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Mediation role of DNA methylation in association between handgrip strength and cognitive function in monozygotic twins

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Handgrip strength is a crucial indicator to monitor the change of cognitive function over time, but its mechanism still needs to be further explored. We sampled 59 monozygotic twin pairs to explore the potential mediating effect of DNA methylation (DNAm) on the association between handgrip strength and cognitive function. The initial step was the implementation of an epigenome-wide association analysis (EWAS) in the study participants, with the aim of identifying DNAm variations that are associated with handgrip strength. Following that, we conducted an assessment of the mediated effect of DNAm by the use of mediation analysis. In order to do an ontology enrichment study for CpGs, the GREAT program was used. There was a significant positive association between handgrip strength and cognitive function ($\beta = 0.194$, $P < 0.001$). The association between handgrip strength and DNAm of 124 CpGs was found to be statistically significant at a significance level of $P < 1 \times 10^{-4}$. Fifteen differentially methylated regions (DMRs) related to handgrip strength were found in genes such as *SNTG2*, *KLB*, *CDH11*, and *PANX2*. Of the 124 CpGs, 4 within *KRBA1*, and *TRAK1* mediated the association between handgrip strength and cognitive function: each 1 kg increase in handgrip strength was associated with a potential decrease of 0.050 points in cognitive function scores, mediated by modifications in DNAm. The parallel mediating effect of these 4 CpGs was −0.081. The presence of DNAm variation associated with handgrip strength may play a mediated role in the association between handgrip strength and cognitive function.

INTRODUCTION

Cognitive decline, which significantly impacts daily functioning and independence, is prevalent during the aging process [1]. It arises as a result of age-related neurodegenerative processes and can potentially progress to dementia [2]. With the advent of population aging, the incidence rates of mild cognitive impairment (MCI) and dementia are steadily increasing [3]. This poses substantial challenges for healthcare systems, society, and families alike. Cognitive decline is influenced by intricate interplays among environmental, disease-related, and genetic factors, potentially mediated by epigenetic modifications. Despite considerable advances in the field of genetics [4–7], the specific pathogenesis underlying cognitive decline remains to be fully elucidated.

Handgrip strength, as a crucial indicator for monitoring changes in cognitive function over time [8], has been widely utilized due to its non-invasive, convenient, and well-received attributes [9, 10]. A recent study has indicated that weaker handgrip strength was a significant risk factor for a late-life-dementia events in community-dwelling older women [11]. Several population-based prospective cohort studies have found that lower handgrip strength was associated with cognitive decline, regardless of gender, in both males and females [12–14]. Furthermore, recent literature reviews have robustly confirmed the association between handgrip strength and cognitive decline [8, 15]. Handgrip strength has the potential to influence cognitive function by affecting oxidative stress and inflammatory pathways [16]. Moreover, the association between handgrip strength and cognitive function might stem from common pathologies associated with hormone levels and inflammatory biomarkers [17]. As a result, handgrip strength might be associated with cognitive decline through these or related processes.

In contemporary research, there is an extensive exploration of the involvement of epigenetic modifications, particularly DNA methylation (DNAm) variations, in the underlying mechanisms of complex traits and diseases. Ivana Alece Arantes Moreno et al. discovered that the DNAm level of *BDNF* promoters was associated with episodic memory deficits [18]. Di Francesco et al. observed a general elevation in DNAm levels in peripheral blood mononuclear cells from individuals with late-onset Alzheimer's disease, and this increase was found to be associated with inferior cognitive function [19]. In recent years, the association between epigenetic modifications and handgrip strength has garnered attention from researchers. Mark D. Peterson et al. demonstrated that handgrip strength is inversely associated with DNAm age acceleration [20]. However, there has been no research exploring whether DNAm variations associated with handgrip strength mediate the relationship between handgrip strength and cognitive function.

Nowadays, the discordant monozygotic twin design has gained significant prominence as a robust and widely employed methodology in epigenome-wide association studies (EWASs) [21, 22], facilitating the control of individual genetic factors and

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enabling comprehensive investigations into the epigenetic landscape. In this study, we utilized a group of monozygotic twins to examine the possible mediating role of DNAm in the association between handgrip strength and cognitive function using a combination of causal inference testing and mediation analysis.

MATERIALS AND METHODS
Participants
We conducted the present study using data from the Qingdao Twins Registry System. A detailed description of participant recruitment and data collection has been provided previously [23]. Participants who were pregnant or lactating females, those with major diseases, those who were unable to finish the assessment, professional athletes, and monozygotic twins were excluded from the study. Finally, a total of 59 pairs of monozygotic twins were included in this study. Prior to completing the survey questionnaire and health examination, all participants were required to fast for 10–12 h. Blood samples were collected post-fasting. The separated serum and plasma were immediately frozen at −80°C within 30 min to maintain their integrity. The Regional Ethics Committee of the Qingdao Centers for Disease Control and Prevention (CDC) Institutional Review Boards approved this study, and we followed the ethical principles of the Helsinki Declaration. In addition, we obtained prior written informed consent from all participants.

Measurement of handgrip strength
Trained investigators conducted physical examinations, which involved measuring handgrip strength using Nantong-manufactured WCS-100 handgrip dynamometers. Participants were instructed to apply maximum force while squeezing the handle. For each hand, three measurements were made, and the highest value was chosen for analysis.

Measurement of cognitive function
The Montreal Cognitive Assessment (MoCA, Chinese version), a highly reliable and widely accepted tool [24, 25], was used to measure cognitive function in Chinese adults. The MoCA test evaluates multiple cognitive domains, such as attention, naming, delayed recall, language, visuospatial/executive abilities, orientation, and abstraction, and yields a maximum total score of 30 points. To account for the influence of education on cognitive performance, education-adjusted scores were used [26]. A reduction in cognitive scores is indicative of a decline in cognitive capacity.

Reduced representation bisulfite sequencing (RRBS) analysis
In our study, genome-wide DNAm profiling in whole blood of twins was performed using RRBS, yielding 551,447 raw CpGs. RRBS analysis was performed on whole blood DNA by a Chinese biomarker technology company. Concisely, genomic DNA was digested with *Msp*I enzyme to generate CpG-rich short fragments, which were then selected and bisulfite-converted. To obtain methylation data, the bisulfite-converted DNA fragments were used for sequencing, and the resulting raw data were preprocessed, mapped with *Bismark* [27], and smoothed using the R-package *BiSeq* [28]. We established coverage control at the 90th percentile and omitted CpGs displaying methylation β-values < 0.01 or those with more than 10 missing observations. After these quality control, a total of 248,840 CpGs remained for subsequent analyses. To facilitate statistical analysis, we converted the methylation β-value to M-value.

To mitigate the risk of false discoveries that could result from varying methylation profiles of different cell types in whole blood samples [29], we employed the *ReFACTor* method. For our study, we controlled for the influence of cell-type composition on DNAm by included the top five components identified by the method as variables [30] (Table S1).

Statistical analysis
Mediation analyses. The current study used the Causal Inference Test (CIT) to assess the potential mediating role of DNAm in the association between handgrip strength and cognitive function [31]. Each CpG had four models fitted separately, including the following: In Model 1, we examined the association between handgrip strength and cognitive function. To implement the generalized estimating equation (GEE), we consulted the R-package geepack and its corresponding geeglm function. Here, cognitive function served as the outcome, while handgrip strength was the predictor. We took into account variables such as sex, age, cell-type composition, and twin pairing; In Model 2: we focused on the association between handgrip

strength and DNAm of each CpG through an EWAS. In this model, we used GEE, controlling for cognitive function. DNAm of each CpG was the outcome, and handgrip strength was the predictor. We again adjusted for sex, age, cell-type composition, twin pairing, and cognitive function. We explained intra-pair correlation by setting the ID=family_ID parameter in the model, and intra-cohort correlation was elucidated by setting the corstr = "exchangeable" parameter. The Manhattan plot was generated from the EWAS. To correct for multiple testing, we calculated false discovery rate (FDR) and defined FDR < 0.05 as genome-wide significance. For CpGs with FDR ≥ 0.05, we defined $P < 1 \times 10^{-6}$ as suggestive significance and $1 \times 10^{-6} \le P < 1 \times 10^{-5}$ as weaker-than-suggestive significance. The CpGs with $P < 1 \times 10^{-4}$ were reported as top CpGs of this EWAS.The CpGs that exhibited P values of association < 1×10^{-4} were chosen for further analysis; In Model 3: the association between the DNAm of each CpG and cognitive function was evaluated. This model employed GEE while controlling for handgrip strength. Adjustments were made for gender, age, cell-type composition, twin pairing, and handgrip strength. We selected CpGs with association P values < 0.05 as candidate mediators; In Model 4: we used the R-package mediation to analyze how the DNAm of each CpG mediated the association between handgrip strength and cognitive function [32]. Estimates and 95% confidence intervals (95% CIs) were determined after running 5000 simulations. The average causal mediation effect (ACME), average direct effect (ADE), total impact, and the absolute value of the ratio of mediated effects to direct effects were determined for each CpG. If the P value associated with the ACME for a specific CpG was < 0.05, it was considered that this CpG played a significant mediating role in the association between handgrip strength and cognitive function. Furthermore, for CpG mediators deemed significant, we conducted an assessment of the exposure-mediator interaction, with statistical significance set at P < 0.05. SPSS 22.0 and PROCESS v3.5 were used for parallel mediation analysis. Ultimately, we assessed the potential mediating role of handgrip strength on the association between the DNAm at each CpG and cognitive function.

Region-based analysis. The *comb-p* method was employed to identify differentially methylated regions (DMRs) associated with predictors for the CpGs (P < 0.05) discovered in Model 2 through EWAS [33–35]. DMRs with significant enrichment were identified using a Stouffer-Liptak-Kechris (*slk*) adjusted P value < 0.05.

Ontology enrichment analysis. We used the GREAT program to conduct an analysis of the functional ontology enrichments for CpGs from Model 2 that had association P values < 0.05, as well as the candidate CpG mediators that were found in Model 3 [36]. The detailed setup has been explained in previous articles [35, 37, 38]. The ontology enrichments that were deemed significant were evaluated based on a FDR of <0.05.

RESULTS
Characteristics of participants
The fundamental characteristics of the enrolled individuals are shown in Table 1. The sample comprised 59 pairs of twins, with 33 pairs consisting of males. The participants had a median age of 52 years (95% range: 41–66), a median handgrip strength of 33.26 kg (SD: 11.59), and a mean cognitive function score of 19.65 (SD: 5.30). Handgrip strength demonstrated a significant intra-pair correlation ($r = 0.860$, $P < 0.001$), whereas the intra-pair correlation within cognitive function score was not deemed statistically significant ($r = 0.252$, $P = 0.054$). In addition,the substantial proportion of the clinical indicators demonstrated statistically significant correlations within twin pairs, suggesting the advantageous implications of using a co-twin design.

Association of handgrip strength and cognitive function
Handgrip strength was positively associated with cognitive function in twins ($\beta = 0.194$, $P < 0.001$) in Model 1.

Association of handgrip strength and DNAm while controlling for cognitive function
Based on the visual representation of the Manhattan plot (Fig. 1) and the data presented in Table S2, it was evident that there existed a significant association between handgrip strength and DNAm of 124 CpGs, reaching a statistical significance level of

Table 1. Baseline characteristics of participants

Characteristics	Values	Intra-pair correlation	
		r	P value
Number of twin pairs	59		
Gender, pairs (%)			
Male	33.00 (55.93)	–	–
Female	26.00 (44.07)	–	–
Age, years, M (P2.5, P97.5)	52 (41,66)	–	–
Handgrip strength, kg, mean (SD)	33.26 (11.59)	0.860	<0.001
Cognitive function			
Cognitive function score, mean (SD)	19.65 (5.30)	0.252	0.054
Absolute Δ(Cognitive function score), M (P2.5, P97.5)	4.00 (1.00,16.00)	–	–
Educational level, n (%)			
<high school	38 (32.20)	–	–
high school	53 (44.92)	–	–
>high school	27 (22.88)	–	–
BMI, kg/m2, mean (SD)	24.87 (3.62)	0.665	<0.001
SBP, mmHg, mean (SD)	135.31 (22.09)	0.380	<0.01
DBP, mmHg, M (P2.5, P97.5)	82.00 (62.00,106.20)	0.207	0.115
GLU, mmol/L, M (P2.5, P97.5)	5.50 (3.80,10.73)	0.498	<0.001
CHOL, mmol/L, mean (SD)	4.93 (1.21)	0.542	<0.001
TG, mmol/L, M (P2.5, P97.5)	1.19 (0.20,5.66)	0.584	<0.001
HDLC, mmol/L, M (P2.5, P97.5)	1.32 (0.67,2.71)	0.743	<0.001
LDLC, mmol/L, mean (SD)	2.86 (0.91)	0.501	<0.001

Continuous variables were presented as mean (standard deviation (SD)) or median (P2.5, P97.5); Categorical variables were presented as numbers with percentages

Cognitive function score was corrected by education level

BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, GLU fasting glucose, CHOL total cholesterol, TG triglyceride, HDLC high-density lipoprotein cholesterol, LDLC low-density lipoprotein cholesterol

$P < 1 \times 10^{-4}$ in Model 2. There was an negative association between handgrip strength and the DNAm of 85 CpGs. The majority of these 85 CpGs were located at/near EBF3 ($n = 8$) ($\beta = -0.02$), WNK2 ($n = 7$) ($\beta = -0.03$), SNTG2 ($n = 7$) ($\beta = -0.03$), TYW1B ($n = 5$) ($\beta = -0.24$), CCDC144A ($n = 4$) ($\beta = -0.18$), NEUROG3 ($n = 4$) ($\beta = -0.03$), PLEKHA2 ($n = 4$) ($\beta = -0.03$), RXRA ($n = 4$) ($\beta = -0.47$), FOXA1/ TTC6 ($n = 3$) ($\beta = -0.03$), and FOXA2 ($n = 3$) ($\beta = -0.03$). The DNAm of 39 CpGs was positively associated with handgrip strength, with the majority of CpGs located at/near FBLN1 ($n = 7$) ($\beta = 0.04$), CELF2 ($n = 4$) ($\beta = 0.07$), GPR157 ($n = 3$) ($\beta = 0.05$), INPP5A ($n = 3$) ($\beta = 0.03$), KRBA1 ($n = 3$) ($\beta = 0.04$), and MFSD6 ($n = 3$) ($\beta = 0.26$).

Table 2 listed 15 DMRs that may be associated with handgrip strength. The pattern of differential methylation was depicted in Fig. 2. The association between handgrip strength and methylation levels differed for 3 DMRs (DMR-C, G, L) compared to the majority of DMRs (DMR-A, D, E-F, J-K, M-N), which exhibited hypermethylation with increasing handgrip strength (Fig. 2).

However, the precise associations between 4 DMRs (DMR-B, H, I, O) and handgrip strength remained unclear. Notably, 7 DMRs falling into FBLN1, EBF3, WNK2, KRBA1, TIMP2, SNTG2, and NEUROG3 also covered the top CpGs listed in Table S2.

For the identified CpGs (P value < 0.05), numerous GREAT ontology enrichments potentially associated with handgrip strength were discovered. These enrichments included p53 pathway by glucose deprivation, ATP synthesis, genes related to the regulation of the actin cytoskeleton, and insulin-mediated glucose transport, etc (Fig. S1).

Association of DNAm with cognitive function while controlling for handgrip strength
In Model 3, there was a significant association ($P < 0.05$) between the DNAm at 13 CpGs and cognitive function (Table S3). These candidate CpG mediators mapped to KCNN3, KRBA1, ST8SIA5, FOXA2, ULK4, SNCB, ALDH1B1, CNTNAP2, and FOXA1, and were mainly involved in dopaminergic neuron differentiation, response to interleukin-6, cell differentiation in hindbrain, and FOXA family transcription factor networks, etc (Fig. S2).
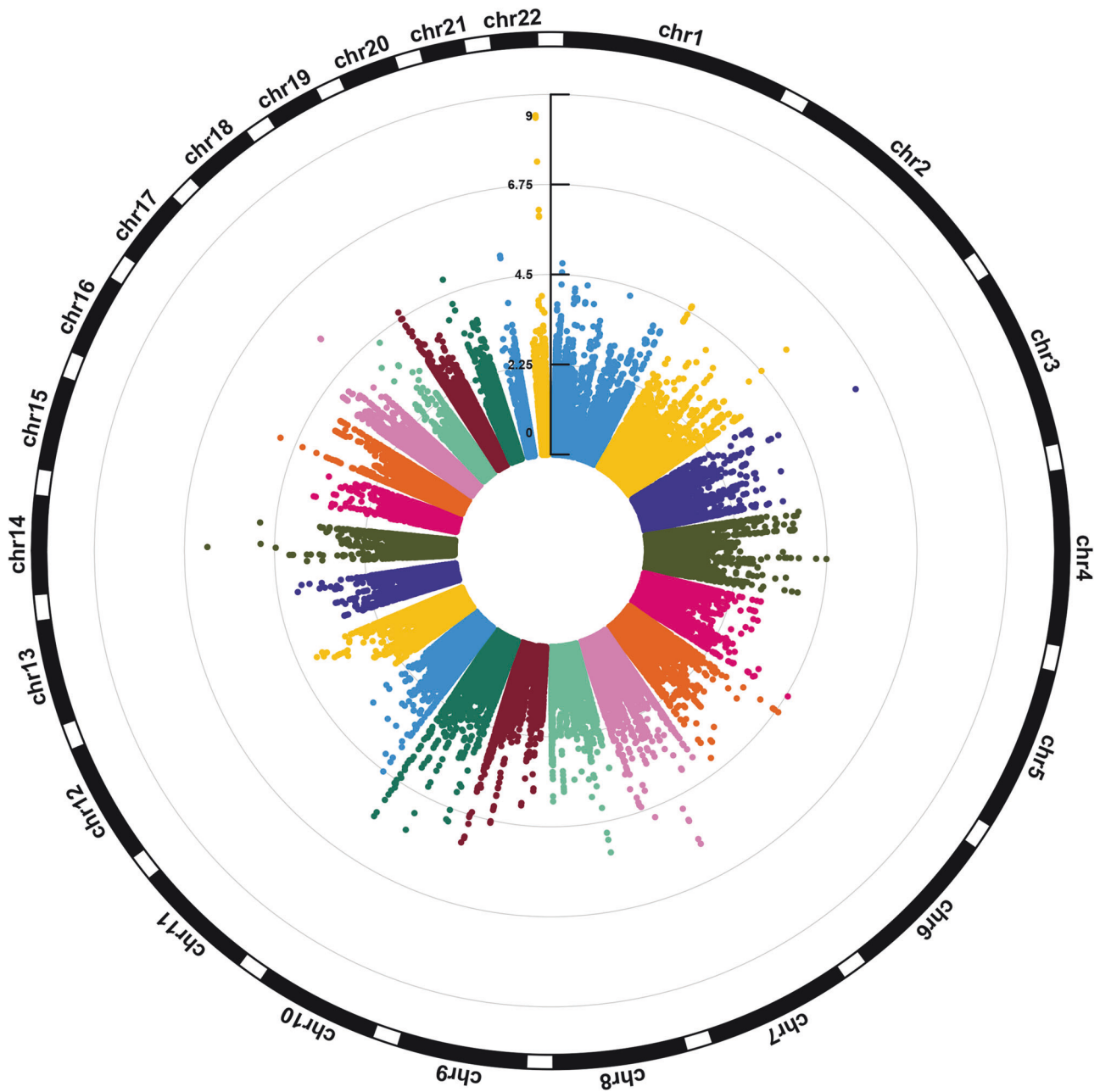
Mediation analysis
In Model 4 (Table 3), we discovered that 3 CpGs mapped to KRBA1 and 1 CpG mapped to TRAK1 could mediate the association between handgrip strength and cognitive function (P value of ACME < 0.05). Nearly all the DNAm of these CpGs was with an ADE of 0.254 and an ACME of −0.050. This indicated that, after averaging the ADE across 4 CpGs and controlling for methylation at these CpGs, a positive association was observed between handgrip strength and cognitive function. In other words, for each 1 kg increase in handgrip strength, there was an associated increase of 0.254 points in cognitive function scores. Similarly, the ACME for these 4 CpGs indicated that the methylation at these CpGs mediated the association between handgrip strength and cognitive function. On average, each 1 kg increase in handgrip strength was associated with a potential decrease of 0.050 points in cognitive function scores, mediated by modifications in DNAm. The results of parallel mediation analysis showed that ADE of these 4 CpGs was 0.285 and ACME was −0.081. It was noteworthy that we observed handgrip strength exerted a positive direct effect on cognitive function, and the DNAm variation at these CpGs negatively mediated the effect of handgrip strength on cognitive function. In other words, the mediating role of DNAm at these CpGs attenuated the positive impact of handgrip strength on cognitive function. Nevertheless, overall, there still exists a significant positive correlation between handgrip strength and cognitive function. In addition, we explored whether handgrip strength mediates the association between the four CpGs mentioned above and cognitive function. However, we found no statistically significant results.

DISCUSSION
Current research widely recognizes low handgrip strength as a risk factor for cognitive function decline. Using a sample of monozygotic twins, we identified four CpGs that mediate the association between handgrip strength and cognitive function. Importantly, our study emphasizes the significance of considering exposure and mediator interactions in epigenetic research.

In EWAS of handgrip strength, genes such as SNTG2, KLB, CDH11, and PANX2, where DMRs were located, could potentially exert substantial effects on handgrip strength. SNTG2 belonged to the syntrophin family and exhibited high expression in the musculoskeletal system [39]. So the suppression of SNTG2 expression might have had an impact on handgrip strength. The downregulation of the KLB gene was associated with skeletal muscle function [40]. CDH11 was known to play a pivotal role in inflammation-related conditions [41], including rheumatoid arthritis, thereby potentially influencing handgrip strength.

Fig. 1 Circular Manhattan plot for epigenome-wide association analysis of handgrip strength. The numbers of chromosome and the -log10 of *P* values for statistical significance are shown. The dots represent the observed CpGs

The functional role of the remaining genes in relation to handgrip strength is currently unknown.

In the process of handgrip strength influencing cognitive function, we observed that the 4 CpG mediators identified were annotated near 2 important genes, namely *KRBA1* and *TRAK1*. In addition, we utilized a causal inference methodology to demonstrate that handgrip strength can induce alterations in the methylation levels of *KRBA1* and *TRAK1*. Three CpG mediators were annotated near *KRBA1*. John Alexander et al. found that *KRBA1* possessed novel stop-gain mutations exclusively in individuals affected by familial early-onset dementia, as compared to those unaffected by the disease [42]. *KRBA1* interacted with glycogen synthase kinase-3beta [42], which mediated neuroinflammation associated with Alzheimer's disease [43]. Therefore, handgrip strength led to alterations in the methylation levels of *KRBA1*, which could subsequently have impacted cognitive

function through an inflammatory pathway. One CpG mediator was annotated near *TRAK1*. It was predicted that *TRAK1* enabled γ-aminobutyric acid receptor binding activity and myosin binding activity and was associated with severe neurodevelopmental disorders [44]. We hypothesized that the elevated methylation levels of *TRAK1* induced by low handgrip strength affected γ-aminobutyric acid receptor binding activity, subsequently inhibiting acetylcholine synthesis, and ultimately influencing cognitive function.

We found that handgrip strength exerted a positive direct effect on cognitive function, and the DNAm variation negatively mediated the effect of handgrip strength on cognitive function. Several studies refer to the situation where the direction of the indirect effect is opposite to that of the direct effect as a "suppressing effect" [45–48]. In other words, the mediating effect of DNAm variation attenuated the positive impact of handgrip

Table 2. The results of annotation to significant differentially methylated regions for handgrip strength

DMR	Chromosome	Start (bp)	End (bp)	Length	*slk* corrected *P* value	Gene symbol
DMR-A	chr22	45,948,412	45,948,733	13	5.37E-10	*FBLN1*
DMR-B	chr10	131,770,987	131,771,418	36	3.75E-04	*EBF3*[a]
DMR-C	chr9	95,947,588	95,947,708	16	2.05E-03	*WNK2*
DMR-D	chr7	149,389,727	149,389,874	16	2.95E-03	*KRBA1*[a]
DMR-E	chr20	23,636,303	23,636,792	20	5.79E-03	*CST3*[a]
DMR-F	chr17	76,880,072	76,880,441	16	6.68E-03	*TIMP2*
DMR-G	chr1	244,894,212	244,894,338	12	1.65E-02	*DESI2*[a]
DMR-H	chr6	170,238,404	170,238,669	12	3.04E-02	*LINC00574*[a]
DMR-I	chr2	1,291,214	1,291,472	14	3.31E-02	*SNTG2*
DMR-J	chr8	117,962,019	117,962,182	18	3.60E-02	*SLC30A8*[a]
DMR-K	chr17	20,687,603	20,687,749	12	3.63E-02	*CCDC144NL*[a]*(pseudo)*
DMR-L	chr10	71,331,685	71,331,977	14	4.14E-02	*NEUROG3*
DMR-M	chr4	39,448,528	39,448,977	39	4.22E-02	*KLB*
DMR-N	chr16	63,651,261	63,651,606	23	4.66E-02	*CDH11*[a]
DMR-O	chr22	50,616,620	50,618,057	72	4.85E-02	*PANX2*

[a]Not within the gene; therefore, annotated distance to nearest gene
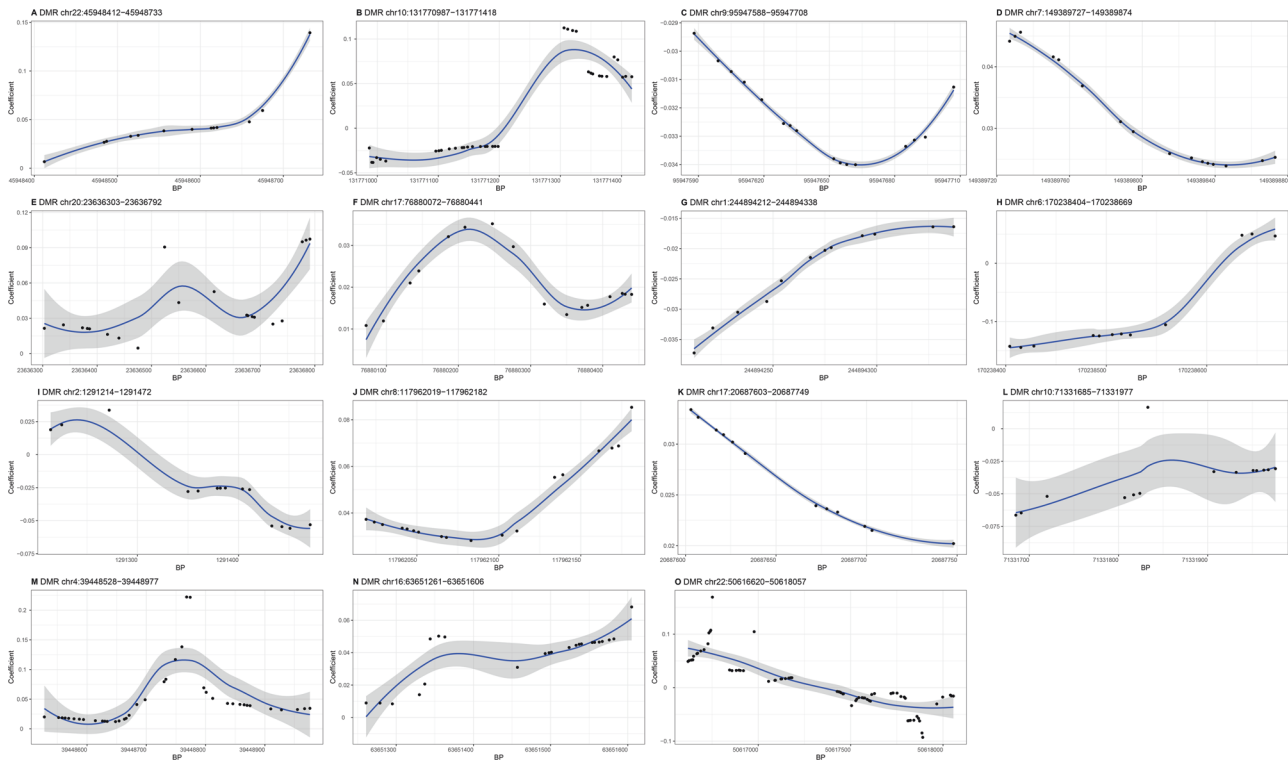
Fig. 2 Differential methylation patterns of the identified differentially methylated regions for handgrip strength. The dots represent the CpGs. The x-axis shows the position of CpGs on chromosome and the y-axis shows regression coefficients. BP, base pair; DMR, differentially methylated regions

strength on cognitive function. Recently, several studies on DNAm variation have also demonstrated suppressing effects [49–51]. Given the complexity of DNAm in regulating gene expression, maintaining genomic stability, responding to environmental and lifestyle factors, and influencing the onset and progression of diseases, we consider this situation to be reasonable.

Our study has several strengths based on our findings. First and foremost, the utilization of a traits-discordant monozygotic twin design in our study has been recognized as a robust approach for detecting and understanding the epigenetic variations associated with complex diseases [22]. Secondly, to the best of our knowledge, this represents the first exploration of the mediating role of DNAm in the association between handgrip strength and cognitive function. Furthermore, considering the diverse genetic constitutions, environmental exposures, and multiple lifestyles across different ethnic populations worldwide, our findings will be particularly valuable in elucidating the underlying mechanisms of cognitive function decline in the Chinese population.

There are a number of limitations that should be acknowledged in our research. First, the limited sample size was attributed to the

Table 3. DNA methylation mediators for the association of handgrip strength and cognitive function

CpGs annotation			Average direct effects			Average mediated effects			The absolute value of the ratio of mediated effects to direct effects
Chromosome	Position (bp)	Official symbol	β	95% CI	P value	β	95% CI	P value	
chr7	149,389,751	KRBA1[a]	0.2586	0.1137–0.4059	0.001	−0.0547	−0.1109 to 0.0037	0.030	21.15%
chr7	149,389,754	KRBA1[a]	0.2606	0.1131–0.4079	0.001	−0.0567	−0.1151 to 0.0047	0.022	21.76%
chr7	149,389,767	KRBA1[a]	0.2591	0.1192–0.4056	0.002	−0.0552	−0.1181 to 0.0075	0.026	21.30%
chr3	42,033,867	TRAK1[a]	0.2358	0.0979–0.3772	0.002	−0.0319	−0.0811 to 0.0001	0.046	13.53%
Parallel mediation effect			0.2854	0.1372–0.4337	<0.001	−0.0815	−0.1572 to 0.0203	–	28.56%

[a]Not within the gene; therefore, annotated distance to nearest gene

challenges encountered in recruiting and identifying eligible pairs of twins. Nevertheless, the use of the trait-discordant twin design in our study demonstrated superior statistical power in comparison to traditional cross-sectional or case-control designs. For handgrip strength and cognitive function, which exhibit a moderate level of heritability, their heritabilities were approximately 0.58 [52] and 0.44 [53], respectively. Consequently, the design of our study allowed for a reduction in the required sample size. The ratio of the minimum sample size between twin designs and conventional case-control designs was approximately 0.4, suggesting that the 118 participants in our study were equivalent to 295 participants in a typical case-control study. Second, although we adjusted for some confounding factors in the mediation analysis, the outcomes may still be influenced by residual confounding. And the possibility of a reverse causal association cannot be entirely ruled out. Third, although we have identified certain DNAm sites, our understanding of their functional implications remains incomplete. Further research is necessary to elucidate the underlying mechanisms involved. Finally, the rate of decline in handgrip strength and cognitive function were not only associated with age span but also with gender [54, 55]. Although we adjusted for age and gender in our study, we were still unable to eliminate the effects they exert. Therefore, future research with larger sample sizes is needed to explore potential gender differences.

In summary, we discovered specific DNAm sites that mediate the association between handgrip strength and cognitive function. Our findings provide important clues for further elucidating the underlying mechanisms of cognitive function decline.

DATA AVAILABILITY
Data available on request from the authors.

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COMPETING INTERESTS
The authors declare no competing interests.

ETHICS
This study followed the Helsinki Declaration and was approved by the Regional Ethics Committee of the Institutional Review Committee of Qingdao CDC.

ADDITIONAL INFORMATION