BRAIN
ORIGINAL ARTICLE

Smaller cingulate grey matter mediates the association between dual-task gait and incident dementia

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Individuals with mild cognitive impairment who have high dual-task gait cost (≥20% slowing in gait speed while performing a cognitive brain-demanding task) are 3-fold more likely to progress to dementia. However, the cortical regions that might explain this association are unknown, which might identify potentially treatable areas.

The aim of the present study was to investigate whether brain grey matter volume loss and motor cortex metabolite levels explain the association between dual-task cost and incident dementia in individuals with mild cognitive impairment.

We included participants with mild cognitive impairment from the Gait and Brain Study Cohort, who had a baseline MRI and were followed up for 9 years with cognitive and gait assessments every 6 months. Gait performance was investigated in four conditions: usual gait, counting backwards by ones, naming animals and subtracting serial sevens. Dual-task cost was calculated as the percentage change in gait speed in dual-task conditions relative to usual gait speed. Data were collected from July 2007 to June 2023.

From the 139 individuals with mild cognitive impairment included at baseline [mean (standard deviation) age, 73 (6) years; 62 (44%) female], 33 (24%) progressed to dementia. Baseline high dual-task cost (≥20%) during counting backwards by ones and naming animals conditions were associated with smaller grey matter volume in several brain structures. A higher ratio of choline to creatine in the primary motor cortex was associated with higher serial sevens dual-task cost. High dual-task cost while counting backwards by ones and naming animals was associated with a 3-fold risk of incident dementia ($P = 0.02$). Mediation analyses revealed that grey matter volume clusters localized in the right anterior and middle cingulate cortices mediated the association between counting backwards by ones dual-task cost and incident dementia (effect: 48%; $P = 0.045$) with no mediation observed in grey matter loss in other brain regions or through motor cortex metabolite levels.

Smaller grey matter volume of the right anterior and middle cingulate cortices explained the association between high dual-task cost and incident dementia in mild cognitive impairment. This result sheds light on the neural mechanisms of cognitive–motor interaction linked with cognitive decline and dementia in mild cognitive impairment and supports the use of gait under dual-tasking as a motor biomarker of dementia.

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Introduction

With the rapid ageing of the global population, dementia remains a major public health concern that has a profound impact on the quality of life of individuals and their families, while also imposing a high economic burden on society.[1] The risk of developing dementia is high in subjects with mild cognitive impairment (MCI), which is a transitional state between normal cognition and dementia, making this group a crucial population for study.[2] However, the MCI population is heterogeneous, with some remaining stable, whereas others progress to dementia.[2] Thus, the challenge is to identify those at highest risk of progression by identifying early clinical manifestations, including motor and cognitive impairments.[3,4] Therefore, the identification of simple, robust and cost-effective markers of higher progression risk is crucial for the early detection and monitoring of dementia.[5]

The dual-task gait test, which involves walking while performing a demanding cognitive task, has shown potential as a functional marker of dementia in individuals with MCI.[6] A high dual-task cost (DTC) of gait, which is the percent change in gait speed between dual-tasking and single-task walking, is associated with a 3.8 times higher risk of progression to dementia while counting backwards by ones, and a 2.4 times higher risk while naming animals.[6] The DTC is frequently linked to specific cognitive domains such as attention, executive functions and working memory.[7-9] This clinical biomarker offers a rapid and reliable measure that can provide a complementary quantitative estimation of the interaction between an individual's cognitive–motor abilities[10] and their global cognitive status.[11] Consequently, the DTC has the potential to contribute to the diagnostic framework for dementia, complementing the standard neuropsychological battery tests and neuropathological biomarkers.

Early dementia is associated with a variety of anatomical changes in specific areas of the brain, particularly in the temporal and frontal lobes,[12,13] where a range of neural insults can be found, including microvascular damage, chronic inflammation and degeneration.[14] These morphological changes can negatively impact both cognition and gait.[15-17] Despite extensive use of DTC in the literature and clinical guidelines that recommend its use,[18] only a few studies have explored the neural mechanisms of DTC in MCI. Our previous work demonstrated that a higher DTC of gait speed, in the subtracting serial sevens condition, was associated with a smaller grey matter volume in the primary motor cortex and a higher choline-to-creatine ratio in this same region measured by proton magnetic resonance spectroscopy.[19] A higher choline-to-creatine ratio might indicate increased membrane turnover and neuroinflammation. In the same cohort, lower grey matter volume in the left entorhinal cortex was associated with a high DTC in the conditions of serial subtraction by ones and sevens.[20] However, these studies were cross-sectional, which limited the establishment of causal inference, and consequently, DTC could not be tested as a potential biomarker of progression to dementia. Moreover, there is a lack of evidence that links the neural substrates of DTC and neurodegenerative processes in people who convert to dementia.[5] Mediation models are developed to facilitate comprehension of the association between DTC and dementia by decomposing the effects and relationships between variables. Therefore, it is crucial to investigate the previously established relationship between high DTC and incident dementia and to elucidate whether grey matter volume loss and altered motor cortex metabolite levels explain these relationships.

The aim of the present study was to investigate the neural basis for the association between poor dual-task performance and incident dementia in MCI. Our main hypothesis was that the association between high DTC and incident dementia would be mediated by reduced cortical grey matter volumes and altered motor cortex metabolite levels.

Materials and methods

Population

Participants with MCI were recruited for the Gait and Brain Study (NCT03020381), at the Aging Brain and Memory Clinic, Parkwood Institute, University of Western Ontario.[6,19,20] Ethics approval was obtained from the local research Ethics Board, and all participants provided written informed consent. Participants were community-dwelling adults free of dementia, aged 65 years and

older, and able to walk 10 m independently without a gait aid at baseline. To be included in the analysis for this study, participants were required to have at least two cognitive assessments; the first was during a baseline visit, and the second was from their last recorded assessment before June 2023. The time between assessments varied because the participants were seen every 6 months. All participants met the Clinical Dementia Rating (CDR)[21] scale score of 0.5 and the Petersen MCI criteria[22] that include: (i) subjective cognitive complaints[23]; (ii) objective cognitive impairment in at least one of the following cognitive domains: memory, executive function, attention and language[4]; (iii) preserved activities of daily living confirmed by a clinician's interviews[24]; and (iv) absence of dementia using criteria from the Diagnostic and Statistical Manual of Mental Disorders[25] (Fourth Edition). Exclusion criteria included inability to understand English, parkinsonism or any neurological disorder with residual deficits, musculoskeletal disorder affecting gait performance, use of psychotropic medications, severe pain affecting mobility, and uncontrolled depressive symptoms. Data collection took place between July 2007 and June 2023.

Medical and cognitive assessment

During each face-to-face visit for the study, a comprehensive participant assessment was performed that included: a medical examination, sociodemographic information, comorbidities or changes in existing conditions, activities of daily living, and medication usage. Global cognition was assessed using a comprehensive neuropsychological battery that was described in detail previously.[6]

Gait assessment

Gait speed was measured using an electronic walkway system with an embedded pressure sensor (Zeno® walkway System, length 600 cm × width 64 cm × height 0.5 cm, and ProtoKinetics Movement Analysis Software). Each participant completed four trials in the following sequence: one for walking at their usual gait speed three times, one trial for counting backwards from 100 by ones, one trial for walking while naming animals, and one trial for walking while subtracting sevens starting from 100. The order of the single and dual-task gait trials was always the same across all participants. There was no instruction to prioritize either the gait or cognitive task during the dual-task trials. To avoid acceleration and deceleration effects, participants began walking 1 m before reaching the electronic walkway and continued walking 1 m beyond the walkway. The reliability of this gait assessment protocol has been established previously at our laboratory.[10]

MRI and spectroscopy data acquisition

Participants underwent an MRI scan at the Centre for Functional and Metabolic Mapping, Robarts Research Institute, Western University, within 1 week of their baseline visit for medical and behavioural assessments. A brain MRI protocol was acquired on a 3 T Siemens Tim Trio scanner, using a 32-channel head coil. Each scan included the acquisition of sagittal 3D T_1-weighted inversion-prepared rapid acquisition with gradient echo (MP-RAGE) anatomical images (repetition time/echo time = 2300/2.9 ms, inversion time = 900 ms, flip angle = 9°, averages = 1, field of view = 256 mm × 240 mm × 192 mm, matrix = 256 × 240 × 160) covering the entire brain. A single voxel (20 mm isotropic) ^{1}H magnetic resonance spectrum (echo time = 135 ms) was acquired from the leg and foot region of the right motor cortex (M1) positioned using the anatomical images (Fig. 1). The details of the spectroscopy acquisition protocol have been described previously.[26,27]

Structural MRI processing

The CAT12 toolbox (Computational Anatomy Toolbox 12, version CAT12.8.2) was used as an extension to SPM12 (Statistical Parametric Mapping, Institute of Neurology, London, UK, version 7771) for image processing. The method involved automated segmentation of each anatomical T_1-weighted image into three distinct tissue classifications: grey matter, white matter and CSF, covering the entire anatomy of the brain. The preprocessing parameters used adhered to the standard defaults for voxel-based morphometry, including comprehensive brain tissue segmentation (with application of bias, noise and intensity adjustments to enhance image quality) and spatial alignment to the Shooting template.[28-30] The resulting grey matter volume images, conforming to the template, were rendered at a resolution of 1.5 mm × 1.5 mm × 1.5 mm. The volume of white matter hyperintensities was also defined as a separate class using the 'expert mode' protocol. Automated quality assurance protocols were applied to all scans, supplemented by manual visual inspections to detect any aberrations. To attenuate individual variations in gyral patterns, we implemented a smoothing operation using a 6 mm full-width at half-maximum Gaussian kernel. Additionally, we set a threshold of 0.1 for absolute grey matter values to include exclusively grey matter regions in the statistical analysis. To compare grey matter volume (predictor) between the high and low DTC groups (outcome), we used the general linear model approach implemented in SPM12. We performed an analysis of covariance (ANCOVA) on the whole brain. Age, sex, educational level, white matter hyperintensities and total intracranial volume were included in the model as covariates. Results from voxel-based morphometry were corrected using threshold-free cluster enhancement (TFCE)[31] with 5000 permutations and for multiple comparisons using the family-wise error method (FWE) with a significance level of $P < 0.05$. Significant clusters were determined with a minimum cluster size of 10 contiguous voxels. We used the Automated Anatomical Labeling version 3 atlas to localize the significant clusters.[32]

^{1}H-Magnetic resonance spectroscopy processing

The methodology used in this work is the same as in our previous work.[19,33] An isotropic voxel measuring 20 mm was placed in the leg and foot region of the right motor cortex (M1) using anatomical imaging (Fig. 1). Spectra were localized with point-resolved spectroscopy (PRESS; repetition time/echo time = 2000/135 ms, voxel size = 8 cm^3), obtaining both water-suppressed and unsuppressed spectra. Spectral processing and analysis were conducted using the software fitMAN (https://github.com/dwong263/MAGIQ/wiki/FITMAN-Overview) integrated into a custom graphical user interface using the IDL programming language (version 5.4, Research Systems Inc.).[34] Line shape correction was performed using the QUECC approach,[35] which includes quantification improvement by converting lineshapes to the Lorentzian type (QUALITY deconvolution)[36] and eddy current correction (ECC). Residual water signals were removed from each water-suppressed spectrum using a Hankel singular value decomposition algorithm. The resulting metabolite spectrum was fitted in the time domain using a Levenberg–Marquardt minimization routine, incorporating a template of prior knowledge of metabolite line shapes. The following metabolites were included in the prior knowledge template used to fit the water-suppressed data from the primary motor cortex (M1): *N*-acetylaspartate (NAA), choline (Cho), creatine (Cr), myo-inositol

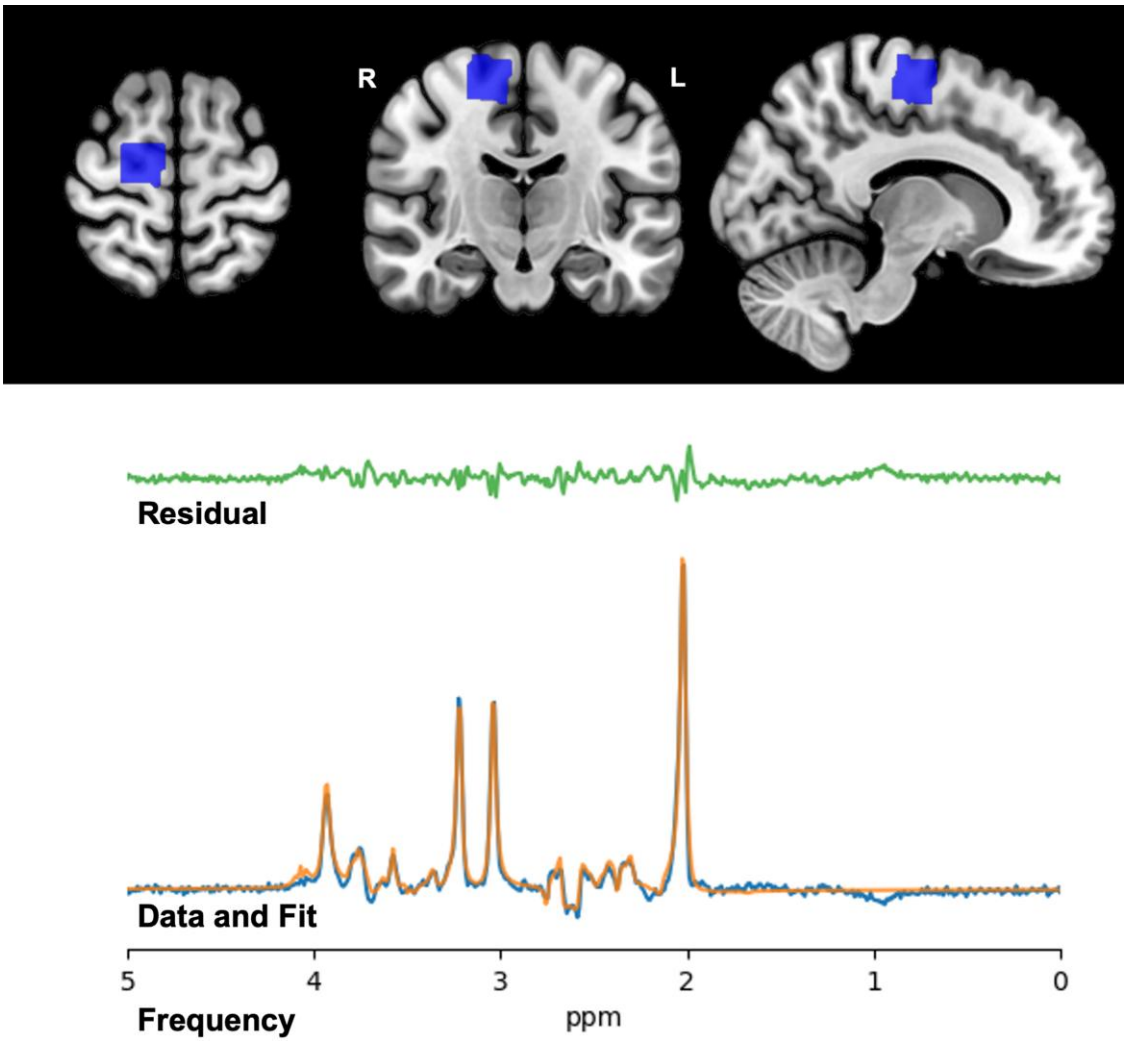

Figure 1 Voxel localization in the right primary motor cortex and magnetic resonance spectrum. *Top*: Voxel position is superimposed on axial, coronal and sagittal T_1-weighted images of one participant. *Middle and bottom*: Representative ^{1}H-magnetic resonance spectroscopy data from the motor cortex in one subject. ^{1}H-Magnetic resonance spectra acquired at 3.0 T (echo time/repetition time = 135 ms/2000 ms) were fitted using in-house software (fitMAN). Data are shown with the fitted spectrum superimposed. The residual is shown in the *middle panel*. ppm = parts per million.

(Myo), glutamate (Glu), glutamine (Gln) and glucose (Glc). Fitted metabolite peak areas were normalized to the unsuppressed water peak area, corrected for T_1 and T_2 relaxation times, and are reported in units of millimoles per litre (Supplementary Fig. 1). The NAA/Cr, Cho/Cr and NAA/Cho ratios were also calculated because they are indicative of neuronal integrity, cellular membrane turnover and energy metabolism, and the balance between neuronal health and membrane turnover, respectively.

To compare groups dichotomized according to high versus low DTC, ANCOVA was used to analyse absolute metabolite concentration and metabolite ratios, while controlling for age, sex, educational level and Montreal Cognitive Assessment score.

Main outcome

Progression to dementia was the primary outcome and was determined objectively by a clinician principal investigator of the Gait and Brain Study during the follow-up visits using the criteria described in the DSM4[25] and the CDR[21] Global score ≥1.

Predictor

Dual-task gait speed cost (DTC) attributable to a cognitive task while walking was calculated as follows: DTC = [(single-task gait speed – dual-task gait speed) / single-task gait speed] × 100.[11] The DTC was dichotomized based on a cut-off established in our previous study.[6] A DTC value of ≥20% indicated a high DTC, whereas a value of <20% indicated a low DTC.

Mediators

To identify variables as potential neural substrates or mediators of the association between DTC and incident dementia, we created a model that included regional grey matter volumes and motor cortex metabolite levels or ratios. The mediators were the significant grey matter volume clusters identified between the high and low DTC groups in the neuroimaging analysis and the metabolite concentrations or ratios that differed between high and low DTC groups.

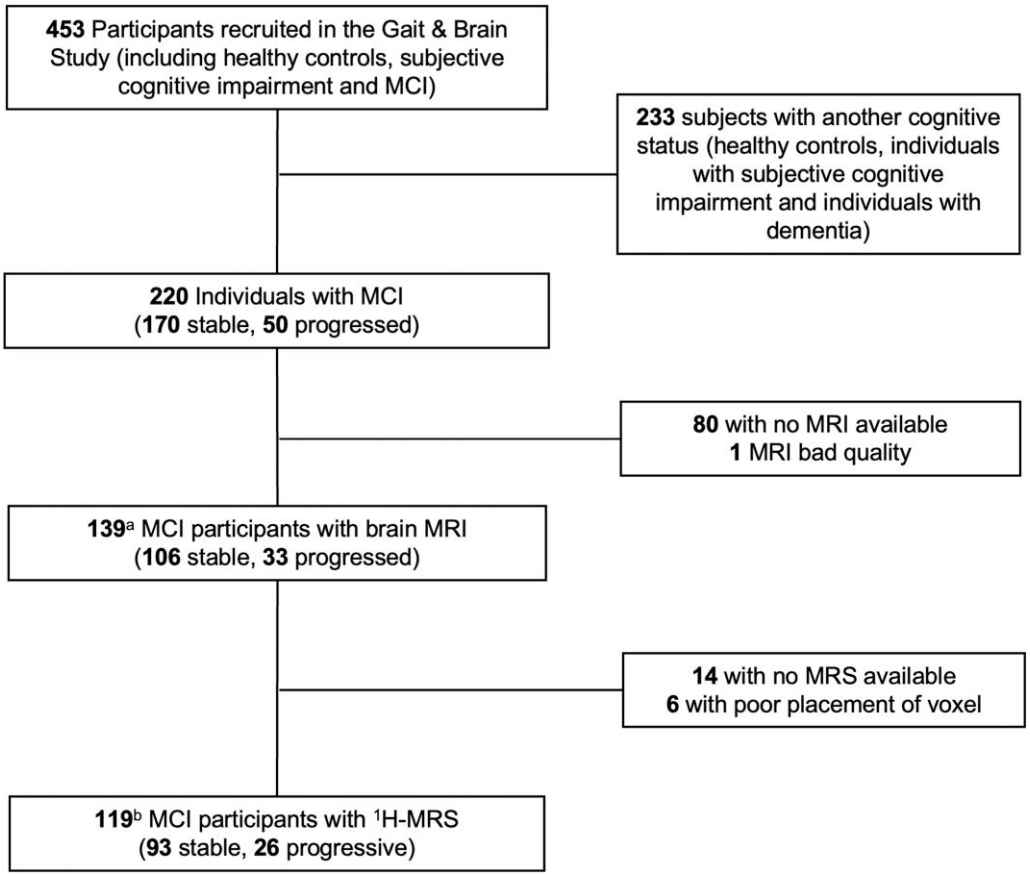

Figure 2 Study flow chart. MCI = mild cognitive impairment; MRS = magnetic resonance spectroscopy. [a]One participant did not perform the naming animals dual-task gait and another did not perform the serial sevens dual-task gait. [b]Six participants who had a placement of the voxel of interest in the left hemisphere instead of the right were included in the analysis.

Statistical analysis

To compare participants, baseline characteristics were stratified according to their conversion status (stable MCI versus progressed MCI) and Mann–Whitney U-tests for continuous variables or χ^2 tests for dichotomous variables were performed. Demographics and clinical characteristics were summarized using either means and standard deviations (SDs) or frequencies and percentages, as appropriate.

To confirm the longitudinal associations found in our previous study,[6] we used Cox proportional hazards regression modelling, entering conversion to dementia and time to conversion in the statistical model. Longitudinal associations were assessed using hazard ratios in Cox regression models both unadjusted and adjusted for age, sex, years of education and number of comorbidities. A *P*-value of <0.05 was considered statistically significant.

To assess whether regional grey matter volume and metabolite level concentrations in M1 (mediators) explained the association between DTC (predictor) and incident dementia (outcome), a mediation analysis was performed using the medmod and jaMM packages within Jamovi (version 2.3; https://www.jamovi.org and https://jamovi-amm.github.io/; Supplementary Fig. 2). The mediation effect calculated with linear regression was assessed quantitatively through a bootstrap procedure with 1000 samples to compute bias-corrected 95% confidence intervals (CIs) to determine the statistical significance of the mediation pathway or indirect effect. This method inherently accounts for variability and provides robust estimates, yielding results that are more accurate and less affected by sample size.[37] The model was adjusted for the number of comorbidities and the follow-up duration until last observation or conversion to dementia. We used the false discovery rate with Benjamini–Hochberg-adjusted *P*-value to correct for multiple comparisons.

Results

Characteristics at the baseline

At baseline, 139 individuals with MCI [mean (SD) age, 73 (6) years; 62 (44%) female] completed brain MRI scans. Of these, 106 remained stable, whereas 33 progressed to dementia (24%, 11 female). The average time between baseline measurement and conversion to dementia was 31 (21) months. Spectroscopy data from M1 were available for 119 subjects (93 stable MCI and 26 progressed to dementia). One participant did not perform the naming animals task, and one did not perform the serial sevens task (Fig. 2).

Table 1 presents the participant characteristics stratified by progression to dementia. No differences were found between groups for sex, age and number of comorbidities. At baseline, participants who progressed to dementia had lower body mass index, higher depressive symptoms evaluated by the Geriatric Depression Scale-15 (GDS-15) and lower global cognitive scores than those with MCI who did not progress.

Table 1 Baseline characteristics of participants stratified by progression to dementia

Characteristic	Full sample (*n* = 139)	Stable MCI (*n* = 106)	Progressed to dementia (*n* = 33)	P-value
Age, mean (SD), years	73.3 (6)	72.9 (7)	75.2 (5)	0.07
Female, *n* (%)	62 (44)	51 (48)	11 (33)	0.14
Number of comorbidities, mean (SD)	5.3 (3)	5.1 (3)	5.8 (3)	0.14
BMI, mean (SD), kg/m^2	27.4 (5)	27.8 (5)	25.9 (4)	0.046*
Educational level, mean (SD), years	13.8 (3)	14.0 (3)	13.5 (3)	0.43
Cognitive tests, mean (SD)				
MMSE score	27 (2)	27.6 (2)	25.4 (3)	<0.001*
MoCA score	23 (3)	23.7 (3)	21.3 (3)	<0.001*
RAVLT	4.7 (3)	5.1 (3)	3.1 (3)	0.04*
TMT A	42 (16)	40 (14)	49 (17)	0.004*
TMT B	152 (281)	140 (309)	186 (173)	0.44
Geriatric Depression Scale-15[a]	3.0 (3)	2.7 (3)	3.8 (2)	0.004*
Gait speed, mean (SD), cm/s				
Single task	114 (20)	118 (20)	100 (17)	<0.001*
Counting backwards	108 (25)	113 (24)	93 (19)	<0.001*
Naming animals	100 (25)	104 (25)	83 (19)	<0.001*
Serial sevens	96 (27)	100 (27)	80 (20)	<0.001*
Dual-task cost, mean (SD)				
counting backwards	5.5 (12)	5.0 (12)	6.9 (12)	0.32
naming animals	13.1 (15)	12.3 (14)	16.0 (17)	0.20
serial sevens	16.5 (16)	15.7 (16)	19.0 (16)	0.17
Subjects with high dual-task cost, *n* (%)				
Counting backwards	15(11)	8 (8)	7 (21)	0.03*
Naming animals	40 (29)	27 (25)	13 (41)	0.10
Serial sevens	51 (37)	35 (33)	16 (50)	0.08
Brain tissue volume, mean (SD), ml				
Total intracranial	1507 (165)	1497 (162)	1537 (171)	0.26
Grey matter[b]	605 (60)	610 (59)	589 (51)	<0.001*
White matter[b]	480 (61)	479 (62)	486 (59)	0.39
CSF[b]	414 (86)	403 (80)	453 (92)	0.003*
White matter hyperintensities[c]	6.5 (7.9)	5.5 (7)	9.5 (9)	0.006*

Note that only participants with an MRI scanner available at baseline were included in the analysis. BMI = body mass index; DTC = dual-task cost; calculated as [(single-task gait value – dual-task gait value)/single-task gait value] × 100; GDS-15 = Geriatric Depression Scale-15; MMSE = Mini-Mental State Examination; MCI = mild cognitive impairment; MoCA = Montreal Cognitive Assessment; RAVLT = Rey Auditory Verbal Learning Test; TMT A = Trail Making Test A; TMT B = Trail Making Test B. The GDS-15 scores ranging from 0 to 15, where lower scores denote minimal depressive symptoms and higher scores indicate more severe depression.
*Statistically significant value ($P < 0.05$).
[a]Three missing data in the stable MCI group. A score of less than five is considered normal and indicates the absence of depressive symptoms.
[b]Grey matter, white matter and CSF were adjusted on total intracranial volume.
[c]Volume of white matter hyperintensities was normalized for total intracranial volume and $\log_{10}$-transformed to obtain a normal distribution.

Participants who progressed to dementia had slower gait speed in all conditions (Table 1). The progressed group had a greater number of subjects with high DTC while counting backwards by ones ($P = 0.03$), but they did not differ significantly from the stable MCI during the naming animals ($P = 0.10$) and serial sevens tasks ($P = 0.08$).

There was no difference between the groups for intracranial and white matter volumes (Table 1). Participants who progressed to dementia showed smaller global grey matter volume ($P < 0.001$), higher CSF volume ($P = 0.003$) and higher volume of white matter hyperintensities ($P = 0.01$) at baseline.

The baseline smaller grey matter volume clusters located in the bilateral anterior cingulate cortex and temporal lobes were associated with progression status (Supplementary Fig. 3 and Supplementary Table 1). No significant differences in baseline metabolite concentrations or ratios were observed between the groups (results not shown).

Longitudinal analysis: association between dual-task performance and incident dementia

High counting backwards by ones DTC [hazard ratio (HR) (adjusted), 3.0; 95% CI, 1.13–7.49; $P = 0.02$] and naming animals DTC (HR, 2.68; 95% CI, 1.19–5.97; $P = 0.02$) were associated with earlier progression to dementia in MCI (Fig. 3 and Table 2). However, the association between serial sevens DTC and progression to dementia (HR, 2.17; 95% CI, 1.0–4.79; $P = 0.05$) did not reach significance after adjusting for covariates (age, sex, years of education and number of comorbidities).

Neural substrate of the dual-task cost

Adjusted ANCOVA indicated that high counting backwards by ones DTC was associated with smaller grey matter volume in several brain regions in comparison to those with low counting backwards by ones DTC. These regions included five clusters located in the right anterior and middle cingulate cortices, right fusiform gyrus and right cerebellum (lobule VI and crus I) and in the left lingual gyrus, precuneus, calcarine fissure, supplementary motor area, middle cingulate cortex and cerebellum (lobule VI and crus I) (Fig. 4A and Supplementary Table 2). No significant differences in metabolite concentration were observed in M1 when comparing high and low counting backwards by ones DTC (Supplementary Table 3).

High naming animals DTC was associated with a smaller grey matter volume in clusters located in the left cerebellum, lingual gyrus, calcarine fissure, middle occipital, superior parietal, precuneus and posterior cingulate cortices and in the right postcentral gyrus and cuneus in comparison to those with low naming animals DTC (Fig. 4B and Supplementary Table 2). Participants with a high naming animals DTC (in comparison to those with low naming animals DTC) exhibited a higher level of glucose [2.9 (1.2) versus 2.3 (1.4) mmol/l; $P = 0.01$] and a lower NAA/Cho ratio [6.0 (0.7) versus 6.3 (0.8); $P = 0.042$; Supplementary Table 3]. No other significant differences in metabolite concentrations were observed based on high versus low naming animals DTC.

High serial sevens DTC did not show any significant difference in structural grey matter volume in comparison to the participants with low serial sevens DTC. High serial sevens DTC was significantly associated with a higher Cho/Cr ratio [0.31 (0.03) versus 0.29 (0.03); $P = 0.042$] and a lower NAA/Cho ratio in comparison to low serial sevens DTC [5.9 (0.7) versus 6.4 (0.8); $P = 0.001$; Supplementary Table 3].

Mediation analysis

The mediation analysis (Fig. 5 and Table 3) showed that the association between counting backwards by ones DTC and incident dementia was mediated by the grey matter volume of the right anterior and middle cingulate cortices. The indirect effect accounted for 48% of the total effect of counting backwards by ones

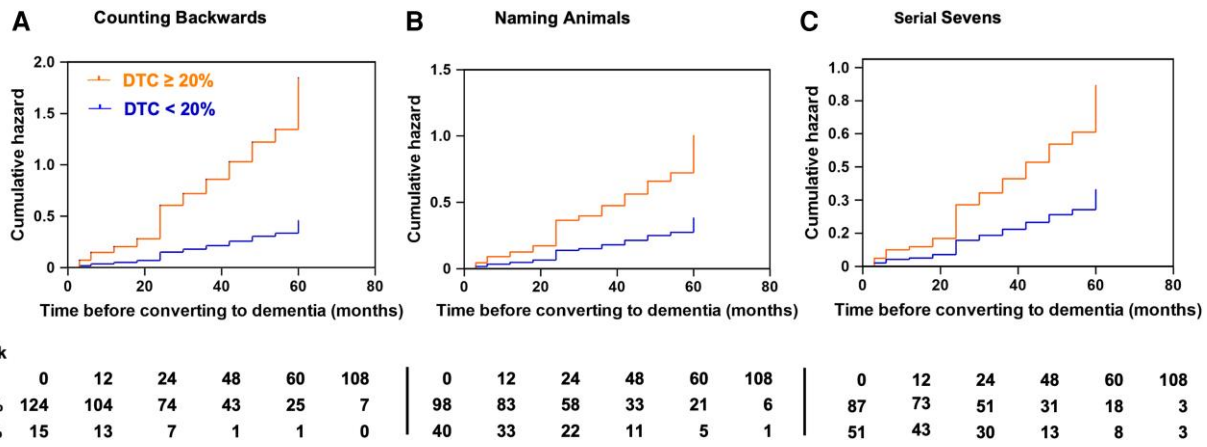

Figure 3 Cumulative hazard ratio for progression to dementia for low and high dual-task cost in gait speed. $n = 139$ for **A** and $n = 138$ for **B** and **C**.

Table 2 Cox proportional hazard regression of the association of dual-task gait cost with incident dementia

Task	Model unadjusted			Model adjusted[a]		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Counting backwards	4.00	1.55 to 9.23	0.002*	3.05	1.13 to 7.49	0.02*
Naming animals	2.63	1.23 to 5.57	0.01*	2.68	1.19 to 5.97	0.02*
Serial sevens	2.36	1.12 to 5.06	0.02*	2.17	1.00 to 4.79	0.05

*Statistically significant values ($P < 0.05$).
[a]Adjusted on age, sex, educational level and number of comorbidities.

DTC (total effect: 0.19, $P = 0.02$; indirect effect: 0.09, adjusted $P = 0.045$; direct effect: 0.10, $P = 0.23$). The indirect effect remained significant after adjusting for the baseline number of comorbidities and time to conversion but it was attenuated and no longer statistically significant when adjusted for global cognition (i.e. Mini-Mental State Examination).

No other mediation effects of the brain structures of interest, including the metabolite concentration levels of choline, were found.

Discussion

This study confirms that DTC is a significant indicator of incident dementia[6] and provides new insights regarding the neural substrate of this association. Participants with MCI with high baseline counting backwards by ones DTC who progressed to dementia exhibited smaller grey matter volume in the right anterior and middle cingulate cortices. No other neural changes, including motor cortex metabolite levels and reduced grey matter volume in other brain areas, mediated the association between dual-task performance and incident dementia.

Consistent with the literature, the anterior cingulate is one of the first structures to lose grey matter volume during the course of dementia, in addition to the more commonly reported changes in the bilateral temporal lobes.[38-41] Therefore, high DTC might signal early degeneration of the anterior cingulate. The anterior cingulate is implicated in cognitive function, including conflict resolution, task selection, error detection and motivation, which are essential for dual-task gait performance.[42-44] The middle cingulate cortex, located more caudally, is involved in generating intentional movements and resisting the potentially interfering effects of automatic subroutines.[45,46] Therefore, a smaller grey matter volume in these areas might explain why individuals who progress to dementia experience difficulties during dual-tasking.[47,48] These areas are crucial for executive functions and successful cognitive–motor interaction.

Participants with high serial sevens DTC showed an elevated ratio of choline to creatine in M1. Choline is present mainly in white matter and is found predominantly in glial cells. It is associated with membrane turnover, neuroinflammation and dysregulation of the cholinergic system.[49,50] Several studies have determined that higher levels of Cho/Cr ratio indicate an increased risk of developing clinical dementia.[16,51] However, some studies have not found this association.[17,52] The inconsistent results in the literature might be attributable to differences in voxel placement. Our voxel was localized to M1, which is an important motor cortical area, whereas others have examined the hippocampus and the posterior cingulate cortex. Additionally, the cognitive status of the population studied at baseline varies across studies (i.e. MCI subtype or a 'free of dementia' population). We found that elevated choline levels in M1 were associated with a high DTC. Although choline did not mediate the relationship between DTC and dementia, these findings suggest that choline levels could be a promising avenue to explore when evaluating whether anticholinesterase drugs improve gait performance. Indeed, these treatments are already used to improve cognitive symptoms in subjects with neurodegenerative disorders.[53] Furthermore, a low NAA/Cho ratio in individuals with high DTC suggests reduced neuronal integrity and higher cellular proliferation in M1.[17] These observations provide insight into the mechanisms that alter motor cortex metabolite levels in both cognitive and gait decline.

Only the DTC from the counting backwards by ones condition was shown to mediate the relationship between DTC and risk of dementia significantly. We did not observe the same effect with the naming animals and serial sevens conditions, despite the fact that these conditions were also associated with reduced grey matter volume and/or change in metabolite levels. This absence of mediation could stem from the difficulties that individuals with MCI have in performing dual tasks as the level of complexity increases.[54] Tasks with a very high cognitive demand, such as serial sevens subtractions, are often performed inadequately by MCI

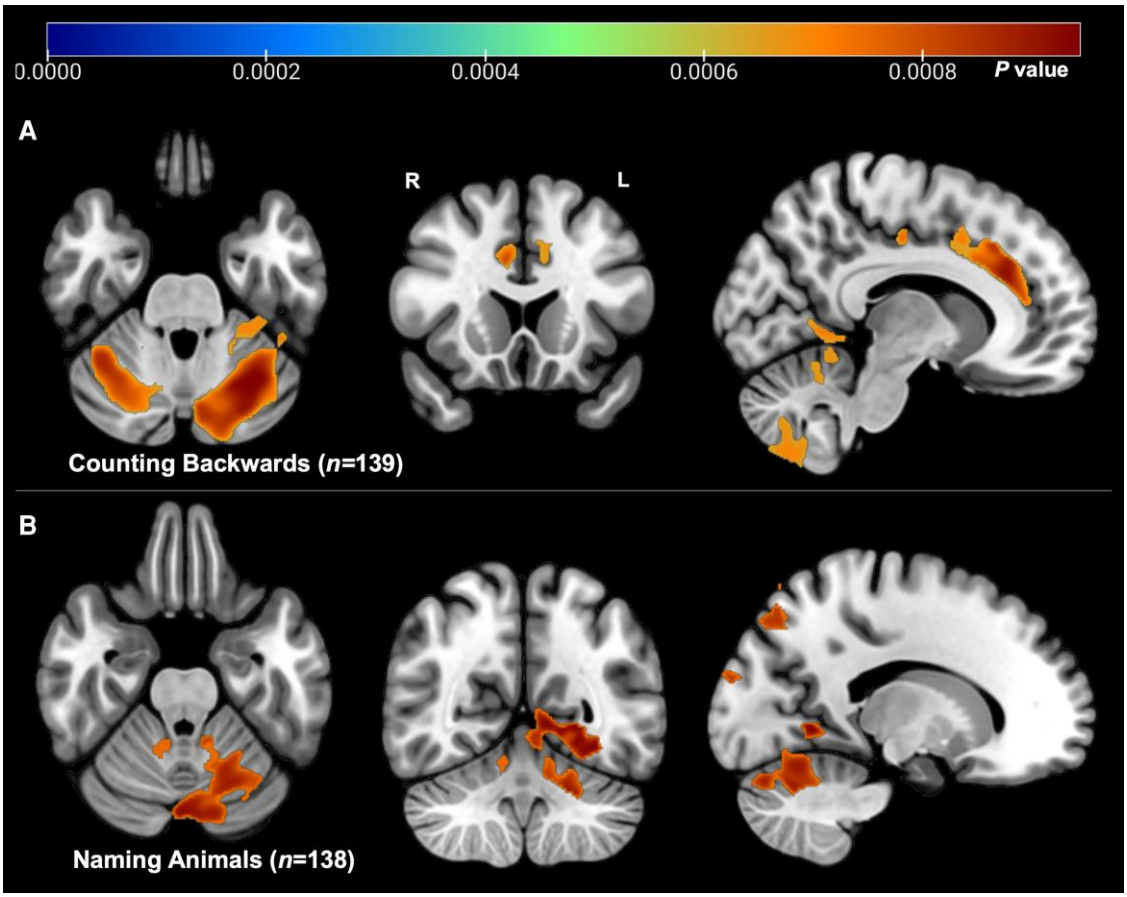

Figure 4 Cross-sectional analysis showing differences in grey matter volume according to the load of dual-task cost. Adjusted for age, sex, educational level, white matter hyperintensities and total intracranial volume. Significant clusters indicated that individuals with high dual-task cost (**A** for counting backwards and **B** for naming animals condition) had a decrease of grey matter volume with $P < 0.05$, family-wise error corrected for multiple comparison.

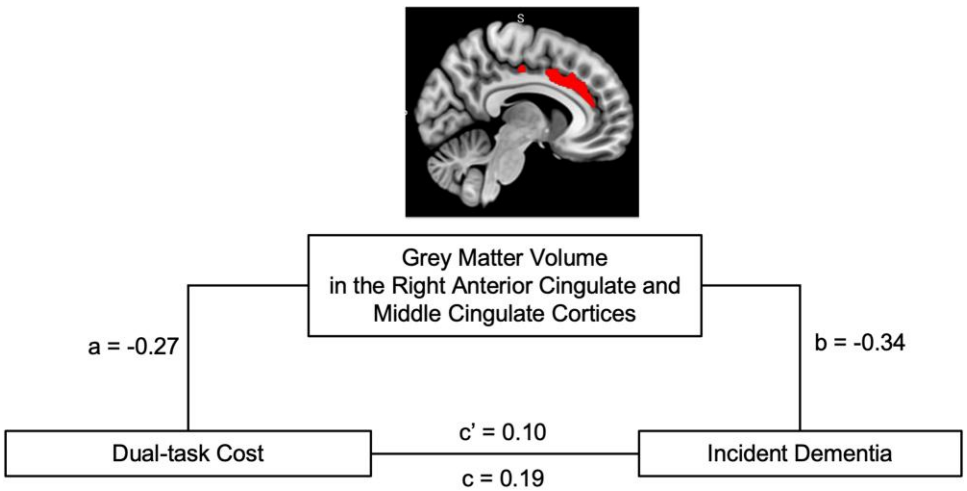

Figure 5 Illustration of the association of dual-task cost with dementia and mediation analysis. Indirect effect ($P = 0.01$): ab (a, association between counting backwards dual-task cost and grey matter volume; and b, association between grey matter volume and incident dementia); c, total effect; c′, direct effect ($P = 0.23$): association between dual-task cost and incident dementia.

participants, who may discontinue the challenging cognitive task and paradoxically increase their gait speed.[55]

To our knowledge, this is the first study to establish a potential role for the anterior and middle cingulate cortices as a neural mechanism linking dual-task performance and higher risk of dementia in the MCI population. Additionally, we provide the largest cohort of spectroscopy data exploring this association. The strengths of this study include its well-characterized cohort,

Table 3 Mediation analysis for the counting backwards dual-task cost

Potential mediator	Total effect (c)	Direct effect (c′)		Indirect effect (ab)		Mediation
	B (95% CI)	B (95% CI)	Percentage of total	B (95% CI)	Percentage of total	*P*-value adjusted with FDR
Unadjusted						
Right anterior and middle cingulate cortex	0.194* (0.04 to 0.49)	0.101 (−0.09 to 0.37)	52	0.093 (0.22 to 0.09)	48	0.045*
Left cerebellum	0.194* (0.04 to 0.49)	0.150 (−0.02 to 0.44)	77	0.044 (−0.01 to 0.13)	23	0.16
Left lingual and precuneus	0.194* (0.04 to 0.49)	0.151 (−0.03 to 0.44)	78	0.043 (−0.02 to 0.14)	22	0.16
Left SMA and middle cingulate cortex	0.194* (0.04 to 0.49)	0.140 (−0.04 to 0.42)	72	0.054 (−0.01 to 0.15)	28	0.16
Right cerebellum and fusiform	0.194* (0.04 to 0.49)	0.159 (−0.01 to 0.45)	82	0.035 (−0.02 to 0.12)	18	0.16
Adjusted on time to conversion and number of comorbidities						
Right anterior cingulate and middle cingulate cortex	0.168* (0.01 to 0.46)	0.074 (−0.13 to 0.33)	44	0.094 (0.03 to 0.22)	56	0.05*
Left cerebellum	0.168* (0.01 to 0.46)	0.134 (−0.05 to 0.42)	80	0.034 (−0.02 to 0.11)	20	0.16
Left lingual and precuneus	0.168* (0.01 to 0.46)	0.134 (−0.05 to 0.42)	80	0.034 (−0.03 to 0.12)	20	0.25
Left SMA and middle cingulate cortex	0.168* (0.01 to 0.46)	0.113 (−0.08 to 0.39)	67	0.055 (−0.01 to 0.16)	33	0.25
Right cerebellum and fusiform	0.168* (0.01 to 0.46)	0.141 (−0.04 to 0.43)	84	0.027 (−0.03 to 0.10)	16	0.25

Mediation analysis with counting backwards dual-task cost as the independent variable, dementia incidence (dichotomous) as the dependent variable, and a given neuroimaging parameter as the mediator. The model is presented as unadjusted and adjusted for time to conversion (or follow-up) and number of comorbidities. FDR = false discovery rate; SMA = supplementary motor area.
*Statistically significant values were adjusted by FDR to account for multiple comparisons.

longitudinal design with extensive follow-up, and application of standardized and systematized analyses, including active and continuous monitoring of the baseline cohort. In addition, dual-task gait assessment provides the opportunity to adjust to different levels of difficulty for the secondary task.[5] However, the attenuation of the indirect effect upon adjustment for baseline cognitive performance indicates that cognitive status plays a crucial role in the observed relationship between dual-task gait, grey matter volume and conversion to dementia and indicates that the baseline cognitive status could be a mediator in this pathway. Additionally, it is important to note that none of the participants exhibited untreated or significant depressive symptoms, as evaluated by the Geriatric Depression Score-15, which could also impact gait.

Limitations

The spectroscopy voxel was placed manually, and placement was assessed using a heatmap (Supplementary Fig. 1). All voxel placements were assessed using an atlas,[32] and those located in the M1 and supplementary motor area regions were included. However, six subjects had a voxel placed in the left M1, whereas the others had it placed in the right M1. Future studies should examine other voxel localizations. For instance, targeting a voxel in the anterior cingulate cortex might have been more sensitive to changes in metabolites, because this structure is the primary hub of cognitive–motor interaction in individuals who progressed to dementia. Lind *et al.*[56] has shown previously that the increase in choline levels in the anterior cingulate cortex is negatively correlated with visuo-spatial working memory performance in older adults. Finally, it would be valuable to investigate specifically whether white matter tract abnormalities and vascular damage represented by the white matter hyperintensities are associated with DTC.

Conclusion

The anterior and middle cingulate cortices are crucial structures that link dual-task gait performance and incident dementia in subjects with MCI. The present study offers new insights into the neural mechanisms of DTC and explains why some individuals experience faster progression to dementia and might point to potential treatable areas. Future studies should explore how vascular damage and white matter tract integrity might also potentially mediate DTC and dementia to advance knowledge on gait performance as a behavioural and reliable marker of dementia.

Data availability

The study data supporting the findings are available on reasonable request via the corresponding author.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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