXT DNA methylation across nine CpG sites | NR        | yes                   | Emotional perspective task: Negative association between OXT DNA methylation and activity in the right superior temporal sulcus during emotional perspective taking; Emotion attribution task: negative association in the emotion attribution between OXT DNA methylation and activity in: right STS, right fusiform gyrus/middle occipital gyrus, right inferior frontal gyrus; left fusiform; Negative association between OXT DNA methylation and grey matter volume in right fusiform gyrus |
| Resmini et al., 2016 | Spain         | Cushing’s syndrome cases, n=32; controls, n=32
Cases: mean age 45 years; controls, mean age 44 years | FKBP5 - intronic regions | NR        | yes                   | Positive association between Intron 2 region 2 CpG3 DNA methylation and bilateral hippocampal volume |
| Ismaylova et al., 2017 | Canada        | Healthy participants
Cases 40(14M)
At time of blood sampling: 27
At time of saliva/buccal sampling: mean 33.7 years | SLC6A4 – promoter mean methylation score of 10 CpG sites (blood/saliva/buccal ) | NR        | yes                   | Positive associations between SLC6A4 promoter DNA methylation and grey matter volumes, which varied by tissue type: Blood: right superior frontal grey matter; Saliva: left superior frontal gyrus; Buccal cells: left superior frontal gyrus, left inferior frontal gyrus, right ACC. Positive association between blood SLC6A4 DNA methylation and RS connectivity between: RLP and bilateral frontal poles and SFG; RLP and left lateral occipital cortex. Positive associations between buccal SLC6A4 promoter DNA methylation and RS connectivity between the RLP with right lateral occipital cortex and right angular gyrus, ACC, right frontal pole and mPFC |
| Study Authors and Data Source | Study Design and Participants | Candidate Gene and CpG Sites | Epigenetic Technique | Imaging Technique | Neuroimaging Findings |
|-------------------------------|-------------------------------|-----------------------------|---------------------|-----------------|----------------------|
| Wiemerslage et al., 2017, Sweden | Orexigenia among healthy participants, Cases, n=23(all M), Cases, mean age 26 years | Candidate: KLF13-cg07814318 probe methylation | 3T fMRI resting state and task: virtual high or low-calorie food | SPM8 | Negative associations between KLF13 DNAm and neural activity in response to images high calorie food in comparison to images of low-calorie foods in; left claustrum and insula, bilateral cingulate gyrus, right precentral gyrus and claustrum, right MFG, SFG and medial FG. Positive associations between DNAm and RS activity in the right caudate and putamen, left lingual gyrus and fusiform gyrus. |
| Ismaylova et al., 2018, Canada | Fronto-limbic brain responses to sadness/fear, Cases, n=96 monozygotic twins (21M pairs), Cases, mean age 15 years | Candidate: SLC6A4 – promoter CpG 5-14 | 3T fMRI task: Emotional face processing task | SPM8/CONN toolbox | Sad condition: Positive association between SLC6A4 DNAm and OFC activation. Positive association between methylation and functional connectivity between left amygdala and the ACC and the left amygdala and the right OFC. Fearful condition: positive association between SLC6A4 methylation and functional connectivity between ACC and both the left amygdala and left insula. |
| Freytag et al., 2017, Germany/Switzerland | Healthy cohort n=533(222M) of whom 514 have imaging, Mean age at MRI: mean 22.9 years; mean age at blood sampling 23.9 years | Epigenome-wide: Illumina 450 array | 1.5T/3T Structural, T1 | FreeSurfer | Independent component analysis (ICA) applied to decompose methylation profiles. Negative association between the second independent component (ICA2) DNAm and cortical thickness. ICA2 DNAm partially mediates the relationship between chronological age and global cortical thickness. Negative association between ICA2 and F6 (identified through exploratory factor analysis (EFA) of variation in cortical thicknesses); F6 accounts for 4% of variance in cortical thickness measures - spatial pattern comprising mainly temporal areas (loadings >0.3) with highest loadings in L and R temporal poles and entorhinal cortices. |
