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Impact of adult-onset multiple sclerosis on MRI-based intracranial volume: A study in clinically discordant monozygotic twins

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ABSTRACT

Objective: Intracranial volume (ICV) represents the maximal brain volume for an individual, attained prior to late adolescence and remaining constant throughout life after. Thus, ICV serves as a surrogate marker for brain growth integrity. To assess the potential impact of adult-onset multiple sclerosis (MS) and its preceding prodromal subclinical changes on ICV in a large cohort of monozygotic twins clinically discordant for MS.

Methods: FSL software was used to derive ICV estimates from 3D-T1-weighted-3 T-MRI images by using an atlas scaling factor method. ICV were compared between clinically affected and healthy co-twins. All twins were compared to a large healthy reference cohort using standardized ICV z-scores. Mixed models assessed the impact of age at MS diagnosis on ICV.

Results: 54 twin-pairs (108 individuals/80female/42.45 ± 11.98 years), 731 individuals (375 non-twins, 109/69 monozygotic/dizygotic twin-pairs; 398female/29.18 ± 0.13 years) and 35 healthy local individuals (20male/31.34 ± 1.53 years). In 45/54 (83 %) twin-pairs, both clinically affected and healthy co-twins showed negative ICV z-scores, i.e., ICVs lower than the average of the healthy reference cohort ($M = -1.53 \pm 0.11$, $P<10^{-5}$). Younger age at MS diagnosis was strongly associated with lower ICVs ($t = 3.76$, $P = 0.0003$). Stratification of twin-pairs by age at MS diagnosis of the affected co-twin (≤30 versus > 30 years) yielded lower ICVs in those twin pairs with younger age at diagnosis ($P = 0.01$). Comparison within individual twin-pairs identified lower ICVs in the MS-affected co-twins with younger age at diagnosis compared to their corresponding healthy co-twins ($P = 0.003$).

Conclusion: We offer for the first-time evidence for strong associations between adult-onset MS and lower ICV, which is more pronounced with younger age at diagnosis. This suggests pre-clinical alterations in early neurodevelopment associated with susceptibility to MS both in individuals with and without clinical manifestation of the disease.

Abbreviations: ICV, intracranial volume; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; MSSS, Multiple Sclerosis Severity Score; SCNI, subclinical neuroinflammation.

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1. Introduction

Intracranial volume (ICV) correlates to the maximal brain volume an individual achieves during brain growth up to late adolescence (Adams et al., 2016; Bethlehem et al., 2022; Mills et al., 2016). ICV remains constant throughout adult life and is not altered by adult-onset disorders affecting the brain, thus serving as a measure for maximal brain size before disease onset (Buckner et al., 2004). ICV serves as a surrogate marker of brain growth's integrity during neurodevelopment and is considered a suitable proxy of brain reserve (Buckner et al., 2004; van Loenhoud et al., 2018). ICV can be assessed reliably using 3D T1-weighted MRI sequences (Buckner et al., 2004).

The development and growth of the brain consists of various key components, such as synaptic pruning, which have been shown to be impacted by different elements of the immune system (Coulthard et al., 2018; Morimoto and Nakajima, 2019). Therefore, early immune perturbations in the brain, like infections or autoimmunity, could change the architecture of the brain (Zengeler and Lukens, 2021; Han et al., 2021; Tanabe and Yamashita, 2018). Neuroinflammatory changes during brain development can lead to long-lasting structural and functional alterations (Tanabe and Yamashita, 2018). The impact of neuroinflammation on ICV has been demonstrated in pediatric multiple sclerosis (MS), where disease onset is closer in time to critical neurodevelopmental stages. Children with MS have been reported to have altered brain growth trajectories, resulting in lower ICVs and brain volumes than would be expected for their age and sex (Aubert-Broche et al., 2014; Aubert-Broche et al., 2017; Banwell, 2019; Bartels et al., 2019; Kerbrat et al., 2012; Fenu et al., 2018; Weier et al., 2016). Lower brain volumes were observed in children with MS even shortly after first clinical presentation (Aubert-Broche et al., 2017; Bartels et al., 2019; Giorgio et al., 2017; Weier et al., 2016), suggesting alterations in brain growth possibly even before clinical disease onset.

There is consensus that MS is associated with neuroinflammation and neurodegeneration from the early stages of radiological and clinical onset (Filippi et al., 2016; Rocca et al., 2017; Thompson et al., 2018), which is preceded by a prodromal phase characterized by subtle subclinical neuroinflammation (Buscarinu et al., 2020; Lebrun-Frenay et al., 2020; Makhani and Tremlett, 2021; Tremlett and Marrie, 2021). Incidental white matter lesions and diffuse brain parenchymal changes and atrophy have been described in clinically healthy individuals at familial risk for MS, which may reflect the earliest prodromal phase (Beltrán et al., 2019; De Stefano et al., 2006; Gerdes et al., 2020; Xia et al., 2017). Investigating the impact of adult-onset MS on ICV is challenging due to the heterogeneity of genetic and environmental confounders. The potential impact of adult-onset MS on earlier brain growth as a possible indicator of preclinical disease activity during neurodevelopment remains to be elucidated.

We aimed to evaluate MRI-based ICV as a surrogate marker of pre-disease brain growth in an ideally matched cohort of monozygotic twins clinically discordant for MS and compared these to a large reference cohort of healthy individuals (Human Connectome Project S1200 release), while incorporating a local cohort of adult healthy controls.

2. Materials and methods

2.1. Ethics committee approval

All investigations including MRI analysis in the MS-TWIN STUDY and our local control cohort were approved by the local ethics committee at Ludwig-Maximilian University of Munich (MS-TWIN STUDY, project-ID 267-13). All participants provided written informed consent according to the principles of the declaration of Helsinki (Goodyear et al., 2007).

2.2. Participants

Fig. 1 provides a synopsis of all cohorts included in this study.

2.3. Multiple sclerosis twin cohort

The MS twin cohort is part of the MS-TWIN STUDY and represents a cohort of monozygotic twins with clinical discordance for MS. The MS-TWIN STUDY is a monocentric study conducted at the Institute of Clinical Neuroimmunology, University Hospital LMU, Munich, Germany. Recruitment started in May 2012 and is ongoing; samples used for the present study were collected until December 2017. Table 1 summarizes basic clinical characteristics. Previous publications on different subsets from the MS-TWIN STUDY investigated immune profiles in CSF (N = 8 twin-pairs) and blood (N = 43 twin-pairs; N = 61 twin-pairs) (Beltrán et al., 2019; Gerdes et al., 2020; Ingelfinger et al., 2022), DNA methylation patterns (N = 45 twin-pairs) (Souren et al., 2019), altered lipid signaling (N = 73 twin-pairs) (Penkert et al., 2020), the role of the gut microbiota as a disease trigger (N = 34 twin-pairs) (Berer et al., 2017) as well as an broadened T cell reaction to EBV in MS (N = 34 twin-pairs) (Schneider-Hohendorf et al., 2022). In this manuscript we investigated ICV which has never been assessed in the MS-TWIN STUDY before.

Inclusion criteria for study participation were met if one co-twin of a monozygotic twin-pair had a diagnosis of MS (MS-co-twin) according to the revised McDonald criteria (Thompson et al., 2018), whereas the co-twin sibling was clinically healthy (H-co-twin). Exclusion criteria were treatment with corticosteroids in the last three months before study inclusion and any MR-related contra-indications. Monozygotic twin-pairs clinically discordant for MS (N = 54) underwent a detailed interview, neurological examination, blood sampling, and MRI examinations on the same 3 T MR scanner (Magnetom Skyra, Siemens Healthineers). Medical records including prior MRI scans were obtained and reviewed to confirm the diagnosis of MS. As previously described (Beltrán et al., 2019; Gerdes et al., 2020), Subclinical neuroinflammation (SCNI) were detected in a subset of clinically healthy co-twins, reflected either by suspicious lesions in MRI and/or inflammatory changes in CSF, indicating a possible prodromal stage of MS in this high risk cohort; (the concordance rate in monozygotic twins is estimated to be up to 20 % (Westerlind et al., 2014). These H-co-twins are labelled SCNI-co-twins, (N = 13), whereas in the rest of H-co-twins no SCNI was detected (truly healthy co-twins N = 23, see Fig. 6). The results on SCNI were inconclusive in some H-co-twins (N = 18), and therefore these H-co-twins were not included in the analyses that examined the effects of SCNI on ICV, to obtain a clearer SCNI dichotomy. EDSS (Kurtzke, 1983) and MSSS39 were used as measures of disease severity in MS-co-twins. EDSS is a clinical neurological examination assessing all functional systems and provides a score to evaluate MS related neurological disability from 0 to 10 and MSSS compares an individual's EDSS to a reference population with a similar disease duration, offering insights into disease severity and prognosis.

2.4. Reference cohort

To calculate population-based standardized z-scores, data from a reference cohort consisting of healthy individuals with no history of neurological and psychiatric disorders, including monozygotic and dizygotic twins and non-twin individuals, retrieved from the Human Connectome Project (HCP, S1200 data release) were used (Van Essen et al., 2012; Glasser et al., 2013). These healthy subjects had reported no parental history of Alzheimer disease, schizophrenia, and Parkinson disease.

2.5. Local control cohort

A local control cohort was included, consisting of healthy individuals

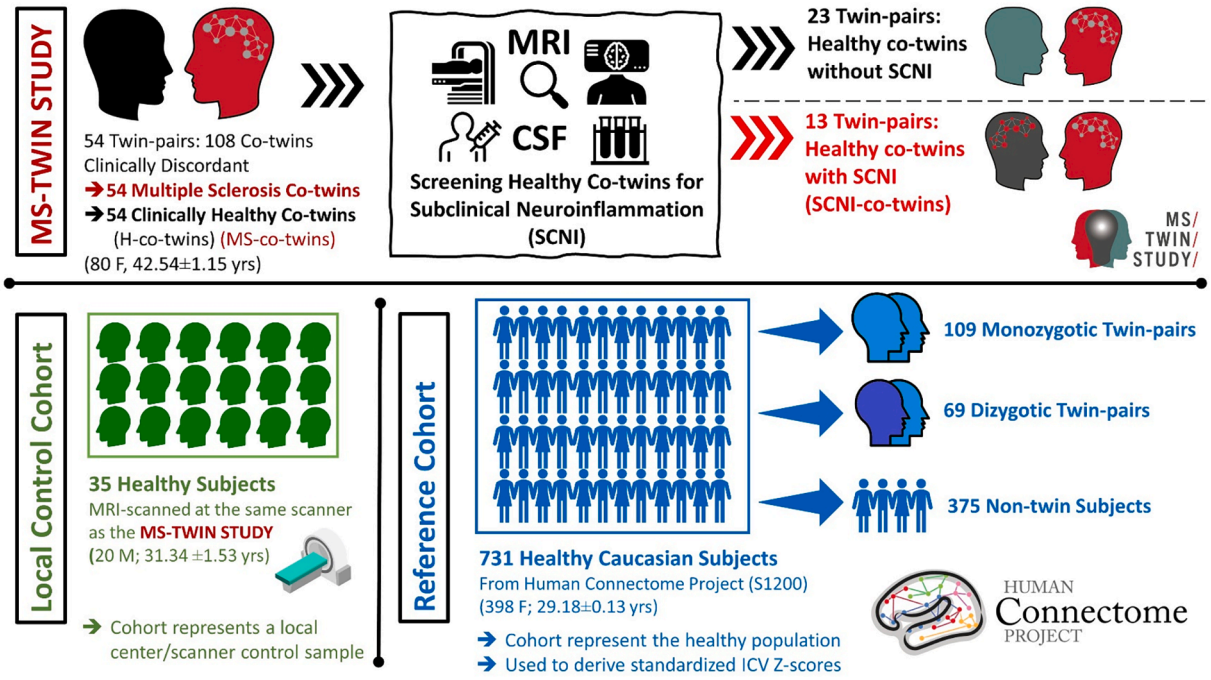

Fig. 1. Synopsis of the Cohorts.

Table 1
Main demographic and clinical characteristics of patients with MS and their healthy co-twins of the MS-TWIN STUDY.

	MSTWIN Cohort (N = 108 co-twins)		Reference Cohort (N = 731)
	Healthy co-twins	Co-twins with MS	
N co-twins	54	54	NT (368), MZ (225), DZ (138)
Female/Male	40/14	40/14	398/333
Mean age at MRI, years (SD)	42.54 (11.98)	42.54 (11.98)	29.18 (3.5)
Range (Median)	21 – 69 (30)	21 – 69 (30)	22 – 36 (29)
Disease course (N co-twins)	Without SCNI (23)	RRMS (43)	
	With SCNI (13)	SPMS (9)	
		PPMS (2)	
Mean age at MS diagnosis, years (SD)	30.8 (8.75)	30.8 (8.75)	
Median	30	30	
Disease Duration at MRI, years (SD)		11.72 (93)	
Mean EDSS (SD)	0.23 (0.5)	2.89 (1.91)	
Range (Median)	0–1.5 (0)	0–8.5 (2.75)	

N = number; EDSS = Expanded Disability Status Scale; MS = Multiple Sclerosis, SD = standard deviation, RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; SCNI = Subclinical Neuroinflammation; MZ = Monozygotic, DZ = Dizygotic, NT = Not twin

who were examined in the same 3 Tesla MRI scanner (Magnetom Skyra, Siemens Healthineers). Part of this cohort was previously published to assess effects of bifrontal transcranial direct current stimulation on brain glutamate levels and resting state connectivity (Mezger et al., 2021).

2.6. MRI data acquisition

MRI for the MS-TWIN STUDY and our local control cohort was performed using a 3 T MR scanner (Magnetom Skyra, Siemens Healthineers) with a 32-channel head coil. Sequence details are provided in the Supplement. The MRI acquisition protocols of the reference cohort, of which we used only the T1-weighted 3D sequence, were previously described (Van Essen et al., 2012).

2.7. Intracranial volume calculation

We calculated the estimated total ICV for each individual using linear registration of a T1-weighted image to a standard atlas space and subsequent computation of the inverse determinant of the affine transformation matrix, which serves as an atlas scaling factor (Buckner et al., 2004) (please see Supplement for more technical details). To calculate the estimated total ICV for every subject, this scaling factor was multiplied by the volume of the MNI atlas (1948.105 cm^3; MNI 152 T1 nonlinear 6th Generation (Grabner et al., 2006). This approach (Buckner et al., 2004) to estimate ICV has been validated using manual measurements which outline cranial cavity on T1-weighted sagittal planes with the aid of structural landmarks such as supratentorial dura margin, the contour of cerebral lobes, foramen magnum, and the craniovertebral junction. ICV estimates based on only one image processing method have been questioned (Katuwal et al., 2016). Thus, we calculated the atlas scaling factor with two separate pre-processing algorithms in FSL (version 6.0) and Freesurfer (version 6.0; https://surfer.nmr.mgh.harvard.edu/) using publicly available pipelines provided by the ENIGMA consortium (http://enigma.ini.usc.edu/). The resulting atlas scaling factors from both methods were highly correlated ($r > 0.85$, $p < 0.0001$; FSL: 0.79 ± 0.01, range = 0.65–1.05; Freesurfer: 0.75 ± 0.01, range = 0.6–0.93). Since a more rigorous semi-automatic quality control was performed on the FSL image processing outputs, we only show statistical results using FSL ICV estimates in this report. Nevertheless, all the regression models and pair-wise comparisons were also calculated using Freesurfer ICV estimates and resulted in same findings as the ones reported here (see Supplementary table 2 for a comparative summary statistic of atlas scaling factors derived using the two methods).

2.8. Statistical analysis

Analyses were performed on three different levels using the following three different outcome variables: 1) individual level: ICV

value; 2) twin-pair level: twin-pair specific ICV ratio calculated for each co-twin (ICV value of the co-twin / average ICV value of the respective twin-pair); 3) population level: standardized ICV z-score for all MS-TWIN STUDY participants and local healthy controls using a sex-matched mean and standard deviation of the reference cohort.

Multiple regression models were used to assess how our ICV outcome variables on all three levels of analysis (ICV, twin-specific ICV ratio, and ICV z-scores) were modulated by the clinical phenotype of co-twins (MS-co-twins vs. H-co-twins) and the age at diagnosis of MS (in the MS-co-twin of the twin-pair). Models were adjusted for sex and education level (with vs. without higher education, defined as having completed post-secondary education). Body mass index (BMI), as a known risk factor for adult-onset MS, was also added as a covariate to account for possible influence of cardiovascular and metabolic factors in brain development (Song et al., 2020; Morys et al., 2023). Age at time of study or disease duration for MS patients were not included in the models since ICV reaches a maximum before adulthood and has not been shown to change considerably thereafter with age or brain pathology/atrophy (Buckner et al., 2004; Stern et al., 2020; Schippling et al., 2017). An adjusted R-squared value, accounting for the number of predictors in the model, and a Cohen's f^2 and Cohen's *d*, as measures of effect size, are reported for regression models and pairwise group comparisons, respectively.

The ICV z-scores of the MS-TWIN study participants were contrasted against a "z-score = 0", representing the mean of the reference cohort, using one-way Wilcoxon ranked tests. Polynomial regression analyses were performed to investigate how clinical phenotype and age at diagnosis in the twinship affected the likelihood of co-twins having ICV scores comparable to the average of a healthy population. A categorical variable with 4 levels served as the outcome variable, and each co-twin was assigned to one category based on their individual ICV z-score: a) ICV z-scores > 0; b) −1 < ICV z-scores < 0; c) −2 < ICV z-scores < -1, and d) ICV z-score < -2. Sex, education level, and BMI were also controlled.

To evaluate the effects of SCNI, all regression models were also performed separately in two sub-groups of the twin-pairs: 13 twin-pairs with confirmed signs of SCNI versus 23 twin-pairs without signs of SCNI in H-co-twins.

Direct correlations between any two variables were investigated using the Spearman correlation test. Comparisons between groups were performed using Wilcoxon signed ranked tests or chi-squared tests, depending on the outcome variable. For all analyses, the threshold for statistical significance was set at p-value < 0.05. The robustness of p-values against multiple comparisons (performed on three levels with three outcomes) were tested by applying a Bonferroni-adjusted significance threshold of p = 0.017. Nevertheless, in case of explorative post-hoc analysis in smaller sub-groups, the uncorrected p-values were preferred to avoid type II errors resulting from lower statistical power of moderate size samples. Descriptive statistics are shown as mean ± standard error of the mean.

The R language (R Core Team, 2020) in Rstudio environment (RStudio Team, 2020) was used for all statistical analyses and visualizations.

3. Results

3.1. Study Sample

54 twin-pairs (MS-TWIN STUDY: 108 individuals, 80 females, mean age = 42.45 ± 11.98 years) and 35 healthy individuals (local control cohort: 20 males; mean age = 31.34 ± 1.53 years) were included (see Supplementary Fig. 1). As a reference cohort, data of 731 healthy adults from the Human Connectome Project were included (398 female, age range: 22–36 years, 29.18 ± 0.13 years; including 109 monozygotic twin-pairs: 130 female, mean age = 29.4 ± 0.2; and 69 dizygotic twin-pairs: 84 female, 29.17 ± 0.29 years).

3.2. ICV values (individual level)

Supplementary Table 1 provides a summary of the regression models of all three levels of analyses with the different ICV outcome variables. The regression model with ICV values as the outcome variable was significant ($F(5, 102) = 29.34$; $P<10^{-5}$). Clinical phenotype was not a significant predictor of ICV (P = 0.96), indicating comparable ICVs between MS-co-twins (1538.58 ± 22.01 cm^3) and H-co-twins (1541 ± 19.2 cm^3; see Fig. 2*a*). Younger age at diagnosis in a twin-pair was significantly correlated with lower ICV in the MS-TWIN STUDY (r_s = 0.35; P < 0.001; see Fig. 2*b*). Age at diagnosis also was a significant predictor of the model (P = 0.0002), showing a lower ICV value for a clinically healthy twin when MS diagnosis of the co-twin was made at a younger age. ICV values were significantly higher in the group with older age at diagnosis (P = 0.010; CI = [-0.06, −0.008]; Cohen's d = 4; older diagnosis age: 1581.6 ± 22.84 cm^3; younger diagnosis age: 1494.76 ± 15.44 cm^3; see Supplementary Fig. 2a). An interaction term between clinical phenotype and age at diagnosis was not found to be significant (P > 0.05) and failed to improve the model. Sex and education level were significant predictors of ICV value, showing higher ICVs for males and for participants with higher education. A sex by clinical phenotype interaction term was tested in the model, which was not significant (P > 0.05) and did not improve the model.

3.3. Twin-specific ICV ratios (twin level)

A twin-specific ICV ratio as an outcome variable allowed us to directly compare the two co-twins of individual twin-pairs. The regression model was not significant overall ($F(5, 102) = 0.93$; $P = 0.46$), and none of the predictors and control variables were significant (all *P*-values > 0.1). Adding an interaction term between clinical phenotype and age at diagnosis improved the model and resulted in significance ($F(6, 101) = 4.31$; $P = 0.0007$; Cohen's $f^2 = 0.03$); the interaction term was also significant ($P<10^{-4}$). Further post-hoc analyses of the interaction term in the two stratified sub-groups (age at diagnosis ≤ 30 years and > 30 years), revealed significantly lower ICV ratios in the MS-co-twins (0.99 ± 0.0) compared to their corresponding H-co-twins (1.01 ± 0.0; t = -3.1; $P = 0.003$; Cohen's d = 1) only in the subgroup with the younger age at diagnosis (see Fig. 4*a*).

3.4. ICV z-scores (population level)

The regression model with ICV z-scores as the outcome variable was significant ($F(5, 102) = 29.34$; $P<10^{-5}$). The results of this model were identical to those of the model with ICV values as the outcome variable (level of individual analyses; see Table 1 for more statistical details). MS-co-twins and H-co-twins had comparable ICV z-scores (P > 0.1) and younger age at diagnosis in the MS-co-twin of the twin-pair was significantly associated with lower ICV z-scores (P = 0.0003, see Supplementary Fig. 2*b*). In polynomial logistic regression, younger age at diagnosis in a twin-pair was associated with a significantly increased likelihood of having negative ICV z-scores below "-1" and "-2" (P = 0.003 and P = 0.001 for outcome categories "c" and "d"; model's residual deviance = 190.01, AIC = 220.02). Clinical phenotype was not identified as a significant predictor in previous regression models with ICV z-scores as the outcome variable and was therefore not included in this polynomial regression model.

The ICV z-scores of the MS-TWIN STUDY (-1.53 ± 0.11) were significantly lower than the mean of the reference cohort (i.e., z-score of "0"; $P < 10^{-5}$; MS-co-twins (-1.53 ± 0.11; $P<10^{-5}$) and H-co-twins (-1.53 ± 0.11; $P<10^{-5}$); Fig. 5). The ICV z-score was lower regardless of the age at diagnosis. A strong significant interaction term between sex and age at diagnosis in the regression model prompted us to run post-hoc tests on sub-groups formed based on these two factors. Female co-twins had significantly negative ICV z-scores, irrespective of age at diagnosis (-2.1 ± 0.11; $P < 10^{-5}$ versus −2.06 ± 0.14; $P<10^{-5}$), whereas male co-twins

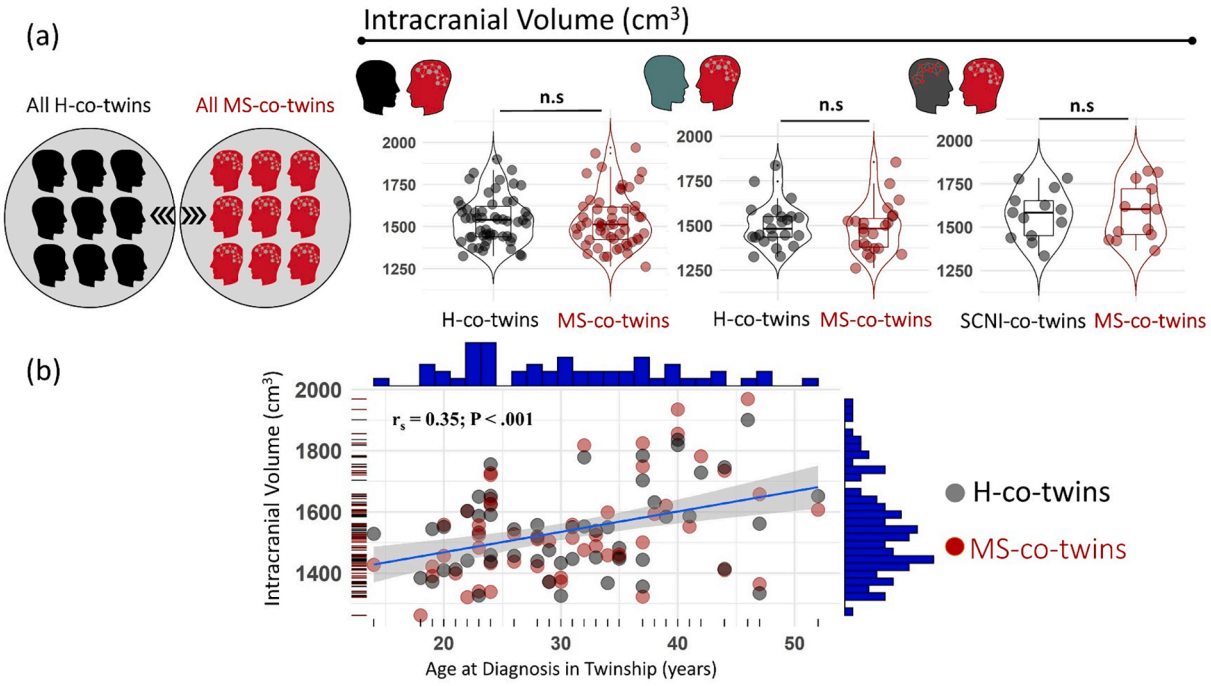

Fig. 2. **(a)** Violin plots compare intracranial volume (ICV) between H-co-twins and MS-co-twins of the MS-TWIN STUDY. Points represent individual co-twins and the box-plot shows the median for each group. ***Left Panel:*** comparing ICV between all H-co-twins with all MS-co-twins showed no significant difference ($P = 0.96$); ***Middle Panel:*** comparing all H-co-twins with all MS-co-twins only in twin-pairs where H-co-twins had no subclinical neuroinflammation (SCNI; truly healthy H-co-twins) showed no significant difference ($P > 0.05$); ***Right Panel:*** comparing all H-co-twins with all MS-co-twins only in twin-pairs where H-co-twins expressed confirmed SCNI (SCNI-co-twins) showed no significant difference ($P > 0.05$). **(b)** Scatter-plot with a regression line between ICV and age at diagnosis in a twin-pair. Histograms represent the distribution of the variables on the axis parallel to them. A younger age at diagnosis in a twin-pair was significantly correlated with lower ICV in the MS-TWIN STUDY ($r_s = 0.35$; $P < 0.001$). (**n.s**: not significant, $P > 0.05$).

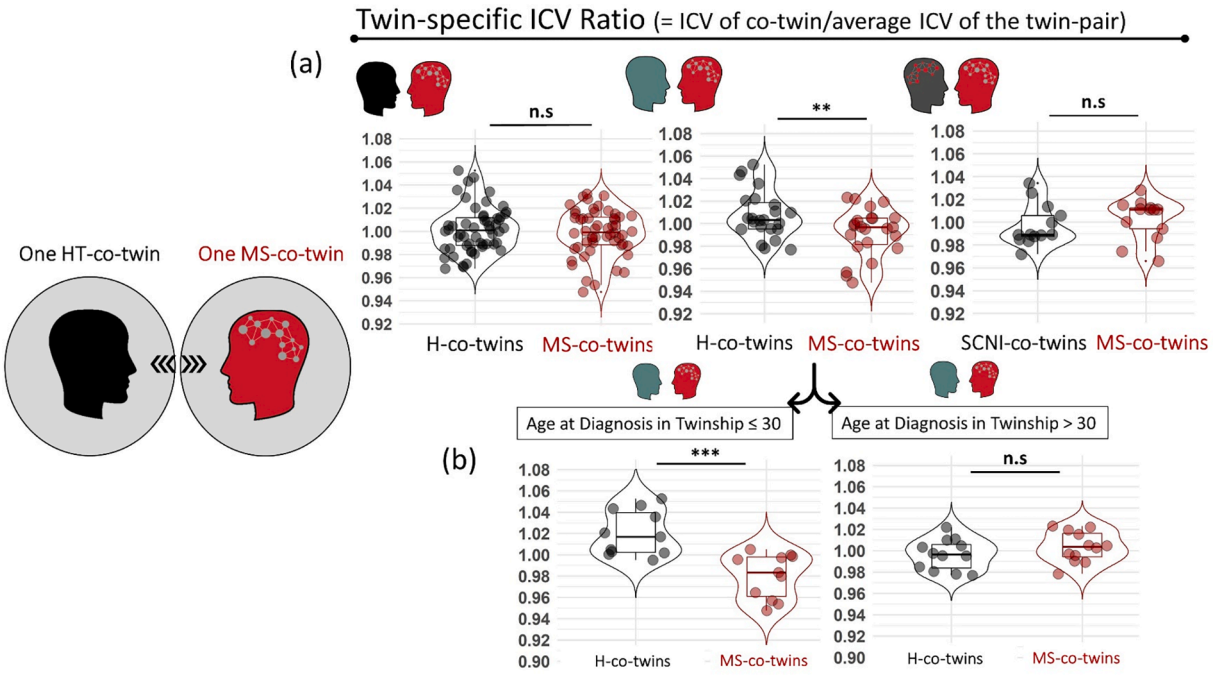

Fig. 3. Violin plots comparing ICV between H-co-twin and MS-co-twin of the same twin-pair, by mean of a twin-specific ICV ratio (ICV of a co-twin/average ICV of the twin-pair). Points represent ICV ratios of individual co-twins and the box-plot shows the median for each group. ***Left Panel:*** Comparison includes all twin-pairs in the MS-TWIN STUDY and shows comparable ICV ratios between MS-co-twins and their corresponding H-co-twins ($P > 0.05$); ***Middle Panel:*** Significantly higher ICV ratios for H-co-twins without SCNI in comparison with their corresponding MS-co-twins ($P < 0.01$); ***Right Panel:*** Comparable ICV ratios between SCNI-co-twins and their corresponding MS-cotwins ($P > 0.05$). **(b)** Stratifying the twin-pairs including H-co-twins without SCNI based on age at diagnosis in the MS-co-twins: Higher ICV ratios in H-co-twins compared to their corresponding MS-cotwins, only when the patients are diagnosed at a younger age (≤30 years old; $P < 0.001$). (**n.s**: not significant, $P > 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

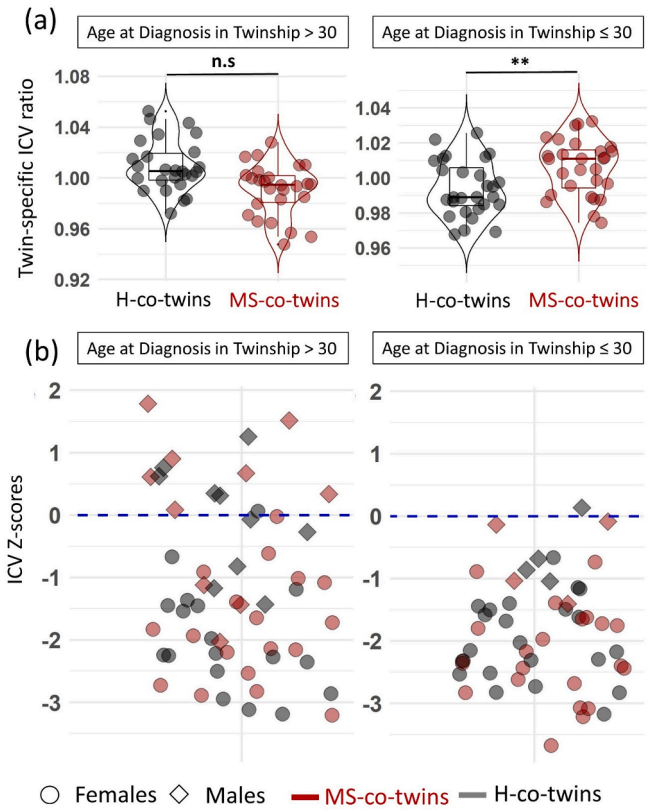

Fig. 4. Twin-pairs of the MS-TWIN STUDY are stratified into two sub-groups based on the age at diagnosis in the MS-co-twins (≤30 and > 30 years old). **(a)** Violin plots comparing ICVs between the H-co-twin and MS-co-twin of the same twin-pair using the twin-specific ICV ratio (=ICV of a co-twin/average ICV of the twin-pair). Points represent ICV ratios of individual co-twins and the box-plot shows the median for each group. Only in the younger age at diagnosis sub-group the H-co-twins had higher ICV ratios compared to their corresponding MS-co-twins (P < 0.01). **(b)** Points indicate the standardized ICV z-scores of individual co-twins of the MS-TWIN STUDY (calculated using the ICVs of the reference cohort). The dashed-blue line shows "ICV z-score = 0", representing the average ICV of the reference cohort. In 45/54 twin-pairs, both MS- and H-co-twins had negative ICV z-scores, meaning lower ICVs than the average ICV of the healthy population. Only 14/108 co-twins (including 13 males and 13 co-twins from the age at diagnosis > 30 sub-group) had positive ICV z-scores. (**n.s**: not significant, P > 0.05; **: P < 0.01). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with younger age at diagnosis in their twin-pair only showed significantly negative ICV z-scores (-0.64 ± 0.19; P = 0.02). Males with older age at diagnosis in their twin-pair had ICV z-scores comparable to z-score of "0" (0.04 ± 0.24; P = 0.73; Fig. 4*b* and *Supplementary Fig. 2c*).

ICV z-scores for our local healthy control participants were comparable to "0″ on one-way Wilcoxon test and no difference was found to the reference population (P > 0.1). The same comparison was performed for monozygotic and dizygotic co-twins of the reference cohort which showed ICV z-scores comparable to "0" for both types of twins (P > 0.1). ICV values of the MS-TWIN STUDY were found to be lower than those of the monozygotic co-twins of the reference cohort (W = 9758; P = 0.012; 95 %; CI = [-0.042, −0.005]; Supplementary Fig. 3). These findings make it less likely for the significantly negative ICV z-scores in the MS-TWIN STUDY to be due to a scanner-related effect or an effect related to twinship.

3.5. ICV and subclinical neuroinflammation

Twin-pairs with confirmed SCNI in the H-co-twins and those without detection of SCNI were comparable in terms of disease duration, age at diagnosis, intra-twin-pair lesion difference score (Schmidt et al., 2012) (see Supplement) EDSS and MSSS (P-values > 0.4). ICV values and z-scores were comparable between MS-co-twins and H-co-twins with or without SCNI (Figs. 2 and 5*a*). On the twin-level, a significant model was found only in the sub-group without SCNI ($F(6, 39) = 4.62$; $P = 0.001$; Cohen's $f^2 = 0.12$), where H-co-twins had higher ICV ratios compared to their corresponding MS-co-twins ($P<10^{-4}$). These higher ICV ratios in the H-co-twins without SCNI were more prominent when the corresponding MS-co-twin had a younger age at diagnosis, highlighted by a significant interaction between clinical phenotype and age at diagnosis ($P = 0.0002$) in this sub-group (Fig. 3*b*).

4. Discussion

To investigate the potential impact of adult-onset MS on prior brain growth, we assessed MRI-derived ICV as a surrogate marker of premorbid maximal brain volume in an ideally matched cohort of monozygotic twins clinically discordant for MS in comparison to a large reference cohort from the Human Connectome Project and local healthy controls. In 45/54 (83 %) twin-pairs, both clinically affected and healthy co-twins showed ICVs lower than the average of the healthy reference cohort (M = -1.53 ± 0.11, P < 10–5). Younger age at time of MS diagnosis was strongly associated with lower ICVs (t = 3.76, P = 0.0003). Our findings suggest disease-related preclinical alterations in brain development prior to the onset of MS in adulthood. The monozygotic twin study design allowed us to identify preclinical disease states in the clinically non-affected, at-risk individuals based on imaging or laboratory manifestations. Reduced ICV underscores a decrease in maximum attained brain volume and therefore likely an even earlier disease onset than assumed.

Genes involved in immunity and inflammation are implicated in brain growth and development of various subcortical brain structures, as reported by a recent genome-wide association study (GWAS (Satizabal et al., 2019). The lower ICVs found in our study cannot be solely attributed to twinship or scanner-related factors since such low ICVs were not observed in our local healthy control sample or in the healthy co-twins of the reference cohort. Moreover, co-twins of the MS-TWIN had lower ICVs when compared to monozygotic co-twins of the reference cohort. While clinically discordant, twin-pairs in our MS-TWIN study share a genetic and environmental susceptibility to MS. Our findings could also indicate an association between susceptibility to MS and lower ICV, as a surrogate marker of brain growth. Since ICV reaches a maximum during adolescence and does not change thereafter (Buckner et al., 2004), our findings are suggestive of preclinical alterations during brain growth in patients with adult-onset MS as well as in their healthy co-twins.

A reduced head and brain size due to failure of age-expected brain growth has been reported for children with pediatric-onset MS, even at first clinical presentation (Aubert-Broche et al., 2014; Bartels et al., 2019; Kerbrat et al., 2012; Fenu et al., 2018). There is also evidence of impaired brain growth in other pediatric demyelinating diseases, including monophasic demyelination, even without chronicity or severe clinical outcomes (Bartels et al., 2019; Aubert-Broche et al., 2017; Weier et al., 2016). In our study, the diagnosis of adult-onset MS at a younger age was strongly associated with lower ICV in both co-twins. All 14 clinically healthy co-twins with comparable ICVs to the average of a healthy population (z-scores > 0) belonged to twin-pairs in which MS was diagnosed at an age above 30 years.

In our study, healthy co-twins with no signs of SCNI had higher ICVs than their corresponding MS-co-twins, while healthy co-twins with signs of SCNI had comparable ICVs to their MS-co-twins. A recent scoping review (Mortazavi et al., 2021) summarized 16 MRI studies demonstrating focal white matter lesions and diffuse brain parenchymal alterations in a substantial proportion of healthy individuals with familial risk of MS. This report is the first to show similarity in ICV as an outcome

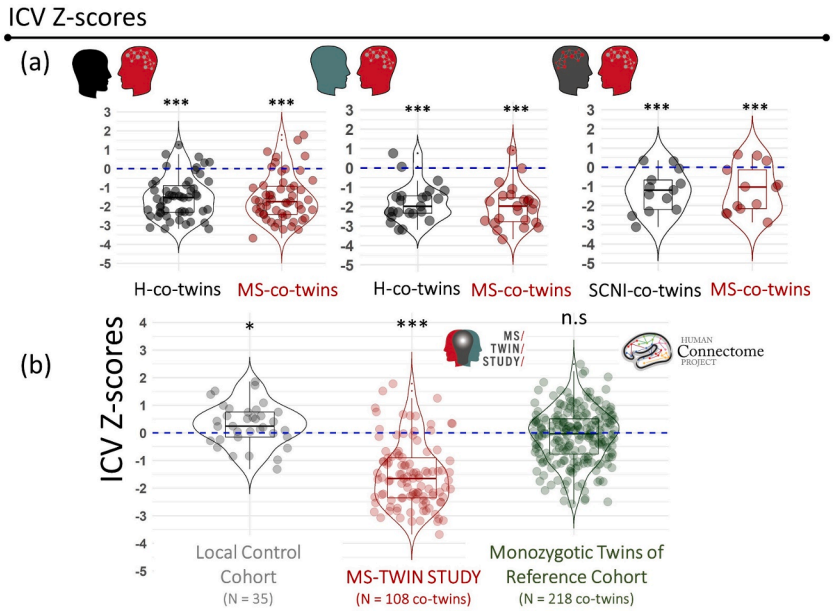

Fig. 5. **(a)** Violin plots comparing ICV z-scores between H-co-twin and MS-co-twin of the MS-TWIN cohort. Points indicate ICV z-sores of individual co-twins and the box-plots show the median for each group. ***Left Panel:*** all twin-pairs are included; ***Middle Panel:*** only twin-pairs with H-co-twins without SCNI (truly healthy co-twins) are included; ***Right Panel:*** Only twin-pairs with SCNI-co-twins are included. The H-co-twins and MS-co-twins had comparable ICV z-scores in all three panels. Points indicate ICV z-sores of individual co-twins. The dashed-blue line shows "ICV z-score = 0", representing the average ICV of the reference cohort. The H-co-twins and MS-co-twins in all three panels had significantly negative ICV z-scores (Ps < 0.001), meaning lower ICVs than the average ICV of the healthy population **(b)** ICV z-scores of the local control cohort, the co-twins of the MS-TWIN STUDY, and the monozygotic co-twins of the reference cohort. Points indicate the ICV z-score of individual persons/co-twins of each cohort. The dashed-blue line shows "ICV z-score = 0", representing the average ICV of the reference cohort, against which each cohort in this panel is compared. The co-twins of the MS-TWIN cohort had significantly negative ICV z-scores ($P < 0.001$). The local control cohort had slightly positive ICV z-scores ($P < 0.05$), suggesting that the lower ICVs in the MS-TWIN cohort compared to the healthy cohort are not due to a scanner-related effect. Also, the monozygotic co-twins of the reference cohorts had ICV z-scores comparable to "0" ($P > 0.1$), which suggest that the low ICVs is the MS-TWIN STUDY is not merely a twin-related phenomenon. (**n.s**: not significant, $P > 0.05$; **: $P < 0.01$; ***: $P < 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of brain growth between patients with MS and healthy individuals with confirmed subclinical traces of the disease.

There are limitations to our study. First, our study design was cross-sectional and we could not directly examine the trajectory of brain growth. Second, disease modifying and steroid treatments were not accounted for, but this should not impact ICV, as ICV does not change after reaching its maximum in adolescence. Third, the exact contributions of genetic and environmental risk factors of MS to the low ICVs found were not disentangled.

In conclusion, lower intracranial volumes in patients with adult-onset MS and their healthy monozygotic co-twins compared to a reference cohort of healthy adults suggest an impact of adult-onset MS on brain growth during childhood or adolescence, possibly related to a clinically silent prodromal disease stage and/or genetic and environmental risk factors of MS. Our findings encourage the understanding of neurological disorders in general, and MS in specific, as a continuum, entailing very early clinically silent periods which could affect development of the brain, also in healthy individuals who remain clinically unaffected by the disease, but are susceptible to it. Future studies with a longitudinal design will need to disentangle the contributions of genetic and environmental risk factors of MS to intracranial volume.

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Author contributions

L.A.G, T.K, B.E.W, and M.M contributed to the conception of the study. L.A.G, T.K, D.K, K.A, S.S, and B.E.W were involved in requirement and data acquisition. L.A.G and B.E.W assessed the clinical data. M.M, D. K, Ö.H, F.P, and A.S were involved in the analyses of the MRI data. M.M, L.A.G, D.K, and B.E.W wrote the manuscript.

CRediT authorship contribution statement

Matin Mortazavi: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Lisa Ann Gerdes:** Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Writing – original draft, Writing – review & editing. **Öznur Hizarci:** Data curation, Project administration. **Tania Kümpfel:** Conceptualization, Data curation, Funding acquisition, Resources. **Katja Anslinger:** Formal analysis. **Frank Padberg:** Data curation, Funding acquisition, Supervision. **Sophia Stöcklein:** Data curation, Funding acquisition, Investigation, Project administration. **Daniel Keeser:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Software, Supervision, Writing – original draft, Writing – review & editing. **Birgit Ertl-Wagner:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial

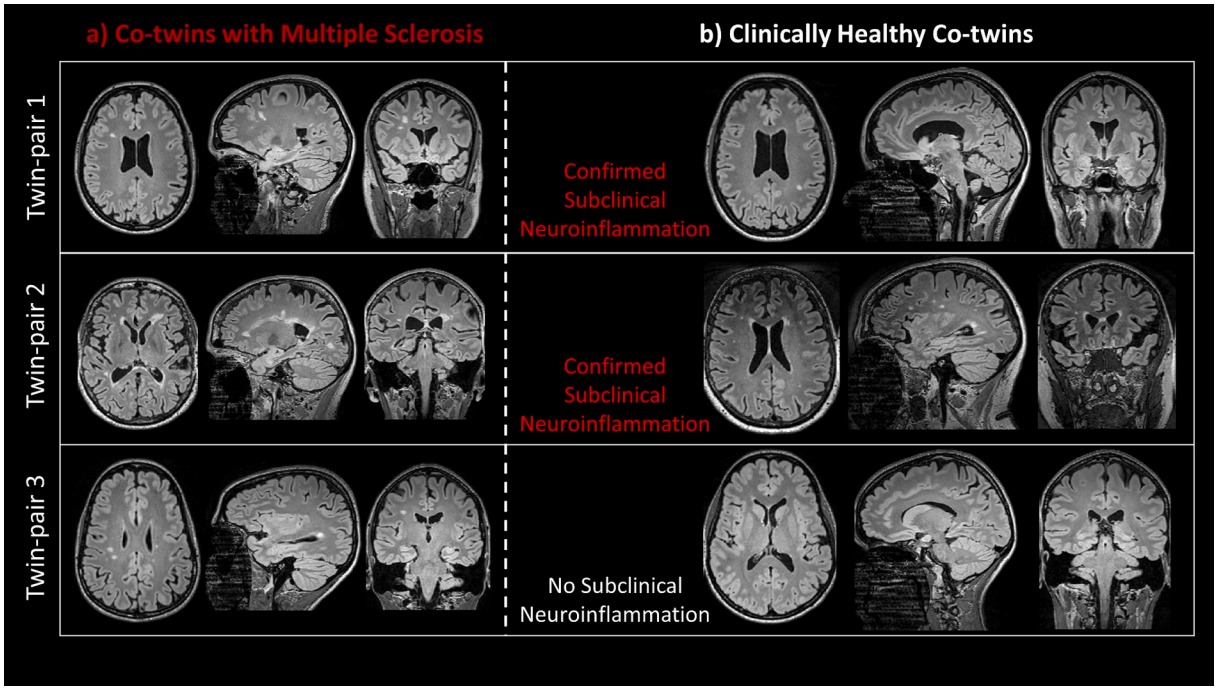

Fig. 6. FLAIR scans of 3 twin-pairs from the MS-TWIN STUDY cohort in transversal, sagittal, and coronal views. Left column (a) shows co-twins diagnosed with MS and right column (b) shows their corresponding clinically healthy co-twins. Twin-pair 1 (upper row; female, 42 years old): healthy co-twin with confirmed subclinical neuroinflammation indicated by suspicious MRI lesions (sagittal scan: lesion in corpus callosum; coronal scan: periventricular lesion) and inflammatory changes in CSF (oligoclonal bands in CSF positive, in serum negative). Twin-pair 2 (middle row; female, 67 years old): healthy co-twin with subclinical neuroinflammation indicated by highly suspicious MRI lesions (periventricular lesions in all three scans), CSF was not available for this individual. Twin-pair 3 (lower row; female, 32 years old): healthy co-twin with no subclinical neuroinflammation, determined upon inspection of MRI scans and CSF.

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2024.103597.

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