Epigenome-wide association study of left-handedness for different tissues and ages

Veronika Odintsova (v.v.odintsova@vu.nl)
VU University Amsterdam https://orcid.org/0000-0002-9868-6981
Matthew Sudermann
MRC Integrative Epidemiology Unit, Bristol Medical School, Population Health Science, University of Bristol, Bristol BS8 1TH, UK
Fiona Hagenbeek
Vrije Universiteit Amsterdam https://orcid.org/0000-0002-8773-0430
Doretta Caramaschi
MRC Integrative Epidemiology Unit, Bristol Medical School, Population Health Science, University of Bristol, Bristol BS8 1TH, UK
Jouke-Jan Hottenga
Vrije Universiteit https://orcid.org/0000-0002-5668-2368
René Pool
Department of Biological Psychology, Vrije Universiteit Amsterdam, 1081 BT Amsterdam, The Netherlands
Conor Dolan
VU University Amsterdam
Lannie Ligthart
Department of Biological Psychology, Vrije Universiteit Amsterdam, 1081 BT Amsterdam, The Netherlands
Catharina van Beijsterveldt
VU University Amsterdam
Gonneke Willemsen
VU University
Eco de Geus
Vrije Universiteit
Jeffrey Beck
Avera Institute for Human Genetics
Erik Ehli
Avera Institute for Human Genetics https://orcid.org/0000-0002-7865-3015
Gabriel Cuellar-Partida
University of Queensland https://orcid.org/0000-0001-7648-4097
David Evans
University of Queensland https://orcid.org/0000-0003-0663-4621
Sarah Medland
QIMR Berghofer Medical Research Institute https://orcid.org/0000-0003-1382-380X
Caroline Relton
MRC Integrative Epidemiology Unit, Bristol Medical School, Population Health Science, University of Bristol, Bristol BS8 1TH, UK
Dorret Boomsma
VU University Amsterdam https://orcid.org/0000-0002-7099-7972
Jenny van Dongen
Vrije Universiteit Amsterdam, Amsterdam, The Netherlands https://orcid.org/0000-0003-2063-8741

Article

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Abstract

We report the first epigenome-wide association study of left-handedness, a trait with low heritability for which epigenetic mechanisms have been proposed as an underlying etiological mechanism. A region-based meta-analysis of whole blood genome-wide DNA methylation data from two cohorts (3,914 adults) identified differentially methylated regions annotated to BLCAP (20q11.23), a negative regulator of cell growth involved in apoptosis, NNAT (20q11.23), involved in brain development, and IAH1 (2p25.1), which encodes an acyl esterase. CpGs located in proximity to the SNPs from the largest GWAS of handedness were more strongly associated with left-handedness than other differentially methylated positions. In longitudinal whole blood samples, cord blood, and buccal cells from children (N = 1,967), the association with handedness displayed moderate stability across age, but little consistency across cell types. These findings suggest new candidate loci associated with handedness.

Main

Handedness, defined as the preferential use of one hand over the other, is established early in life and represents a highly stable trait that is thought to be accompanied by changes in brain[1], corticospinal tract[2], peripheral innervation and vascularization of arm skeletal muscles[3], arm dynamics[4], and possibly the immune system[5]. Laterality is already observable in very early stages of development: fetuses demonstrate coordinated hand movements at 8–12 weeks post-conception with more right than left arm movements in 85% of fetuses[6–8]. In children and adults, the prevalence of left-handedness is about 10%[9]. Handedness clearly clusters in families, but its inheritance pattern is not clear and the heritability of handedness is relatively low: approximately 25% in twin studies (with 95% confidence intervals (CI) ranging from 11 to 30%[10–12]) and 11.9% (95% CI: 7.2–17.7) based on autosomal Identity By Descent (IBD) information from closely related individuals in the UK Biobank[13]. Early genetic hypotheses on the development of hand preference incorporated a component of randomness[14,15]: depending on inherited alleles a person would be right-handed or have an equal chance of being either left- or right-handed. This randomness has also been referred to as “developmental instability”, or “fluctuating asymmetry”[16,17]. Such randomness could explain monozygotic twin discordance[17] that has been reported in some twin studies[18–22], although very early studies did not confirm zygosity by DNA testing.

Candidate genes associated with handedness and brain and spinal asymmetry include leucine rich repeat transmembrane neuronal 1 (*LRRTM1)*[23], LIM domain only 4 (*LMO4)*[24,25], neuronal differentiation 6 (*NEUROD6)*[24,26–28], proprotein convertase subtilisin/kexin type 6 (*PCSK6*)[29–31] and the androgen receptor gene (*AR*)[32–34]. However, genome-wide association studies (GWASs) found no support for these candidate genes[35–38]. Recently, the largest genome-wide association study to date, which included more than 1,5 million right-handed and 194,000 left-handed individuals, found 41 loci associated with left-handedness[13]. The results of functional analyses suggested involvement of neurogenesis and the central nervous system and brain tissues, including hippocampus and cerebrum, in the etiology of left-handedness. Multiple variants were close to genes involved in microtubule functions that form part of the cytoskeleton, and play a role in neurogenesis, axon transport[39], and brain asymmetry[40]. The variance of handedness explained by SNPs (SNP heritability) on the liability scale was 3.45% in this meta-analysis[13]. The estimate in UK Biobank was 5.9%[13].

Partly because of the limited success of genetic association studies, epigenomic mechanisms have been proposed[41–44]. In epigenome-wide association studies (EWAS), association tests are performed for several hundred thousands of CpGs (cytosine-phosphate-guanine nucleotide base pairing) to identify differentially methylated positions (DMPs) associated with a trait. Approaches that test associations across multiple nearby correlated CpGs to identify differentially methylated regions (DMRs)[45], or that combine multiple CpGs into DNA methylation scores[46,47] help improve power by combining the effects of multiple CpG sites and reducing the number of conducted tests. The predictive value of DNA methylation by construction of individual methylation scores has been shown for several outcomes, e.g. body mass index[48,49]. Epigenetic variation could be one pathway to connect the hypothesized random component of handedness, and contribute to asymmetrical gene expression in the two brain hemispheres[50] and the spinal cord[51]. The existence of an early determination of fetal hand movement asymmetry was supported by a genome-wide DNA methylation in the right and left part of the fetal spinal cord from six samples obtained between 8 and 12 weeks post conception that detected asymmetrically methylated CpG islands of several genes[51].

At present, no epigenome-wide association studies of handedness have been performed, and the role of DNA methylation in handedness has only been examined in small candidate-gene studies[52,53]. Here we analyzed DNA methylation data and left-handedness from two cohorts – the Netherlands Twin Register (NTR) and Avon Longitudinal Study of Parents and Children (ALSPAC).

Both cohorts include methylation data in children and adults. We excluded ambidextrous and mixed-handed persons, and treated handedness as a dichotomous trait (left or right-handed). First, we performed a meta-analysis of DNA methylation data from adults in these two large cohorts (total sample size=3,914) to identify differentially methylated positions (DMPs) and differentially methylated regions (DMRs) associated with left-handedness. Next, we performed additional analyses in which we 1) examined if the epigenetic signal for left-handedness was enriched near previously reported GWAS loci[13]; 2) examined methylation differences between left and right-handed twins from discordant monozygotic (MZ) twin pairs; and 3) characterized the longitudinal and cross-tissue similarity of the genome-wide epigenetic signal associated with left-handedness using data from children. Finally, we created methylation scores and estimated their predictive value over and above polygenic scores (**Fig.1**)

Results

Epigenome-wide association meta-analysis of left-handedness

The epigenome-wide association study (EWAS) of left-handedness meta-analysed data from adults (N=3,914) with DNA methylation data in peripheral blood (Illumina, 450k) from NTR (N=2,682, 37% male, mean age at methylation 36.5, standard deviation (SD) 12.7) and ALSPAC (N=1,232, 31% male, mean age at methylation 48.98, SD 5.55). **Tables 1-2** and **S1-S4** display the characteristics of the participants included in this study. In EWAS discovery cohorts, the prevalence of left-handedness was 12% in NTR, and 8% in ALSPAC. The prevalence of left-handedness increased from 10% in individuals born before 1960's to 13.7% born after 1960's (see **Table S3**).

We tested 409,562 CpGs with adjustment for age, sex, smoking status, body mass index (BMI), measured or estimated cell proportions, and technical covariates. Genome-wide EWAS test statistics from each cohort separately, and from the meta-analysis showed no inflation (**Tables S5-11**). All the p-values were higher than the epigenome-wide Bonferroni-corrected threshold of 1.22×10^{-7} and the False Discovery Rate (FDR) q-value of 0.05 (**Fig.S1**). The CpGs with lowest p-values in meta-analysis ($p<1 \times 10^{-5}$) are shown in **Table 3**. Six of eight CpGs were located near transcription start sites on different chromosomes: in the *LRCC2* gene on chromosome 3, in the *ATP6V1B2* gene on chromosome 8, in the *CKAP4*, *GALNTR6*, and *UNC1198* genes on chromosome 12, in the *C13orf18* gene on chromosome 13, in the *MBD2* gene on chromosome 18, and in the *NTSR1* gene on chromosome 20.

The DMR meta-analysis detected two DMRs associated with left-handedness (**Fig.2a-b, Table 4**): one on chromosome 20 (*BLCAP*, *NNAT*; 16 CpGs; p-value adjusted for multiple testing (p_{adj}) =0.00004), and one on chromosome 2 (*IAH1*; 7 CpGs; p_{adj}=0.03). Both DMRs were within CpG islands, 15 of 16 CpGs in the first DMR and 6 of 7 CpGs in the second DMR were hypomethylated in left-handers. These DMRs were not detected in the individual cohorts. One DMR was detected in ALSPAC adults on chromosome 1 (*AMPD2*, 4 CpGs, p_{adj}=0.031) that did not show associations in the meta-analysis. The follow-up of CpGs from DMRs associated with left-handedness in meta-analysis was done in previous EWAS conducted on other traits and reported in **Table S12.**

GWAS follow-up

We tested the overlap of our EWAS meta-analysis results with findings from the most recent GWAS meta-analysis of handedness[13]. CpGs located within 1 Mb window of SNPs associated with left-handedness (at $p < 5 \times 10^{-8}$) were on average more strongly associated with left-handedness in the EWAS meta-analysis than the other tested CpGs (ß=0.027, p =0.04). The effect was weaker when less stringent GWAS p-value cut-offs were applied (i.e. SNPs with $p<1 \times 10^{-6}$, and SNPs with $p<1 \times 10^{-5}$). Importantly, in a control analysis with a trait that is not associated with handedness, we did not see this stronger association for CpGs located near SNPs associated with type 2 diabetes in GWAS[54] (ß =0.005, p=0.265) (see **Table S13**, **Fig. S2a-d**).

Discordant MZ twins

In NTR, the DNA methylation datasets included 1,279 adult monozygotic (MZ) twins with blood samples and 710 MZ children with buccal samples. We found that 21% (N=133 pairs) of the adult MZ twin pairs and 24% (N=86 pairs) of the MZ twin children were discordant for handedness (**Tables S1-S2**). Characteristics of MZ discordant twins are presented in **Table S4**. In both groups, we performed an MZ discordant within-pair EWAS analysis, comparing right- and left-handed twins. Within-pair analyses of DNA methylation of left and right-handed twins did not find association at DMPs or DMRs in blood or buccal samples at Bonferroni or FDR threshold (**Fig.S1q-t, Tables S14-17**). We compared the methylation results obtained in discordant MZ twins to the EWAS meta-analysis results. To avoid sample overlap, we repeated the EWAS meta-analysis after exclusion of the MZ discordant twin pairs. Correlations of the effect sizes of the top 100 CpGs were weak in adults ($r^{\text{MZ disc adults blood–Meta-analysis}}$=0.189, p=0.06; $r^{\text{Meta-analysis–MZ}}$

disc adults blood=0.188, p=0.06, α = 0.0003) and children ($r^{MZ\ disc\ children\ buccal–Meta\text{-}analysis}$=0.134, p=0.18; $r^{Meta\text{-}analysis–MZ\ disc\ children\ buccal}$=0.252, p=0.01) (**Fig.S3**). There were few overlapping CpGs among the top 100 CpGs ranked on ascending p-value from the within-pair analyses and other analyses (**Fig.S4**).

Longitudinal analysis

While handedness is a stable trait, DNA methylation can vary over time[55]. We analyzed DNA methylation in ALSPAC offspring measured in cord blood at birth, and in peripheral blood at 7, 17, and 24 years old (N~1021, **Table 2** and **Table S2**) to examine the association between DNA methylation and left-handedness throughout childhood and adolescence. The correlations between the top 100 CpG effect sizes from EWASs of handedness performed at different time points were moderate to strong (r=0.355; p=0.0002 to r =0.578, p=1.2 ´ 10^{-10}), except for a weak correlation between top 100 CpGs effect sizes at 17 years and the same CpGs at age 24 years ($r^{ALSPAC17\text{-}\ ALSPAC24}$ =0.079; p =0.435) (**Fig. S3**). There were no overlapping CpGs amongst the top 100 CpGs between analyses at different time points (**Fig. S4**). Correlations between top CpG effect sizes between ALSPAC adults (mothers and fathers) and offspring at birth were strong negative ($r^{ALSPACadults\text{-}\ ALSPACatbirth}$ =-0.68; p = 7.2 ´ 10^{-15}) (**Fig.S5**), and between ALSPAC adults and offspring at 7, 17, 24 years were weak (r from -0.006 to 0.141, p >0.0003). No CpGs passed a Bonferroni correction at any time point (**Tables S18-25**).

DNA methylation in buccal cells

In NTR, buccal DNA methylation data (measured with the EPIC array at 787,711 CpG sites) were available in children (N=946, mean age 9.5, SD=1.85). Top 100 CpG effect sizes in EWAS of handedness in buccal cells had weak correlations with effect sizes in blood in the meta-analysis (from r=0.086, p=0.39 to r=0.179, p=0.07), NTR adults (from r =0.193, p=0.05 to r=0.268, p=0.007), ALSPAC adults (from r =-0.008, p=0.94 to r=-0.04, p=0.95), and ALSPAC children at different ages (from r=-0.384, p=7.9 ´ 10^{-05} to r=0.312; p=0.002). The EWAS did not detect associations of DMPs with left-handedness (**Table S26-27**). Four DMRs in buccal cell DNA were associated with left-handedness: on chromosomes 8 (6 CpGs; p_{adj}= 9.14 ´ 10^{-06}), 9 (10 CpGs; p_{adj}=0.039), 12 (2 CpGs; p_{adj}=0.04), and 22 (2 CpGs; p_{adj}=0.035) (**Table 4. Fig.S6d-g**). These DMRs did not overlap with DMRs detected in the analyses of blood methylation data. Sixteen of 18 CpGs from these regions were hypomethylated in left-handed children.

Sensitivity analyses

Above we reported the DNA methylation and left-handedness association study with adjustment for prenatal and postnatal factors that influence DNA methylation as was shown in previous studies: smoking and BMI in adults[49,56], and gestational age, birth weight and prenatal maternal smoking[57,58] in children. These characteristics were reported to be associated with handedness in studies of prenatal maternal smoking[59,60], gestational age[61,62], birth weight[63], BMI[64], and smoking[65]. We examined if the EWAS results for handedness differ without taking these factors into account. Across all analyses, the correlations between the effect sizes of the top 100 CpGs were strong between the models with and without adjustment for these factors (r ranged from 0.99 to 1), and overlaps of the top 100 CpGs were substantial (32 to 87 CpGs). Adjustment for the factors increased the number of DMRs associated with left-handedness in meta-analysis (1 DMR without adjustment and 2 DMRs with adjustment), and in EWAS in children (2 DMRs without adjustment, and 4 with adjustment in buccal cells in NTR).

Handedness methylation scores

To examine if variation in handedness can be predicted by DNA methylation levels across multiple CpGs, methylation scores (MS) were created. These were based on EWAS summary statistics in NTR to predict into ALSPAC, and on ALSPAC summary statistics to predict into NTR given the following p-value thresholds for inclusion of CpGs: p < 1 ´ 10^{-1}, p < 1 ´ 10^{-3}, p < 1 ´ 10^{-5} (**Fig.1, Table S28, Fig. S7**). To estimate the variance explained by MS above genetic variants, polygenic scores (PGS) were created based on the summary statistics from the handedness GWAS of Cuellar-Partida et al.[13] with exclusion of NTR, ALSPAC and 23andMe cohorts (N_{GWAS}=196,419). Since four scores were tested (3 methylation scores, one polygenic score), we applied Bonferroni correction for four tests (α=0.05/4=0.0125). The results are summarized in **Tables S29-30**. MS did not predict left-handedness in NTR and ALSPAC adults, or in children at 7-9 years old and did not explain variance over and above the variance explained by the PGS in the combined model (R^2_{MS} from -0.17 to 1.28%, R^2_{PGS} from 0 to 0.48%). The largest amount of explained variance was in ALSPAC at 7 years old for the MS based on CpGs with a p < 1 ´ 10^{-5} (R^2_{MS}=1.28%, p=0.1, N_{CpGs}=7).

Discussion

We have performed an epigenome-wide association study of left-handedness including left and right-handed individuals from two population-based cohorts from the Netherlands and the UK. In the meta-analysis, combining the NTR and ALSPAC adult cohorts, two DMRs were associated with left-handedness. The first DMR (genomic location: chr20q11.23, 36,148,679:36,149,022) is located within the 5'UTR of the BLCAP apoptosis inducing factor (*BLCAP*) gene and nearby the transcription start site (TSS1500) of the neuronatin (*NNAT*) gene. *BLCAP* encodes a protein that reduces cell growth by stimulating apoptosis. *NNAT* is involved in brain development and nervous system structure maturation and maintenance. CpGs from this region were previously associated with myalgic encephalomyelitis and chronic fatigue syndrome, preterm birth, obesity, metabolic parameters, and arm fat mass (DXA scan measurement). The second DMR (genomic location: chr2p25.1, 9,614,471:9,614,744) is located within the isoamyl acetate hydrolyzing esterase 1 (*IAH1*) gene. The *IAH1* gene encodes an acyl esterase and is associated with neonatal inflammatory skin and bowel disease, and a disease with an inborn error of leucine metabolism (3 methylglutaconic aciduria type 1). CpGs from the region were previously associated with gestational age, bone mineral density, metabolic parameters, and schizophrenia. Some of these traits have been previously associated with handedness in epidemiological studies, e.g. BMI[64] and gestational age[61,62], for which we adjusted in our analyses. Previous analysis of the genetic correlations between left-handedness and 1,349 complex traits using LD-score regression did not reveal any genetic correlations at FDR <5%, but suggestive positive correlations were observed with neurological and psychiatric traits including schizophrenia[13].

Even though no DMPs were identified after correction for multiple testing, the high-ranking CpGs can be of potential interest. The second-ranking CpG cg09239756 (genomic location: chr12, 106,642,360) is located near the cytoskeleton associated protein 4 (*CKAP4*) gene. This gene mediates the anchoring of the endoplasmic reticulum to microtubules. Microtubules are an important cytoskeleton component that play a role in neuronal morphogenesis and migration, and axon transport[39]. Microtubules have been widely discussed in association with handedness[17,35] and brain anatomical asymmetry[40], and genes involved in microtubule pathways were enriched in the GWAS of handedness[13]. Moreover, in our enrichment analysis, we found that CpGs located within a 1Mb window from SNPs associated with left-handedness from the GWAS meta-analysis by Cuellar-Partida et al. were more strongly associated with left-handedness in our meta-analysis compared to CpGs outside of this window.

Hand movements together with other lateralized movements and molecular signs of lateralization are observed at very early stages of human development in the uterus[51,66–68]. Therefore, DNA methylation differences associated with hand preference are expected to emerge early in development. While DNA methylation at some CpGs in the genome changes throughout the lifespan[55], the DNA methylation signal associated with left-handedness was moderately consistent from birth throughout the lifespan: DMP effect sizes were correlated in ALSPAC individuals from birth to 24 years old, although genome-wide significance for DMPs was not reached. Consistency in DNA methylation signal associated with left-handedness at different time-points may indicate that the pattern for left-handedness is conserved through the lifespan.

Several DMRs were detected in buccal cells in children around 9 years old (genomic locations: chr8, 22, 9, and 12) after correction for multiple testing. Annotation of these regions implicate the following protein coding genes: the plectin gene (*PLEC1)*, the myoglobin gene (*MB*) gene, the elongator complex protein 1 gene (*ELP1*), the actin binding transcription modulator gene (*ABITRAM*), and the WNK lysine deficient protein kinase 1 gene (*WNK1*). The CpGs in the regions are mostly hypomethylated in left-handed individuals. The genes encode for proteins that participate in cytoskeleton functions, chromatin organization, development of neurons, and metabolism. CpGs from DMRs in buccal cells have been previously associated with other phenotypes: CpGs from the DMR on chromosomes 8 were previously associated with myalgic encephalomyelitis, chronic fatigue syndrome, multiple sclerosis, and gestational age; CpGs from the DMR on chromosome 9 were reported in association with bone mineral density, tissue mass of the arm (DXA scans measurement), and multiple metabolic parameters. Interestingly, some of these phenotypes were also associated with CpGs from the blood meta-analysis.

A difference in handedness preference in MZ twin pairs has always fascinated parents of twins and twins themselves: how can children with almost identical genes differ for such a prominent trait? Handedness discordance in identical twins was described a long time ago[18–21], and the percentage of MZ discordant twins were reported as 20% of 3,486 MZ twins in East Flanders[22], and 19% of 1,724 MZ twins from a London twin study[37]. We observed that 21% of adult twins and 24% of young MZ twins were discordant for handedness. Different hypotheses have been proposed for handedness discordance: 1) strongly significant unique environmental effects and low heritability[10–12]; 2) adverse prenatal factors [69,70]; 3) atypical brain lateralization[43,71]; 4) epigenetic mechanisms (e.g.,

genomic imprinting)[43]; 5) late-split twinning[69], and 6) mirror twinning[72]. However, we did not detect strong methylation differences between discordant MZ twins. Our discordant MZ twin analysis may be underpowered to detect small DNA methylation differences[73], as it included only 133 MZ discordant adult twin pairs and 86 child twin pairs.

There is a growing interest to improve the prediction of traits with use of other omics data than SNPs, like DNA methylation[46]. Even though single CpGs did not individually reach statistical significance in our EWAS, combining information across multiple CpGs into an overall methylation score can be a more powerful approach to capture variation in handedness. Given the low heritability of handedness (~25%[10,11]), it is expected that non-genetic factors could play role. We calculated methylation scores as weighted sums of the individual's methylation loci beta values of a pre-selected number of CpG sites. However, the predictive value of polygenic and methylation scores for handedness was low, which likely reflects that current GWAS and EWAS analyses for handedness are still underpowered. Future larger GWAS and EWAS studies of handedness are expected to result in more powerful scores.

Our multi-cohort epigenome-wide association study can be summarized in several key steps presented in **Figure 3**. We examined DNA methylation data in different tissues (whole blood, cord blood, buccal cells) and ages (from birth tto adulthood). The limitations of the study are related to available tissues, handedness measurements, differences in platforms used for DNA methylation (Illumina 450k, EPIC), and study power. Specifically, the primary tissues of interest for handedness would be brain[2,35], spinal cord[51], and arm muscle tissues[4] that were not available in our cohorts. The difference in left-handedness rates among children born before and after 1960 may be due a move away from forcing right handedness prior to 1960[74]. We accounted for this trend by including age (which correlates almost perfectly with birth year in these samples) as a covariate in the analyses, however, it should be noted that the forced use of the right hand in older generations may render the phenotype definition of handedness less precise.

We reported an EWAS of left-handedness in large population-based cohorts, and examined performance of methylation scores and polygenic scores. Despite the plausible rationale of multiple genetic and non-genetic factors that may be acting via epigenetic pathways to influence the development of handedness, only two DMRs on chr2p25.1 and chr20q11.23 were associated with left-handedness in our meta-analysis, but we did not find prediction of left-handedness by methylation scores. Future EWAS studies, DNA methylation in other tissues related to motor activity and central nervous system would be a valuable addition to our findings.

Methods And Subjects

Overview. The primary epigenome-wide association study (EWAS) of handedness was performed in two cohorts with DNA methylation data in whole blood (Illumina, 450k): NTR adults (N=2,682 individuals including twins, mean age at methylation 36.5, standard deviation (SD) 12.7), and ALSPAC adults (N=1,232, mean age at methylation 48.98, SD 5.55). EWAS analyses were performed in each dataset separately, and summary statistics were combined in the meta-analysis (N=3,914) testing 409,563 CpGs. Further, we tested whether EWAS signal was enriched nearby loci detected in the previous GWAS on handedness[13]. We carried out within-pair twin analysis in MZ twins discordant for handedness (N_{adults}= 133 twin pairs, $N_{children}$= 86 twin pairs). Secondary analyses were performed in different tissues: in cord blood and peripheral blood in ALSPAC children (N=1,021 with DNA methylation data at birth, at 7, 17 years old, Illumina 450k chip, and/or at 24 years old, Illumina EPIC array) and in buccal cells in NTR children (N=946 twins, mean age 9.5, SD 1.85, Illumina EPIC array). We performed EWAS analyses in each dataset and examined correlations between the effect sizes of top CpGs (defined as CpGs with the lowest p-value) in each analysis. Finally, we created and tested polygenic and DNA methylation scores for left-handedness. The study methodology and design are presented in **Fig. 1** and **Fig. 3**.

Subjects. *Primary analysis.* The adult NTR Biobank cohort[75] included twins, parents of twins, siblings of twins and spouses of twins, and had DNA methylation data from blood samples, as previously described[76]. Complete data on handedness and DNA methylation from blood samples were available for 2,682 individuals (mean age 36.5, SD = 12.7), of whom 2,486 were twins/triplets and 196 were parents, siblings or spouses of twins. All subjects provided written informed consent. The study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance FWA00017598; IRB/institute codes, NTR 03-180).

The ALSPAC cohort[77–79] is a population-based birth cohort. All pregnant women living in the geographical area of Avon (UK) with expected delivery date between 1 April 1991 and 31 December 1992 were invited to participate. Approximately 85% of the eligible population was enrolled, totaling 14,541 pregnant women who gave informed and written consent. The study website contains details

of all the data that are available through a fully searchable data dictionary and variable search tool (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). The ALSPAC adult group consisted of parents including 1,232 mothers and fathers with mean age 48.98 years, SD 5.55, when blood samples were acquired. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Secondary analysis. The ALSPAC child cohort comprised of 1021 individuals recruited from birth who had information on handedness and DNA methylation profiles. DNA methylation from peripheral blood cells measured at different ages within the Accessible Resource for Integrated Epigenomics Studies (ARIES) project[80]: at birth (N=703), at mean age 7.44 (N=757), at mean age 17.11 (N=759), and at mean age 24.3 (N=442). Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Bristol[81]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies. The NTR child cohort included in the EWAS of buccal cell DNA was part of a project on childhood aggression "Aggression in Children: Unraveling gene-environment interplay to inform Treatment and InterventiON strategies" (ACTION)[82,83]. The ACTION-NTR cohort[84] included 1,235 children for whom epigenome-wide data were successfully assessed, mainly from MZ twin pairs. ACTION included twins who at least once scored high or low on a test score for aggression from the population-based NTR. Complete data on handedness and DNA methylation from buccal samples were available for 946 twin individuals (mean age 9.5, SD 1.8, range = 5-12). The study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance FWA00017598; IRB/institute codes, NTR 03-180). For children, written informed consent was given by their parents.

Handedness. *NTR.* An aggregated variable was made for handedness using all available information on hand preference from Adult Netherlands Twin Register (ANTR) surveys, Youth Netherlands Twin Register (YNTR) surveys and experimental projects to obtain the most accurate value for each person. ANTR surveys were held at different time points and included the question: "Are you right-handed or left-handed?" or "Are you predominantly left-handed or right-handed?". The answer categories were left-handed, right-handed, and both. If there was only one report or only consistent reports, this value was used. In case of inconsistent reports, if there were at least three reports and only one deviating value, the deviating one was excluded and the consistently reported value was used. In all other inconsistent cases, handedness was coded as missing. The YNTR surveys include parent reports as well as self-reports (from age 14 onward), and included questions on hand preference at different ages. In the remaining surveys, handedness was assessed using a single item with 3 categories: left-handed, right-handed, and both hands. Exception was the assessment at age 5 when handedness was assessed based on 7 items (drinking from a cup; eating; throwing a ball; drawing on paper; picking up a coin; combing hair; and thumb sucking while asleep) which were then classified in the same 3 categories as other measurements. The reports at age 2, 5, 14, 16, and 18 (all with categories right-handed, left-handed, and both hands) were checked for consistency across time to obtain handedness based on YNTR surveys. Non-survey projects assessed handedness with 3 categories (left-handed, right-handed, and both hands), except for one project where 7 items were used which were rated on a 5-point scale (writing, throwing, using scissors, toothbrushing, using fork, using spoon, lighting a match). These 7 items were recoded into a single 3 category item using an algorithm similar to the one used for the age 5 questionnaire. Finally, ANTR surveys, YNTR surveys, and projects were combined. The ANTR measurement was used, if not inconsistent with project handedness. If this was not available, YNTR handedness was used, if not inconsistent with project handedness. The final variable was coded left-handed, right-handed, and both hands (that included ambidextrous and mix-handed individuals).

ALSPAC. Child handedness was assessed at 42 months by questionnaire in which the mother was asked which hand the child used to draw, throw a ball, color, hold a toothbrush, cut with a knife, and hit things (6 questions). Responses were scored -1, 0 or 1 for left, either or right, respectively. Those with score sums from -6 to -4 were labelled left-handed and those with sums from 4 to 6 were labelled right-handed. Mothers and fathers were similarly asked which hand they used to write, draw, throw, hold a racket or bat, brush their teeth, cut with a knife, hammer a nail, strike a match, rub out a mark, deal from a pack of cards or thread a needle (11 questions). Responses were scored -1, 0 or 1 for left, either or right, respectively. Those with score sums from -11 to -7 were labeled left-handed and those with sums from 7 to 11 were labeled right-handed. Individuals with scores outside these ranges were labeled ambidextrous or mixed-handed and excluded from this study. Handedness was coded as 1 for left-handed or 0 for right-handed in both cohorts.

DNA methylation. *NTR adults.* The NTR blood DNA methylation data was generated as part of the Biobank-based Integrative Omics Study (BIOS) consortium[76,85]. Blood collection procedures were described previously[75]. DNA methylation was assessed with the Infinium HumanMethylation450 BeadChip Kit (Illumina, San Diego, CA, USA), wet laboratory procedure, preprocessing analyses, and

quality control were performed at the Human Genotyping facility (HugeF) of ErasmusMC, the Netherlands (http://www.glimdna.org/) and have been described previously[76,85]. Only the autosomal methylation sites were analyzed, i.e., a total of 411,169 methylation sites. The percentages of neutrophils, monocytes and eosinophils were used to adjust DNA methylation data for inter-individual variation in white blood cell proportions[76]. Missing probe values (probes with missing values in more than 5% of the sample had been removed) were imputed with the function imputePCA from the package missMDA as implemented in the pipeline for DNA methylation array analysis developed by the Biobank-based Integrative Omics Study (BIOS) consortium[86]

ALSPAC adults and children. The ALSPAC blood collection were generated at different ages. DNA methylation was measured with the Infinium HumanMethylation450 BeadChip Kit and Infinium MethylationEPIC BeadChip (Illumina, San Diego, CA, USA) as part of the ARIES[80]. Wet laboratory procedures, preprocessing analyses, and quality control were performed at the University of Bristol, as previously described[80]. Only autosomal probes were analysed in our study: 838,019 probes (Illumina EPIC human methylation arrays) at 24 years of age, and 471,465 probes (Illumina human methylation 450k arrays) at other ages. Blood cell-type proportions were estimated from DNA methylation profiles using deconvolution algorithms[87], and included in statistical models to adjust for cell type heterogeneity. Batch effects and additional unknown confounding were estimated using surrogate variable analysis (SVA) in *meffil*[88]. DNA methylation outliers were identified as those three times the inter-quartile range from the nearest of the first and third quartiles. Outliers were replaced with missing values.

NTR children. DNA samples were collected from buccal swabs, as previously described[89]. DNA methylation was measured using the Infinium MethylationEPIC BeadChip (Illumina, San Diego, CA, USA)[90], wet laboratory procedure, preprocessing analyses, and quality control were performed by the Human Genotyping facility (HugeF) of ErasmusMC, the Netherlands (http://www.glimdna.org/), as previously described[91]. Only autosomal methylation sites were analyzed, leaving 787,711 out of 865,859 sites for analysis. Cellular proportions of buccal cells were estimated from DNA methylation profiles using the deconvolution algorithm HepiDISH[92]. Cell proportions of epithelium cells and natural killer cells were included in statistical models to adjust for cellular heterogeneity. DNA methylation outliers were identified as those three times the inter-quartile range from the nearest of the first and third quartiles. Outliers were replaced with missing values.

Genotyping. *NTR.* Genotyping in NTR was done on multiple platforms including Perlegen-Affymetrix, Affymetrix 6.0, Affymetrix Axiom, Illumina Human Quad Bead 660, Illumina Omni 1M and Illumina GSA. Quality control was carried out and haplotypes were estimated using PLINK[93]. For each genotype platform, samples were removed if DNA sex did not match the expected phenotype, if the PLINK heterozygosity F statistic was < -0.10 or > 0.10, or if the genotyping call rate was < 0.90. SNPs were removed if the MAF < 1×10^{-6}, if the Hardy-Weinberg equilibrium p-value was < 1×10^{-6}, and/or if the call rate was < 0.95. Subsequently, for each platform, the genotype data was aligned with the 1000 Genomes reference panel using the HRC and 1000 Genomes checking tool, which tests and filters for SNPs with allele frequency differences larger than 0.20 as compared to the CEU population, palindromic SNPs and DNA strand issues. The data of the six platforms was then merged into a single dataset, keeping all quality-controlled SNPs of each platform. For each individual, one platform was chosen. Based on the ~10.8k SNPs that all platforms have in common, DNA Identity By Descent state was estimated for all individual pairs using the Plink and King programs. CEU population outliers, based on per platform 1000 Genomes PC projection with the Smartpca software[94], were removed from the data. Data were phased per platform using Eagle, and then imputed to 1000 Genomes and Topmed using Minimac following the Michigan imputation server protocols[95]. For the polygenic scoring the imputed data were converted to best guess data, and were filtered to include only ACGT SNPs, SNPs with MAF > 0.01, HWE p > 10^{-5} and a genotype call rate > 0.98, and to exclude SNPs with more than 2 alleles. All Mendelian errors were set to missing. 20 PCs were calculated with Smartpca using LD-pruned 1000 Genomes–imputed SNPs that were also genotyped on at least one platform, had MAF > 0.05 and were not present in the long-range LD regions.

ALSPAC. Genetic data were collected from the blood samples obtained in clinic visits. Genotyping was conducted with the Illumina HumanHap550 quad chip for children and the Illumina human660W-quad array for mothers. Quality control measures were carried out and haplotypes estimated using ShapeIT. A phased version of the 1000 genomes reference panel from the Impute2 reference data repository was used, and imputation of the target data was performed with this, using all reference haplotypes. Following imputation, variants were retained only given info scores > 0.8 and minor allele frequency > 0.01. Retained variants were then converted to best-guess genotype calls. To avoid potential confounding due to relatedness, closely related individuals were removed using GCTA with a GRM cutoff of 0.05.

Statistical analysis

Intergroup differences. We tested if there were differences in characteristics that were included in EWAS models (such as age at biosample collection, sex, BMI, smoking status at blood collection for adults, and gestational age, maternal smoking during pregnancy, birth weight for children, cell proportions/percentages in buccal swabs and in blood samples) between left-handed and right-handed individuals by generalized estimating equations (GEE) to accommodate the relatedness among the twins in NTR, and by standard logistic regression in ALSPAC. The R package 'gee' was used with the following specifications: binomial (for ordinal data) link function, 100 iterations, and the 'exchangeable' option to account for the correlation structure within families and within persons. Right- and lefthanded MZ discordant twins were compared with paired t-test for the traits that were not identical in twins (birth weight, BMI, smoking, cell percentages).

Epigenome-Wide Association Analyses. *Primary analyses.* The association between DNA methylation levels and left-handedness was tested for each site under a linear model (ALSPAC) or generalized estimating equation (GEE) model accounting for relatedness of twins (NTR). DNA methylation β-value was the dependent variable, and the following predictors were included in the basic model: handedness (coded as 0=right-handed and 1=left-handed), sex, age at blood sampling, percentage of blood cells (monocytes, eosinophils, neutrophils) for blood samples, sample plate and array row in NTR. In ALSPAC, the predictors were handedness (coded the same way), sex, age at blood sampling, percentage of blood cells, and surrogate variables (n=20) were included as predictors. An adjusted model was fitted to account for BMI and smoking status at blood draw in both NTR and ALSPAC adult cohorts, because BMI and smoking are known to have large effects on DNA methylation in adults[49,56] and were associated with handedness in some studies[64,65]. The primary results reported in the paper are based on the fully adjusted model. The models are described in **Appendix 2**.

Secondary analyses. The same basic models were fitted to the data from ALSPAC and NTR children. For DNA methylation in buccal cells, cell proportions (epithelial cells, natural killer cells) for buccal samples were included instead of percentage of blood cells. As several characteristics showed association with handedness in previous studies[64,65] and effect on DNA methylation[57,58], they were included in adjusted model in children (gestational age and birthweight, see **Appendix 2**).

In the within-pair analysis of discordant MZ twins, paired t-tests were employed to test for methylation differences between the left-handed and the right-handed twins. Paired t-tests were performed in R on residual methylation levels, which were obtained by adjusting the DNA methylation β-values for sample plate, array row, cell proportions in buccal samples (epithelial cells, natural killer cells) in children and sample plate, array row, and percentages of blood cells (monocytes, eosinophils, neutrophils) in adults. Additional covariates, birth weight in children and BMI and smoking status in adults, were added in adjusted model. Age, sex, maternal smoking, and gestational age were not included because these variables are identical in MZ twins.

To account for multiple testing, we considered Bonferroni correction and a False Discovery Rate (FDR) of 5%. The Bonferroni corrected p-value threshold was calculated by dividing 0.05 by the number of genome-wide CpGs tested, and false discovery rate (FDR) q-values were computed with the R package 'qvalue' with default settings. The Bayesian inflation factor (λ) was calculated with the R package Bacon[96] (see **Table S5**).

Meta-analysis. A meta-analysis was performed in METAL[97] based on estimates (regression coefficients) and standard errors from the EWAS of handedness performed with GEE in NTR and linear regression in ALSPAC. NTR and ALSPAC adult cohorts were combined. In total, 409,563 CpG sites present in both cohorts were tested with statistical significance evaluated after Bonferroni correction and at an FDR q-value <0.05.

Comparison of top CpGs from different analyses. We selected methylation sites that overlapped in 13 analyses with adjusted model (meta-analysis, meta-analysis without discordant MZ twins, EWAS NTR adults, EWAS NTR adults without discordant MZ twins, EWAS ALSPAC adults, EWASs ALSPAC at birth, 7, 17, 24 years, EWAS NTR children, EWAS NTR children without discordant twins, and within-pair analyses of discordant MZ twin adults and children) that resulted in 379,924 methylation sites. We calculated Pearson correlations for effect estimates of the top 100 CpGs ranked by p-value from one analysis with the effect estimates of the same CpGs in other analyses. Statistical significance of correlations was assessed after Bonferroni correction for the number of correlations tested: $\alpha = 0.05/(13 \times 13 - 13) = 0.0003$.

Differentially methylated regions. We used the R dmrff library for R[45] to identify regions where multiple correlated methylation sites showed evidence for association with handedness. Dmrff identifies DMRs by combining EWAS summary statistics from nearby CpG

sites with methylation datasets to compute correlations between CpGs (https://github.com/perishky/dmrff). Dmrff was applied in each cohort separately. For the meta-analysis, we calculated CpG site correlations separately for NTR adults and ALSPAC adults (parents) cohorts, and performed DMR meta-analysis. We applied an adjusted p-value that was a p-value multiplied by the total number of tests performed with the number of tests equal to the number of regions for which DMR statistics are calculated. We report significant regions ($p_{adj} < .05$) with at least two methylation sites within a 500bp window. We plotted the DMRs with the coMET R Bioconductor package[98] to graphically display additional information on physical location of CpGs, correlation between sites, statistical significance, and functional annotation (annotation tracks included genes Ensembl, CpG islands (UCSC), regulation Ensembl).

GWAS follow-up. GWAS follow-up analyses were performed to examine whether CpGs within a 1 Mb window of loci detected by the GWAS for left-handedness[13], on average, showed a stronger association with left-handedness than other genome-wide methylation sites (Infinium HumanMethylation450 BeadChip). We obtained a SNP list based on the GWAS meta-analysis without NTR, ALSPAC, and 23andMe by Cuellar-Partida et al[13] (196,419 individuals, N_{SNPs} = 13,550,404), from which we selected all SNPs with a p-value <1.0 $\times 10^{-08}$, <1.0 $\times 10^{-06}$, and <1.0$\times 10^{-05}$, and determined the distance of each Illumina 450k methylation site to each SNP. To test whether methylation sites near GWAS loci were more strongly associated with left-handedness, meta-analysis EWAS test statistics were regressed on a variable indicating if the CpG is located within a 1 Mb window from SNPs associated with handedness (1=yes, 0=no):

$|Zscore| = Intercept + \beta_{category\ x} * Category\ x,$

where |Zscore| represents the absolute Zscore for a CpG from the EWAS meta-analysis of handedness; $\beta_{category\ x}$ represents the estimate for category x, i.e. the change in the EWAS test statistic associated with a one unit change in category x (e.g. being within 1 Mb of SNPs associated with left-handedness). For each enrichment test, bootstrap standard errors were computed with 2000 bootstraps with the R-package "simpleboot". Statistical significance was assessed at α = 0.05. As control analysis the same follow-up was performed using GWAS summary statistics on a trait that is unrelated to handedness – type 2 diabetes in UK Biobank cohort (N=244,890)[54]. GWAS summary statistics were downloaded from GWASAtlas (https://atlas.ctglab.nl/traitDB/3686; 41204_E11_logistic.EUR.sumstats.MACfilt.txt; access on February 1 2021).

EWAS follow-up. To examine previously reported associations for epigenome-wide significant DMRs associated with left-handedness in our study, we looked up CpGs from the regions in the EWAS Atlas (https://bigd.big.ac.cn/ewas/tools; accessed on August 1 2020)[99] and EWAS catalogue (http://www.ewascatalog.org; access on November 1 2020)[100].

Polygenic and methylation scores. *Polygenic scores (PGS)* for handedness were calculated based on the GWAS meta-analysis without 23andMe by Cuellar-Partida et al[13]. To avoid overlap between the discovery and target samples, summary statistics without NTR and ALSPAC were requested (196,419 individuals, N_{SNPs} = 13,550,404). The linkage disequilibrium (LD) weighted betas were calculated using a LD pruning window of 250 KB, with the fraction of causal SNPs set at 0.50by LDpred[101]. We randomly selected 2500 2nd degree unrelated individuals from each cohort as a reference population to calculate the LD patterns. The resulting betas were used to calculate the PGSs in each dataset using the PLINK 1.9 software. All PGSs were standardized (mean of 0 and standard deviation of 1).

Methylation scores (MS) were calculated in NTR based on EWAS summary statistics obtained from ALSPAC, and vice versa, as previously done to create methylation scores for BMI, height, and prenatal smoking[102,103]. We calculated same-tissue same-age DNA-methylation scores based on methylation data from NTR adults (blood) and ALSPAC parents (blood), and cross-tissue DNA-methylation scores based on data from NTR and ALSPAC children, with DNA methylation measured in buccal cells, and blood, respectively (see **Fig.3**). For each individual, a weighted score sum was calculated for left-handedness by multiplying the methylation value at a given CpG by the effect size of the CpG (β), and then summing these values over all CpGs: DNA methylation score (i) = $\beta_1 * CpG_{1i} + \beta_2 * CpG_{2i} \ldots + \beta_n * CpG_{ni}$, where CpG_n is the methylation level at CpG site n in participant i, and β_n is the regression coefficient at CpG_n taken from summary statistics of the EWAS analysis. All methylation scores were standardized (mean of 0 and standard deviation of 1).

We used weights from summary statistics of EWASs in four cohorts: NTR adults, ALSPAC parents, NTR children, ALSPAC children at 7 years old. Subsets of CpGs to be included in methylation scores were selected based on p-value <$1*10^{-1}$, <$1*10^{-3}$, and <$1*10^{-5}$.

Testing predictive value. We analysed the predictive value of the left-handedness polygenic scores and methylation scores in NTR and ALSPAC adult and child cohorts from our EWAS study. To quantify the variance explained by the PGS and MS, we used the approach proposed by Lee et al. where coefficients of determination (R^2) for binary responses are calculated on the liability scale[104]. R^2 is equal to the explained variance divided by the total variance; that is the sum of explained variance and residual (homoscedastic) variance. We first regressed left-handedness on PGS and GWAS covariates (genotype platform, the first ten principal components based on genotype data, and sex) (model 1), and then on GWAS covariates only (model 2) using logistic regression. We calculated variance explained by all predictors in each model. We calculated the predictive value of the PGS by subtracting the difference between the variance explained by the first and the second model. For BMI, it has been shown that DNA methylation predicts the trait over and above a polygenic score based on SNPs[102]. To examine the predictive value of MS and PGS in a combined model, we regressed left-handedness on PGS, MS, genotyping and EWAS covariates (model 3). Next, we regressed left-handedness on the same predictors without MS (model 4) and without PGS (model 5). The difference between explained variance in the third and fourth models gave us an estimate of variance explained by MS. The difference between explained variance of the third and fifth models resulted in an estimate explained by PGS. The equations of all models are provided in **Appendix 2**. Statistical significance was assessed following Bonferroni correction for the number of scores tested (PGS and 3 MS). This resulted in $\alpha=0.05/4=0.0125$, nominal significance at 0.05.

Data availability

The HumanMethylation450 BeadChip data from the NTR are available as part of the Biobank-based Integrative Omics Studies (BIOS) Consortium in the European Genome-phenome Archive (EGA), under the accession code EGAD00010000887. The Infinium MethylationEPIC from NTR are available from the Netherlands Twin Register on reasonable request. DNA methylation data from ALSPAC are available at ALSPAC and can be provided on request. The study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (http://www.bristol.ac.uk/alspac/researchers/our-data).

The code used to perform the meta-analyses are available from the corresponding author on request. The pipeline for the DNA methylation array analysis developed by the Biobank-based Integrative Omics Study (BIOS) consortium are available here: https://molepi.github.io/DNAmArray_workflow/[86]. EWAS summary statistics for the top 100 CpGs are given in Supplemental tables (S14-S27). The full EWAS summary statistics from the meta-analysis with adjusted model is provided in Supplemental tables 31.

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Declarations

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Author information

These authors contributed equally: Veronika Odintsova, Matthew Suderman

Contributions

Study design: J.v.D., D.I.B., C.R., M.S, V.V.O;

Manuscript preparation: V.V.O, M.S., J.V.D;

Meta-analysis: V.V.O, M.S., G.C-P.;

Individual analyses: V.V.O, M.S., R.P., J.J.H, C.D.;

Data collection and laboratory analyses: F.H.; CvB; L.L.; G.W., E.E, J.B., BIOS Consortium;

Manuscript Review: All the authors approved the manuscript.

Conflict of interest statement

GC-P contributed to this study while employed at The University of Queensland. He is now an employee of 23andMe Inc., and he may hold stock or stock options. All other authors report no conflicts of interest.

Supplementary Information

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Tables

Table 1. Characteristics of adult cohorts included in the primary meta-analysis

	NTR adults		**ALSPAC adults**	
	N = 2682		N = 1232	
	LH	LH	LH	RH
N	324 (12%)	2358 (88%)	99 (8%)	1133 (92%)
Age at blood sampling	34.3 (11.2)	36.8 (12.9)	49.1 (5.9)	49.0 (5.5)
Sex				
Males	119 (37%)	783 (33%)	31 (31%)	333 (29%)
Females	205 (63%)	1575 (67%)	68 (69%)	800 (71%)
BMI	24.2 (3.7)	24.2 (3.9)	25.80 (4.3)	26.71 (4.7)
Smoking (current)	65 (20%)	486 (21%)	37 (37%)	424 (37%)

Whole blood DNA methylation. Numbers in EWAS basic models are reported

LH, left-handed; RH, right-handed

Values are presented as mean (SD) or n(%).

Current smokers in ALSPAC were defined as those with cg05575921 methylation below 0.82 (see Methods).

Table 2. Characteristics of the datasets included in secondary analyses

	ALSPAC children (longitudinal)								NTR children	
	at birth N=703		7 years old N=757		17 years old N=759		24 years old N=442		N = 946	
	LH	RH	LH	RH	LH	RH	LH	RH	LH	RH
N	60 (8.5%)	643 (91.5%)	68 (9%)	689 (91%)	69 (9%)	690 (91%)	37 (8.4%)	405 (91.6%)	139(15%)	807(85%)
Age at blood sampling			7.43 (0.1)	7.4 (0.1)	16.9 (1.1)	17.1 (1.0)	24.3 (0.7)	24.3 (0.7)	9.58(1.78)	9.56(1.86)
Sex										
Males	32 (53.3%)	302 (47%)	36 (52.9%)	332 (48.2%)	36 (52.2%)	321 (46.5%)	16 (43.2%)	173 (42.7%)	71 (51%)	412 (51%)
Females	28 (46.7%)	341 (53%)	32 (47.1%)	357 (51.8%)	33 (47.8%)	369 (53.5%)	21 (56.8%)	232 (57.3%)	68 (49%)	395 (49%)
Gestational age	39.6 (1.5)	39.6 (1.4)	39.6 (1.4)	39.6 (1.6)					35.51(2.83)	35.93(2.52)
Birth weight	3567.8 (434.4)	3477.3 (493.6)	3583 (445.4)	3481 (493.3)					2369 (585.2)	2407 (533.5)
Maternal smoking during pregnancy	9 (15.2%)	74 (11.6%)	10 (14.9%)	81 (11.8%)					14(11%)	56(7%)
BMI					22.5 (3.6)	22.5 (3.6)	24.2 (3.6)	24.4 (4.5)		
Smoking (current)					20 (29.4%)	161 (23.6%)	9 (24.3%)	131 (33%)		

NTR: buccal cell DNA methylation. ALSPAC: cord blood and whole blood DNA methylation. Numbers in EWAS basic models are reported

LH, left-handed; RH, right-handed; BMI, body mass index

Values are presented as mean (SD) or n(%).

Current smokers in ALSPAC were defined as those with cg05575921 methylation below 0.82 (see Methods).

Table 3. Top differentially methylated positions from EWAS meta-analysis of left-handedness

CpG	CHR	Position[a]	Gene	Location	Effect size	SE	p-value	FDR	Direction
cg22804475	8	20054597	*ATP6V1B2*	TSS200	-0.0007	0.0002	1.28×10^{-06}	0.197	– –
cg09239756	12	106642360	*CKAP4*	TSS1500	-0.0032	0.0007	1.82×10^{-06}	0.197	– –
cg22541911	12	51785465	*GALNT6*	TSS1500	-0.0010	0.0002	1.90×10^{-06}	0.197	– –
cg13719901	3	46608139	*LRRC2*	5'UTR; TSS200	0.0081	0.0017	2.60×10^{-06}	0.197	++
cg02850812	13	46961666	*C13orf18*	TSS200	-0.0014	0.0003	2.62×10^{-06}	0.197	– –
cg16852837	18	51750955	*MBD2*	1st Exon; 5'UTR	-0.0006	0.0001	3.28×10^{-06}	0.205	– –
cg09893588	20	61340109	*NTSR1*	TSS200	-0.0011	0.0003	9.12×10^{-06}	0.256	– –
cg12402132	12	121148554	*UNC119B*	Gene body	-0.0009	0.0002	9.54×10^{-06}	0.256	– –

Effects are indicated for left-handedness in EWAS meta-analysis adjusted model that included $N_{NTR\ adults}$ = 2663 and $N_{ALSPAC\ adults}$ = 1058.

CpGs with uncorrected p-value < 1.0×10^{-5} are presented. Full list of top 100 CpGs is provided in Supplementary table 2.

CHR, chromosome; SE, standard error; FDR, false discovery rate; TSS200, 200 nt upstream of transcription start site; TSS1500, 1500 nt upstream of transcription start site; 5'UTR, 5' untranslated region;

++ positive direction of effect in each cohort; –, negative direction of effect in each cohort

[a]Genome build Hg19 (build 37).

Table 4. Significant differentially methylated regions associated with left-handedness in meta-analysis and secondary analyses

Cohort	Chromosome	Start	End	n CpGs	Genes	Effect size	SE	p value	p_{adjust}
Meta-analysis									
NTR and ALSPAC adults	chr20	36148679	36149022	16	*BLCAP* *NNAT*	-0.153	0.024	9.80×10^{-11}	4.31×10^{-05}
	chr2	9614471	9614744	7	*IAH1*	-0.102	0.019	7.33×10^{-08}	0.03
Secondary analysis									
NTR 9 years	chr8	145024929	145025064	4	*PLEC1*	-0.056	0.008	1.07×10^{-11}	9.14×10^{-06}
	chr22	36011405	36011843	2	*MB*	-0.119	0.022	4.09×10^{-08}	0.035
	chr9	111696674	111697545	10	*EELP1, ABITRAM*	-0.134	0.024	4.59×10^{-08}	0.039
	chr12	899323	899559	2	*WNK1*	-0.117	0.021	4.69×10^{-08}	0.040

Effects are indicated for left-handedness in EWAS adjusted model: $N_{meta\text{-}analysis}$ = 3721, $N_{ALSPAC\ 7years}$ = 713, $N_{NTR\ children}$ = 866.

SE, standard error; p_{adjust}, p-value multiplied by the total number of tests performed; the number of tests is equal to the number of regions for which DMR statistics are calculated

Figures

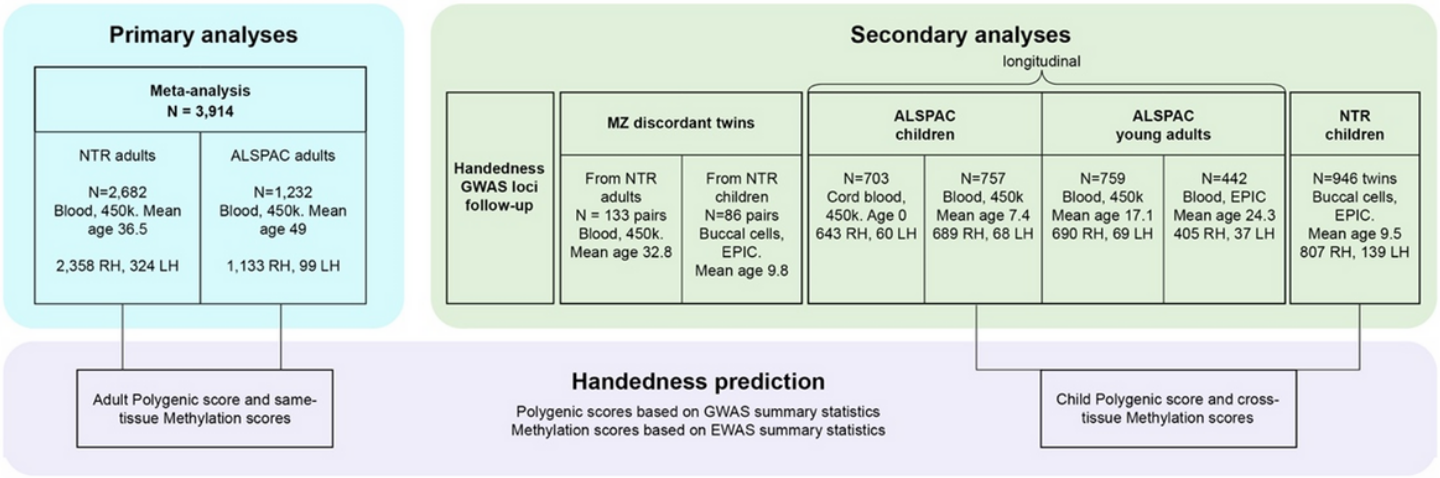

Figure 1

Flowchart of epigenome-wide association study of left-handedness

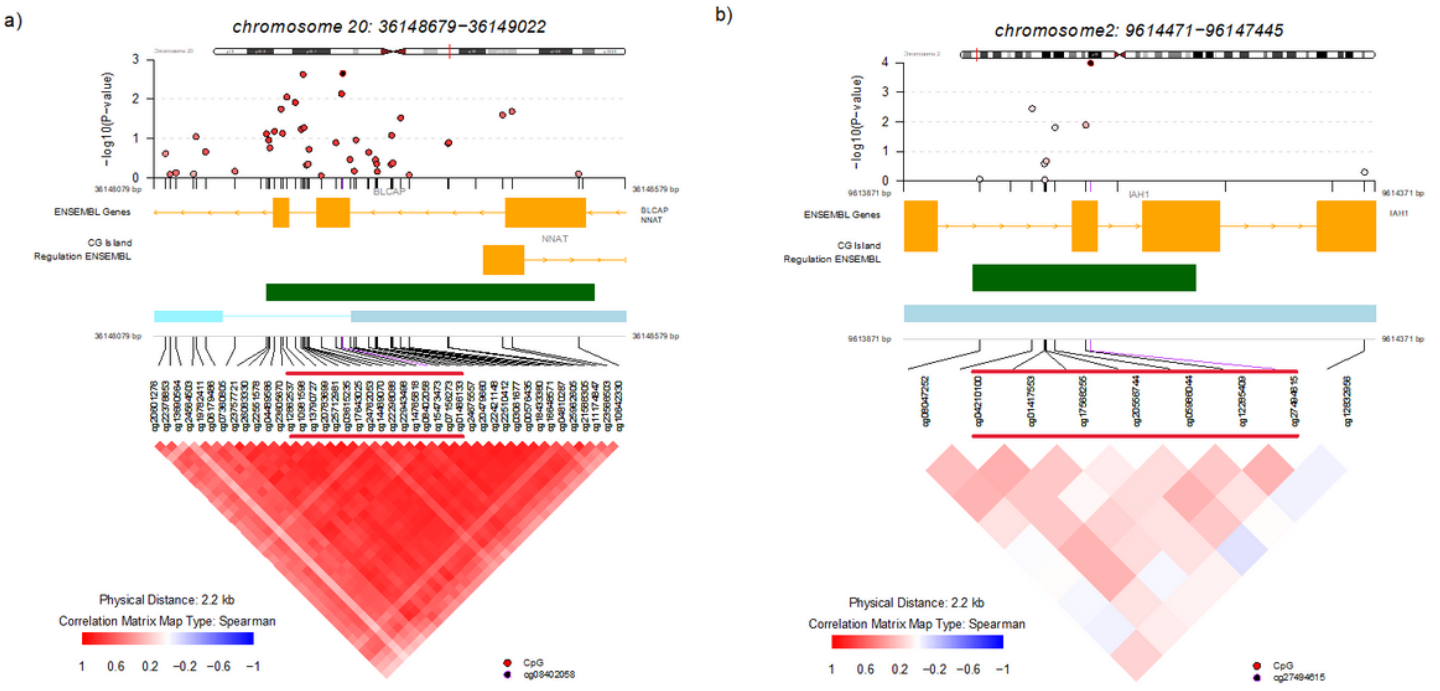

Figure 2

Differentially methylated regions associated with left-handedness in meta-analysis. The top panel of each plot shows the EWAS p-values for all CpGs in the window, with the most strongly associated CpG highlighted. The middle panel shows the genomic coordinates (genome build GRCh37/hg19) and the functional annotation of the region: the ENSEMBL Genes track shows the genes in the genomic region (orange); the CpG Island track shows the location of CpG islands (green); the Regulation ENSEMBL track shows regulatory regions (blue). CpGs from DMR associated with handedness are indicated with red lines above the correlation heatmap.

More detailed information on the regions is provided in Table 4. The bottom panel shows the Spearman correlation between methylation levels of CpGs in the window. a) DMR at chromosome 20; b) DMR at chromosome 2

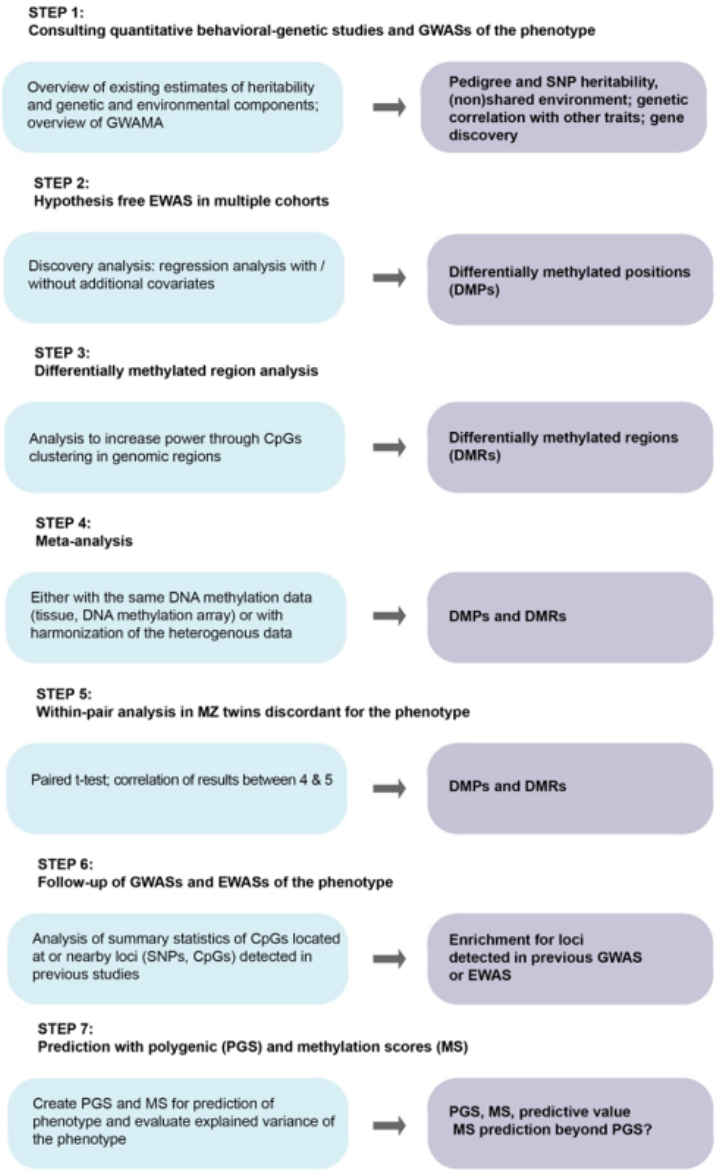

Figure 3

Methodology of the multi-cohort epigenome-wide association study of a phenotype

Supplementary Files

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- SupplementaryTablesS1S4.xlsx
- SupplementaryTablesS512.xlsx
- SupplementaryTablesS1427.xlsx
- SupplementaryTableS30.xlsx
- SupplementaryTableS31.csv.csv
- SupplementaryInformation.pdf