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DNA methylation at birth and fine motor ability in childhood: an epigenome-wide association study with replication

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ABSTRACT

Lower fine motor performance in childhood has been associated with poorer cognitive development and neurodevelopmental conditions such as autism spectrum disorder, yet, biological underpinnings remain unclear. DNA methylation (DNAm), an essential process for healthy neurodevelopment, is a key molecular system of interest. In this study, we conducted the first epigenome-wide association study of neonatal DNAm with childhood fine motor ability and further examined the replicability of epigenetic markers in an independent cohort. The discovery study was embedded in Generation R, a large population-based prospective cohort, including a subsample of 924 ~ 1026 European-ancestry singletons with available data on DNAm in cord blood and fine motor ability at a mean (SD) age of 9.8 (0.4) years. Fine motor ability was measured using a finger-tapping test (3 subtests including left-, right-hand and bimanual), one of the most frequently used neuropsychological instruments of fine motor function. The replication study comprised 326 children with a mean (SD) age of 6.8 (0.4) years from an independent cohort, the INfancia Medio Ambiente (INMA) study. Four CpG sites at birth were prospectively associated with childhood fine motor ability after genome-wide correction. Of these, one CpG (cg07783800 in GNG4) was replicated in INMA, showing that lower levels of methylation at this site were associated with lower fine motor performance in both cohorts. GNG4 is highly expressed in the brain and has been implicated in cognitive decline. Our findings support a prospective, reproducible association between DNAm at birth and fine motor ability in childhood, pointing to GNG4 methylation at birth as a potential biomarker of fine motor ability.

Introduction

Fine motor skills involve the fine control of fingers and hands and are important for activities such as playing an instrument, writing, cutting, and opening boxes. Efficient fine motor control requires a cascade of neuronal activities in order to manipulate objects or perform specific tasks appropriately. These motor movements also entail the age-appropriate development of related physical skills, such as core trunk control and shoulder strength, as these provide a stable base from which the arm and hand can then move with control, as

well as the smaller muscles of the hands. In turn, the efficiency of fine motor skills influences the quality of manual task outcomes as well as the speed of task performance [1]. Children with poor fine motor skills have difficulties developing appropriate independence in 'life' skills, for instance, getting dressed and feeding themselves, which has social implications within the family and peer relationships.

The early development of fine motor ability underlies and precedes cognitive functioning later in life [2–8]. Repetitive rapid finger tapping is a standard test of fine motor control of the upper extremities. Hubel et al. refer to the finger-tapping test (FTT) as an efficient and precise measure of tapping speed and kinetics [9], and therefore one of the most frequently used neuropsychological instruments of utility in research and clinical studies of motor performance [10]. Lower performance in fine motor ability is a neuropsychological deficit that transcends diagnostic boundaries. Abnormal fine motor skills have been associated with a range of neurodevelopmental disorders, such as autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD), as well as cognitive decline in ageing adults [11–16]. Mental disorders, including ASD and ADHD, can be considered as the extremes of quantitative traits rather than dichotomizing the continuous variation of symptoms [17–19]. Identifying the underlying mechanisms that give rise to individual differences in fine motor ability, in particular during developmentally sensitive periods of childhood, provides opportunities to understand typical neurodevelopment and cognitive function. It can also lead to new insights into the molecular processes underlying risks for related neurodevelopmental disorders in the general population [20,21].

Previous research has shown that fine motor ability is partially under genetic influence, with twin and familial heritability ranging from 41% to 86% on different fine motor tests [22,23]. Environmental factors that shape motor development may occur very early in life, for example, exposure to adverse intra-uterine environments, such as higher placental vascular resistance, malnutrition, and stress, are associated with poor motor ability, including FTT performance [24–30]. Yet, how these influences affect early fine motor development at a molecular level remains unclear. Epigenetic processes, such as DNA methylation (DNAm), have emerged as a potential molecular system of interest, as they regulate gene activity in response to both genetic and environmental influences [31]. DNAm at birth is particularly of interest, as it (i) may be a good proxy of genetic, biological, or prenatal environmental factors relevant to the fine motor development [31,32], while the epigenetic signal becomes 'noisier' in later life; and (ii) growing evidence shows that DNAm at birth associates more strongly with certain neurodevelopmental outcomes that also feature fine motor deficits, such as ADHD and social communication deficits [33,34], compared to DNAm patterns measured concurrently in childhood (i.e., prospective > cross-sectional associations). As such, DNAm patterns at birth – but not in childhood – may also mediate risk for developmental outcomes in later life (e.g., DNAm alterations at birth may impact early neurodevelopment and downstream behavioural phenotypes that persist despite changes in DNAm patterns per se).

Previous epigenetic studies have linked DNAm of candidate genes at birth, such as the glucocorticoid receptor gene *NR3C1*, to poorer motor performance during early development [35–37]. However, evidence from epigenome-wide association studies (EWASs) is limited. We are aware of only one EWAS, which identified associations between cord blood DNAm at a cytosine-phosphate-guanine (CpG) site in *SPTBN4* and infant motor function, measured using a composite score of general (fine and gross) motor skills [38]. Given that research to date implicates more fine then gross motor skills as indicators of cognitive development [39,40], it is important to focus specifically on fine motor ability. Yet, to our knowledge, no study has examined epigenome-wide DNAm patterns associated with fine motor ability in childhood. Furthermore, it is unclear to what extent current findings replicate in independent samples, which is important for identifying reliable biomarkers.

In light of these gaps, we performed an EWAS examining long-term prospective associations between DNAm at birth (cord blood) and fine motor ability in childhood, measured by FTT at age 10 years, leveraging data from a large

population-based prospective cohort, the Generation R Study (GenR). We further tested whether our findings can be replicated in an independent birth cohort. To verify the relevance of FTT to higher-order cognition in the general paediatric population, we also examined the prospective association between FTT performance and later cognitive function in adolescence (age 14 years). Finally, we investigated whether slower FTT performance mediated the association between lower DNAm at birth and lower cognitive functioning in the general paediatric population.

Methods

Study population

Detailed information for each cohort is described in the **Supplementary Methods**. Primary analyses were conducted using data from GenR, a population-based prospective cohort from early foetal life onwards, based in Rotterdam, the Netherlands. The design and sample characteristics of GenR have been described in detail elsewhere [41]. The EWAS analyses included a subsample of 1,396 European-ancestry singletons with available DNAm data at birth. Of these, 924 ~ 1069 children had data on fine motor ability at a mean (SD) age of 9.8 (0.3) years and relevant covariates. In addition, we included 2813 participants with cognitive assessment at a mean (SD) age of 13.6 (0.3) years. A flowchart of sample selection is described in **Figure S1**.

The replication analyses were performed in an independent population-based prospective cohort, the INfancia Medio Ambiente (INMA) study in Spain [42]. We included 326 European singletons with complete data on cord blood DNAm at birth and fine motor ability at a mean (SD) age of 6.8 (0.4) years.

DNA methylation

Briefly, DNAm from cord blood was measured using the Illumina Infinium HumanMethylation450 BeadChip in both cohorts. Pre-processing and normalization were performed in R with the CPACOR [43] workflow in GenR and the Bioconductor package minfi [44] in INMA. DNAm levels are characterized by beta (β) values ranging from 0 (no methylation) to 1 (full methylation). CpG sites with values outside the 25th percentile − 3 × interquartile range (IQR) and the 75th percentile+3 × IQR of the distribution were identified as outliers and winsorized.

Finger Tapping Test

In GenR, the fine motor ability was measured using a computerized finger-tapping task during an assessment visit at the age-10 years. The task was programmed in Python using modules from the PsychoPy toolbox (version 1.90.3), as previously described [45,46]. Briefly, children were instructed to perform five 10-second finger tapping trials in which they tapped their index fingers on a button as quickly as possible using their (1) right, (2) left, (3) both (alternating), (4) right and (5) then left hand. For the right- and left-hand conditions, we calculated the average number of taps across two trials on the same hand. For the alternating condition, the total number of taps was calculated as bimanual outcome.

In INMA, fine motor ability was measured during the age-7 assessment visit using a computerized finger-tapping task administered with E-Prime 2.0 [47], first completing two trials with the preferred hand for practice, then two more trials with the non-preferred hand. The children used a standardized method by pressing a key in a joystick board; it was attached to the desk in order to avoid slips while doing the task. We calculated the mean number of taps of same-hand trials.

Cognitive Functions

Cognitive functions were measured by the Wechsler Intelligence Scale for Children-Fifth Edition (WISC-V) at 14 years of age in GenR [48]. Four core subtests (i.e., Vocabulary, Matrix Reasoning, Digit Span, and Coding) were selected to produce four domain-specific index scores: verbal comprehension, fluid reasoning, working memory, processing speed. Trained research assistants administered four subtests. T-scores based on Dutch norm for all subtests were summed and converted to an estimated Full-Scale Intelligence Quotient (FSIQ) score.

Covariates

Covariates for epigenetic associations in both cohorts included: child sex, maternal age at

delivery (in years), smoking during pregnancy (binary categorization of 'no smoking/quit in early pregnancy' vs. 'smoked throughout pregnancy'), gestational age at delivery (in weeks), child age at the fine motor assessment (in years), and estimated cell-type proportions estimated using a cord blood cell type reference panel [49]. In addition, we adjusted for technical covariates (i.e., batch effect only in GenR, as ComBat was applied to remove batch effect in INMA), and handedness as measured by the Edinburgh Handedness Inventory (EHI) in GenR. In INMA, handedness was defined as a laterality index calculated with the relative difference between scores obtained with preferred and non-preferred hand, expressed as a percent of the sum.

Further details on DNAm, finger tapping test, cognitive functions, and handedness are provided in Supplementary Methods

Statistical analysis

Epigenome-wide association study (EWAS) analysis
Robust linear regression was applied to investigate the prospective associations between neonatal DNAm and fine motor ability in childhood. DNA methylation β values at each CpG site were specified as the predictor and right-, left-hand or bimanual FTT score as the outcome adjusted for all covariates. In order to DNAm values were z-standardized to facilitate interpretation and comparison across two cohorts. Both DNAm β value and FTT outcomes were z-standardized to facilitate interpretation and comparison across two cohorts. Probes were annotated using the meffil R package [50], enhanced using the University of California Santa Cruz (UCSC) Genome Browser, based on genome build hg19. P-values were adjusted for genome-wide significance using false discovery rate (FDR) adjustment using the Benjamini–Hochberg method [51] and only sites with FDR-corrected $q < 0.05$ were considered statistically significant.

Follow-up analyses
Follow-up analyses were first performed to further characterize identified top CpGs (FDR q < 0.05) as

follows. (1) We examined the concordance between methylation in peripheral blood and brain using two independent online tools based on post-mortem data: the blood–brain concordance tool (http://epigenetics.essex.ac.uk/bloodbrain/) from 75 adults characterizing whole blood versus 4 brain regions (superior temporal gyrus, prefrontal cortex, entorhinal cortex, and cerebellum); and BECon (https://redgar598.shinyapps.io/BECon/) from 16 adults with 3 brain regions (inferior temporal gyrus, anterior prefrontal cortex, and parietal cortex). (2) Gene expression profiles were probed across 53 human tissues using GTEx database from the FUMA portal (https://fuma.ctglab.nl/). (3) Look-up of methylation quantitative trait loci (mQTL) was performed using the largest mQTL database to date to explore potential genetic influences (https://mqtldb.godmc.org.uk/).

Additionally, to verify the relevance of FTT to cognitive functions, we examined the associations of the FTT score with FSIQ and 4 subdomain index scores of cognition using linear regression. The analyses were adjusted for sex, age of cognitive assessment, handedness, child ethnicity, maternal age, and education. Last, a candidate gene follow-up analysis was performed on results extracted from a previous EWAS of infants' motor ability for CpG sites annotated to *SPTBN4*, in order to examine associations with DNAm at this previously implicated gene ($n = 36$ CpGs). P-values were adjusted for gene-level significance using the Benjamini-Hochberg method for false discovery rate (FDR), and only methylation sites with FDR-corrected $q < 0.05$ were considered statistically significant.

Replication analyses in INMA
Last, we attempted to replicate our findings in an independent sample ($n = 326$) with complete data on cord blood DNAm at birth and FTT at age 7 from INMA. Robust linear models were run in INMA on the top CpGs identified in GenR, adjusting for covariates as described above.

Mediation analysis
In a post hoc analysis, we examined whether fine motor development mediates the association between DNAm at birth (specifically in CpG sites showing significant replication in INMA) and

cognition in adolescence. To this end, we ran a mediation model using R (package *mediation*) with 99% bias-corrected bootstraps confidence intervals (CIs) using 1000 bootstrapped samples. We partitioned the total effect of replicated CpGs on cognition into a direct and an indirect effect through fine motor development. The indirect effect is the estimated effect of DNAm on cognition through fine motor development, while the direct effect represents the effect of DNAm on cognition scores that is independent of fine motor development.

Data availability

The summary statistics of the EWAS will be made available on figshare https://figshare.com/.

Results

Participants characteristics

Sample characteristics are described in Table 1. Differences in characteristics between children included in our EWAS sample and those who were not included were evaluated using chi-square tests for the categorical and independent t-tests for the continuous variables. Children in the current EWAS sample had a higher gestational age and lower performance on left-hand and bimanual FTT than those not included. Mothers of children in the study were on average older, had higher education, and were less likely to smoke throughout pregnancy compared to mothers of children

who were excluded. Children from INMA showed, on average, higher left- and right-hand FTT performance compared to children in GenR, likely due to differences in the duration of FTT assessment between cohorts (15-second per trial in INMA and 10-second per trial in GenR).

Epigenome-wide Association Analysis

At birth, lower methylation at one CpG (cg07783800) site was prospectively associated with slower childhood right and left hand FTT after FDR correction (q < 0.05; Table 2 and Figure 1). The top site is located in the gene body of *GNG4*, which is a member of the G-protein family and has been associated with cognitive decline [52]. For left-hand FTT, four CpGs surpassed the FDR-correction, including (a) the same site cg07783800 in *GNG4*; (b) cg05136476, annotated to the gene body of *PRKCD*, a gene previously linked to several psychiatric disorders by genome-wide association studies (GWAS), including ASD, ADHD, and schizophrenia [53,54]; (c) cg07638797, annotated to the promotor of *DYRK1A*, a gene localized in the Down syndrome critical region of chromosome 21; variations in this gene have also been linked to neuroticism and Parkinson's disease by GWAS at a genome-wide significant level [55,56]; (d) cg16705073, annotated to *ST3GAL2*, a sialyltransferase gene responsible for sialylation of gangliosides and glycoproteins; knock-out of this gene results in cognitive deficits in mice [57]. We did not observe any significant association for bimanual FTT scores (**Table S1**).

Table 1. Descriptive statistics in two cohorts.

	GenR (N = 925)	INMA (N = 326)
Child characteristics		
Sex, % girls	50.8	48.2
Gestational age in weeks, mean (SD)	40.2 (1.5)	39.8 (1.4)
Prematurity, %	**2.38**	**2.45**
Age at measure of FTT, years, mean (SD)	9.8 (0.3)	6.8 (0.4)
Handedness, laterality quotient, mean (SD)	0.7 (0.5)	0.8 (0.5)
Finger-tapping test (FTT), number of taps, mean (SD)		
Left-hand FTT	38.2 (5.6)	56.6 (8.8)
Right-hand FTT	41.1 (6.6)	66.0 (8.1)
Bimanual FTT (alternating)	35.1 (11.9)	NA
Maternal characteristics		
Age at intake/delivery, years, mean (SD)	32.1 (4.1)	31.7 (4.1)
Sustained smoking during pregnancy, %	11.2	12.6
Educational level, %		
Lower (below university degree)	32.5	68.0
Higher (university degree and above)	67.5	32.0

Note. Prematurity is defined as neonates at less than 37 weeks gestation.

Table 2. Top 10 CpGs derived from the EWAS of the association between neonatal blood DNAm and right and left hand finger tapping test at 10 years in GenR; sorted by ascending p-value (*N* = 925).

CpGs	Chr	Pos	Nearest gene	Effect	SE	*p*	FDR
Outcome: right-hand FTT							
cg07783800	chr1	235803662	**GNG4**	0.17	0.03	5.11E–08	0.023
cg14660125	chr4	89378460	*HERC5*	0.14	0.03	6.95E–07	0.159
cg20790998	chr1	68290436	*GNG12*	−0.18	0.04	1.31E–06	0.171
cg09469355	chr1	2161886	*SKI*	0.16	0.03	1.61E–06	0.171
cg26657045	chr5	60132712	*ELOVL7*	0.17	0.04	2.20E–06	0.171
cg13964781	chr19	14552350	*PKN1*	0.14	0.03	2.29E–06	0.171
cg26095658	chr2	71017887	*FIGLA*	−0.13	0.03	2.65E–06	0.171
cg11849213	chr14	65210900	*PLEKHG3*	0.16	0.03	3.28E–06	0.171
cg04477675	chr6	33168449	*SLC39A7;RXRB*	−0.14	0.03	3.35E–06	0.171
cg23468128	chr2	61108270	*REL*	−0.15	0.03	4.35E–06	0.200
Outcome: left-hand FTT							
cg05136476	chr3	53190709	**PRKCD***	0.15	0.03	1.20E–07	0.026
cg07783800	chr1	235803662	**GNG4**	0.16	0.03	1.70E–07	0.026
cg07638797	chr21	38792116	**DYRK1A**	0.19	0.04	2.12E–07	0.026
cg16705073	chr16	70472678	**ST3GAL2**	0.17	0.03	2.29E–07	0.026
cg10194627	chr13	74568601	*KLF12*	0.21	0.04	7.25E–07	0.067
cg09004351	chr7	5515555	*FBXL18*	0.15	0.03	2.20E–06	0.168
cg02019988	chr3	50311211	*SEMA3B*	−0.13	0.03	9.38E–06	0.404
cg19321991	chr1	213124472	*VASH2*	−0.20	0.04	1.02E–05	0.404
cg02960777	chr7	77562848	*PHTF2*	−0.14	0.03	1.05E–05	0.404
cg19870626	chr1	175889703	*RFWD2**	0.13	0.03	1.07E–05	0.404

Note. FTT: finger-tapping test; Chr: chromosome; Pos: position; Effect, standardized regression coefficients, SE, standardized error. The full model is adjusted for sex, gestational age, age at FTT assessment, handedness, maternal age in take/delivery, maternal smoking during pregnancy, batch effects, estimated cell-type proportions.*Annotation based on University of California Santa Cruz Known Gene fills in the nearest gene within 10 MB.
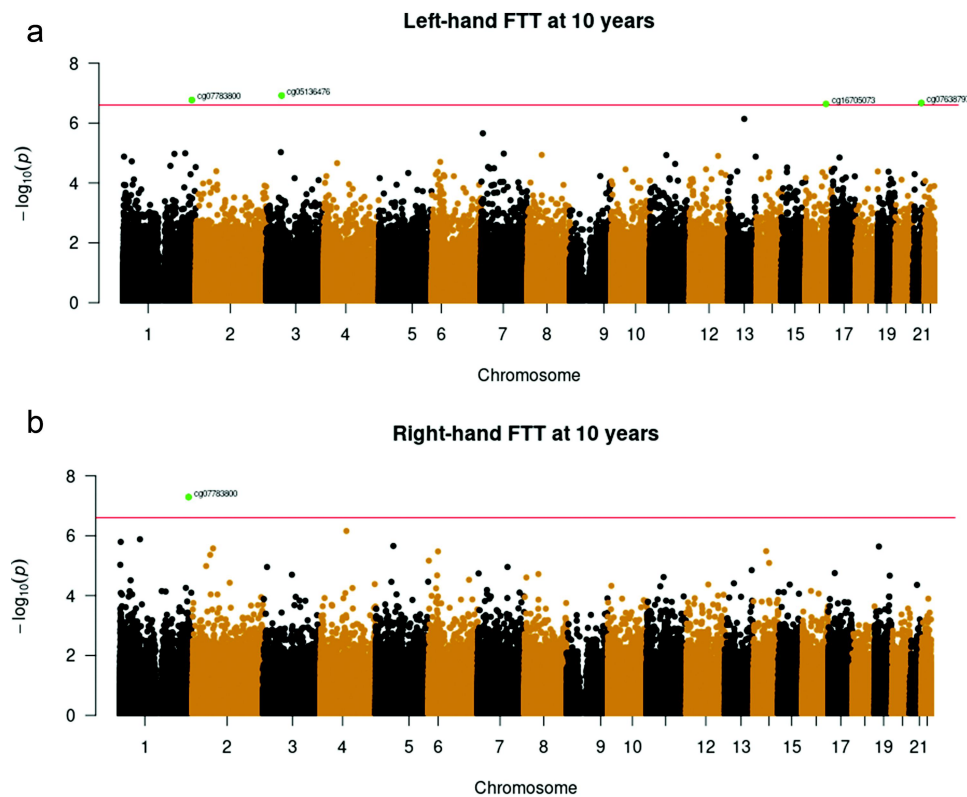
Figure 1. Manhattan plots (Fig.1a. Left-hand; Fig.1b. Right-hand). showing EWAS between cord blood DNA methylation and finger tapping test at 10 years (*N* = 925). The horizontal line indicates a FDR threshold of q < 0.05. Top CpG sites are highlighted in green.

Follow-up analyses

Blood–brain concordance

Using two independent tools, we found modest and inconsistent DNAm patterns across blood versus different brain regions for the four identified CpG sites (**Table S2**). For example, cg07783800 showed a positive correlation between blood and entorhinal cortex ($r = 0.30$) from the blood–brain DNAm comparison tool, but negative correlations across blood versus parietal cortex and inferior temporal gyrus (r = -0.39) from BECon. Despite partial overlap between frontal cortex and anterior prefrontal cortex across tools, cg05136476 showed a positive correlation between blood and frontal cortex ($r = 0.11$), but a negative correlation between blood and anterior prefrontal cortex ($r = −0.23$). These findings suggest that DNAm patterns on FTT-associated CpGs vary across tissues (blood vs. brain tissue) and across specific brain regions.

Gene expression profile across tissues

Next, we assessed gene expression levels across 53 tissues including blood and several brain regions from public GTEx data (**Figure S2**). The *GNG4* gene showed higher expression in the ovary, pituitary gland, and brain, but with low specificity in brain regions. *PRKCD* and *DYRK1A* were highly expressed across different tissues, with lower but consistent expression in brain-related tissues.

Methylation quantitative trait loci (mQTL)

Of the four significant CpGs identified at birth, three (cg05136476 in *PRKCD*, cg16705073 in *ST3GAL2*, cg07638797 in the promoter of *DYRK1A*) were all associated with several known mQTLs (**Table** S3). Only cg07783800 (*GNG4*) was unrelated to known mQTLs. This was further supported by a heritability tool based on data from monozygotic and dizygotic twins [58], showing low additive genetic and low shared environmental influences on DNAm levels at this site ($r = 0.04$ and 0.10, respectively), but high nonshared environmental influences ($r = 0.86$).

Associations between FTT and cognitive functions

We found that slower FTT performance for both right- and left-hand at age 10 years were associated with the lower total score of cognitive function in adolescence (right-hand, $\beta = .20$, $p < .001$); left-hand, $\beta = .20$, $p < .001$). Slower performance in right-hand FTT was associated with less-optimal functioning in all cognitive subdomains except for verbal comprehension, while lower performance in left-hand FFT was associated with poorer processing speed only (**Table S4**).

Mediation analysis

We examined the potential mediation (indirect effect) and found that the association between lower methylation at the cg07783800 site and lower cognitive functioning was mediated by less optimal fine motor development; the indirect effect was significant for the right ($\beta = 0.027$, 95% CI: 0.01, 0.05, $p = 0.009$) and for the left hand ($\beta = 0.019$, 95% CI: 0.01, 0.03, $p = 0.007$). We did not observe a significant direct effect of cg07783800 on cognition.

Candidate gene follow-up analysis

In candidate gene follow-up analysis, none of the CpGs annotated to *SPTBN4* reached gene-level significance after FDR correction (**Table S5**).

Replication analyses in INMA

We sought to replicate the four top CpG sites identified in GenR in an independent birth cohort. Lower methylation at the Cg07783800 site (see Figure 2) was associated with a lower FTT score in both the left- and right-hand in GenR ($\beta_{left} = 0.16$, $SE_{left} = 0.03$, $p_{left} = 1.70E–07$; $\beta_{right} = 0.17$, $SE_{right} = 0.03$, $p_{right} = 5.11E–08$) and was significantly associated with right-hand FTT in the same direction in INMA ($\beta = 0.12$, $SE = 0.05$, $p = 0.023$). This means that a 1 SD decrease in methylation at cg07783800 was associated with a 0.17 SD lower right-hand FTT score in the discovery cohort and a 0.12 SD lower FTT score in the replication cohort. No associations were replicated with left-hand FTT in INMA (**Table S6**).

Discussion

This is the first study to characterize epigenome-wide associations between DNA methylation at birth and fine motor ability in childhood. We highlight three key findings. First, we identified 4 CpG
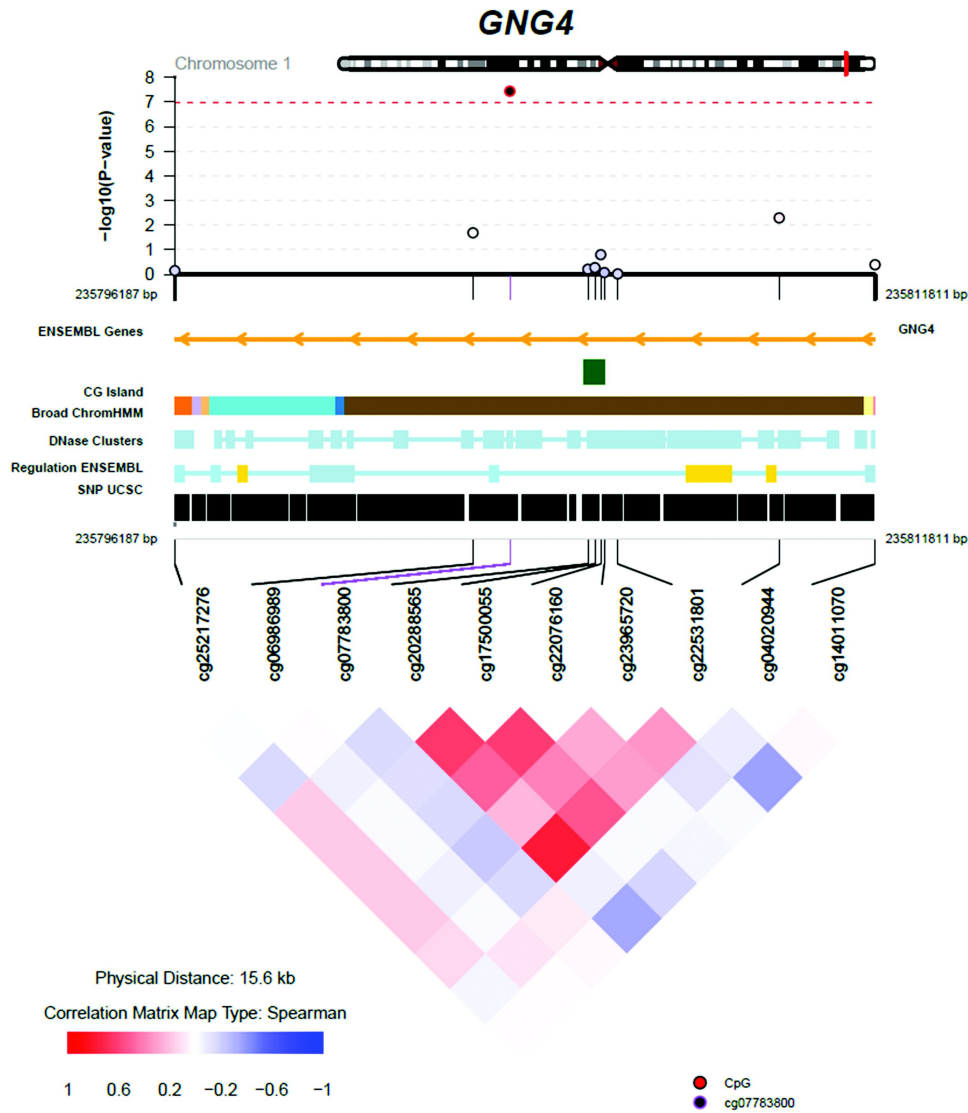
Figure 2. Regional Association Plot for the Top DNA Methylation (CpG) Site cg07783800.

Note. On the top graph, the x-axis depicts the position in base pair (bp) (hg19) for the region of *GNG4*. The y-axis indicates the strength of association from EWAS with FTT. Each circle represents a CpG site. Red dashed line within the graph indicates the genome-wide significance threshold. The regulatory information and correlation matrix of other CpG sites in the region with the top hit are shown below the x-axis. Color intensity marks the strength of the correlation and color indicates the direction of the correlation. The figure was made using the R package coMET.

sites in cord blood that were prospectively associated with a child's fine motor ability, as measured by the Finger Tapping Test (FTT), after genome-wide correction in a large population-based birth cohort. These FTT-associated CpGs map to genes that have been implicated in cognitive functions, neurodevelopmental and neurodegenerative diseases. Second, one CpG annotated to the *GNG4* gene, cg07783800, was replicated in an independent birth cohort, suggesting it might be a valid early epigenetic marker of fine motor ability. Our findings highlight the importance of employing multi-

cohort approaches to replicate epigenetic associations and reduce the risk of false positive discoveries [59]. Third, we found an association between slower FTT and worse cognitive function measured at 14 years of age, supporting the functional relevance of this neuropsychological measure of fine motor control to higher-order cognitive function in the general paediatric population. Furthermore, slower FTT performance in childhood mediated the association between lower DNAm at birth of our replicated CpG site and delayed cognition in adolescence.

Our EWAS at birth identified four CpGs after genome-wide correction. For the top site, cg07783800, we observed that the lower DNAm in this site was associated with less optimal fine motor performance on both hands, which was supported by the replication for right hand results in an independent cohort. This CpG is annotated to the body of the *GNG4* gene, a modulator and transducer of several transmembrane signalling systems that play a role in haemostasis and glucagon response. It has been suggested that its expression variability *in utero* influences cognitive trajectories in ageing [52] *GNG4* is mostly expressed in the cerebellum, hypothalamus, hypophysis (pituitary gland), amygdala, basal ganglia, and cortex. In addition, *GNG4* is expressed in the ovaries and may play a role in the hypothalamus-pituitary-ovarian axis. *GNG4* expression changes over time, with the highest expression during foetal development, decreasing with ageing [52]. Interestingly, cg07783800 was unrelated to known mQTLs, and as such unlikely to be under strong genetic control – a finding further supported by twin data: variation in this site was mainly explained by non-shared environmental influences. Future studies are needed to elucidate whether DNAm at this site reflects and potentially propagates the influence of prenatal environmental exposures on fine motor development.

The other three top CpGs identified in GenR did not replicate in INMA. We thus only briefly mention them here. The CpG cg05136476 is located in the *PRKCD* gene, which regulates the processing of the amyloid precursor protein (APP), a central element in Alzheimer's disease pathophysiology; however, *PRKCD* has also been implicated in ASD [60]. Cg07638797 is annotated to the promotor of *DYRK1A*, a gene localized in the Down syndrome critical region of chromosome 21. This gene plays a critical function in the central nervous system during development and ageing. It controls the differentiation of prenatally formed neurons and has effects on synaptic plasticity. Cg16705073 is annotated to *ST3GAL2* gene products sialyltransferase, which are largely responsible for ganglioside terminal α2–3 sialylation in the brain, synthesizing the major brain gangliosides GD1a and GT1b [61]. Taken altogether, all genes to which the four CpG sites were mapped are expressed in the brain and have been linked to neurodevelopmental and neurodegenerative diseases, including autism and Down syndrome. Impaired neuromotor development is implicated in all of these neurodevelopmental disorders.

We observed a relationship between fine motor skills in childhood and cognitive performance in adolescence. This converges with previous epidemiological studies as well as other lines of evidence, for example neuroimaging studies, which have shown that maturation of the dorsolateral prefrontal cortex and cerebellum may underlie both motor experience and active exploration of the world shaping these cognitive functions [62]. In samples of typically developing children, neuromimaging studies have also shown that regions of the brain that underpin motor functioning are among the first to mature [63]. While difficult to tease apart the directionality of associations, several researchers have posited that fine motor abilities may precede the development of cognitive abilities, starting with Piaget's theory of developmental stages and recently supported by a longitudinal cross-lagged study [7,8]. It is possible that epigenetic mechanisms partly explain the association between less optimal fine development and cognitive functioning. In line with this hypothesis, we found that DNAm levels at birth of our replicated CpG (cg07783800) prospectively associated with cognition in adolescence through fine motor development. Specifically, slower FTT mediated the association between lower DNAm at birth and poorer cognitive functioning during adolescence.

Overall, these findings point to *GNG4* methylation as a promising candidate for future studies investigating the development of fine motor abilities. It will be important in future to test whether *GNG4* methylation may be causally related to motor and cognitive function, through for example the use of advanced causal inference approaches in humans (e.g., Mendelian randomization) as well as experimental models (e.g., epigenetic editing). Furthermore, it will be useful to examine whether information on *GNG4* methylation may add predictive value on top of known risk factors for poor motor development. Of note, the identified effect sizes were small, and

it is unlikely that information on single CpG sites will sufficiently improve predictive performance, in the same way that single genetic variants are insufficient to predict complex traits or disorders. However, the use of polyepigenetic scores (also referred to as methylation risk scores – akin to polygenic risk scores) may help in future to capturing broader DNAm patterns associated with motor ability and help improve predictive power.

Strengths and limitations

This study presents a number of strengths, including its prospective design, objective performance-based measures of child fine motor ability, and the replication in an independent cohort. However, several limitations should be noted. Since subjects included in the discovery phase were not representative of the whole cohort concerning maternal education and ethnicity, the generalizability of findings from the current study may be limited. However, we replicated the top CpG in an independent cohort (INMA), where the education of mothers is quite different than in GenR. While not impossible, reverse causality at this age is unlikely to explain our results, as hand fine motor ability only manifests at later stages of child development. We could not perform causal inference analysis as genome-wide association studies of motor development are unavailable. In addition, epigenetic mechanisms are tissue-specific, and the functional relevance of blood-based DNAm for brain-based phenotypes, such as motor ability, is unclear. As we do not have individual gene expression data in Generation R, we utilized publicly available tools to explore the expression of the annotated genes in other relevant tissues (e.g., the brain). The sample sizes of these tissue databases are often small, limited to adults, and available post-mortem tissue; therefore, caution is warranted when interpreting these results.

Regarding the blood–brain correlations, it is important to note that although currently available blood–brain tools are helpful in exploring cross-tissue concordance, they have several caveats that limit their interpretation, including the use of small samples with mixed clinical presentation and incomplete phenotyping, limited data on relevant brain regions, and the reliance on mainly adult post-mortem samples, which may not generalize to neurodevelopmental processes such as fine motor ability and cognition in childhood. We also note that peripheral DNAm patterns may still be useful and valid markers even in the absence of significant cross-tissue concordance. To illustrate, similarly to our study, DNAm at the cg05575921 site in blood (annotated to the AHRR) – a strong, widely replicated biomarker of smoking exposure which is associated with poor cognitive function and structural brain integrity in adults [64] – shows null-to-weak positive associations with DNAm in the brain using the blood–brain comparison tool, whereas it shows negative correlations with the brain regions tagged by the BECon tool. Finally, this study focused on DNA methylation, which is one of several types of epigenetic mechanisms (e.g., histone or RNA modifications), which may contribute to motor development and the pathogenesis of motor diseases [65].

Conclusions

To our knowledge, we report the first EWAS of fine motor ability. At four CpGs, lower DNAm at birth was prospectively associated with poorer fine motor ability in childhood, as indexed by slower performance on the finger tapping test. All genes corresponding to the identified CpGs have been involved in brain pathways regulating neurodevelopment and neurodegeneration. One site annotated to the GNG4 gene, cg07783800, showed consistent results for both hands in the Generation R study and was replicated for the right hand in the INMA study, showing potential as an epigenetic marker of fine motor development. Furthermore, lower DNAm at cg07783800 was associated with poor cognitive functioning through non-optimal fine motor development. Our findings highlight the importance of employing multi-cohort approaches to replicate epigenetic associations and reduce the risk of false positive discoveries. The results from this study contribute to a better understanding of epigenetic factors associated with fine motor development in childhood and later cognitive function.

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Disclosure statement

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