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Research Article | **Pathology**

Cortex and White Matter of the Cerebral Hemispheres: Anatomical Correlations and Age-Related Changes Measured with Fractal Analysis

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Abstract

Aim. The aim of the present study was to determine the fractal dimension (FD) values of the cortex and white matter of the cerebral hemispheres using fractal analysis of two-dimensional magnetic resonance images, explore the anatomical correlations of the cortex and white matter FD, and study age-associated changes in the cortex and white matter.

Methods. Two-dimensional brain magnetic resonance images of 100 apparently healthy individuals of both genders (44 males and 56 females) aged 18-86 years were studied. Five sections of each participant's brain were selected (4 coronal and 1 axial). After image segmentation, the FD values and sectional areas of the cortex and white matter were determined. Fractal analysis was conducted using a two-dimensional variant of the box-counting method.

Results. The FD values of the cortex and white matter varied across the five brain sections analyzed. Specifically, the cortex exhibited a decrease in the FD, whereas the white matter showed an increase in the FD in the coronal sections along the rostro-caudal direction. The FD values obtained from different sections displayed weak to moderate correlations. Additionally, no significant differences were observed in the FD values of the cortex and white matter between males and females. However, the sectional area values of the cortex and white matter were slightly higher in males as compared to females. Furthermore, a negative correlation was identified between the FD values of the cortex and white matter of the cerebral hemispheres, while sectional areas did not exhibit significant correlations. The cortex FD positively correlated with the gyrification index, whereas the white matter FD showed a negative correlation with this parameter. Additionally, both the FD and sectional area values of the cortex displayed strong and moderate negative correlations with age, respectively. In contrast, the FD and sectional area values of the white matter demonstrated weak negative correlations with age. Males showed stronger correlations of the studied parameters with age across the majority of the analyzed sections compared to females, although these differences were not statistically significant.

Conclusions. In this study, a negative correlation was found between the cortex and white matter FD values, influenced by anatomical factors such as the degree of gyrification. While the cortex FD values significantly decreased with age, age-related changes in the white matter FD values were relatively weak.

Keywords
Brain; Gray Matter; White Matter; Aging; Tomography; Fractals

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Introduction

Neurodegenerative diseases and the development of algorithms for their early and precise diagnosis pose significant challenges in neuroscience. These diseases, such as Alzheimer's disease, are accompanied by morphological changes in brain structures, that resemble those observed in normal aging - deepening and widening of sulci, smoothing of gyri, and the entire surface of the cerebral hemispheres, along with a reduction in cortical thickness and a decrease in overall brain volume [1–3]. Therefore, distinguishing atrophic changes, neurodegeneration, and aging-associated brain changes may cause diagnostic challenges. Atrophic changes in brain structures can be identified in vivo using neurovisualization methods, with structural magnetic resonance imaging (MRI) being the most common [1–9]. However, the assessment of brain magnetic resonance (MR) images in clinical practice is currently mostly subjective and descriptive. Hence, there is a need to develop morphometric methods that would allow for an objective quantitative assessment of brain structures.

Currently, neurovisualization studies of the cerebral hemispheres rely on morphometric methods derived from Euclidean ("traditional") geometry. These methods involve determining linear dimensions, area, and volume of various structures, as well as calculating derived indices based on these measurements. In cerebral hemispheres studies, commonly assessed parameters include volumes of gray and white matter [4–10], cortical thickness [8–10], gyrification index [8–10], depth of sulci [8–10], and the area inside cortical folds (folding area) [10]. However, the irregular and complex spatial configuration of the brain and its structures makes a comprehensive quantitative assessment challenging using classical morphometric methods.

An alternative direction in morphometric research involves the application of fractal analysis, a method rooted in fractal geometry [11]. The key parameter determined through fractal analysis is the fractal dimension (FD), which characterizes the complexity of the spatial configuration of a structure by quantifying the degree of space filling. Over the past few decades, fractal analysis has seen increasing use in studies of brain structures [12, 13]. The cerebral cortex (encompassing the cortical ribbon and its outer surface) [9, 10, 14–25] and the cerebral white matter (including its volume, outer surface - the gray-white matter interface, and digital skeletons) [22–28] have been the most common subjects of investigation. Previous studies have reported a decline in the FD of the cortex [9, 15, 16] and white matter [26, 27] of the cerebral hemispheres during normal aging. Reduced FD values of the cerebral cortex were identified in patients with Alzheimer's disease [17, 18], multiple sclerosis [19, 20], amyotrophic lateral sclerosis [21], mild cognitive impairment, and leukoaraiosis [22]. Changes in the FD of the cerebral white matter were also noted in patients with multiple sclerosis [28] and mild cognitive impairment [23]. The findings of the mentioned studies [9, 15–23, 28] affirm the sensitivity of the FD of the cortex and white matter to age-related and neurodegenerative changes, underscoring the potential utility of fractal analysis for diagnostic purposes.

The challenging aspect of integrating fractal analysis into clinical practice lies in the complexities associated with its implementation. Previous studies [16, 18–28] often employed three-dimensional modifications of this method, typically necessitating the construction of three-dimensional models of the brain. Three-dimensional variants of fractal analysis offer undeniable advantages, enabling the assessment of the spatial configuration of the cortex or white matter as a whole. However, three-dimensional modeling based on MRI results is not always accessible or practical (due to factors such as insufficient image quality, significant distance between sections, as well as lack of software and proficiency in using relevant programs). On the other hand, working with two-dimensional MR images is a daily routine in clinical practice [15]; therefore, the application of two-dimensional modifications of fractal analysis in brain research is unjustifiably infrequent. There is currently a lack of information regarding the age dynamics of FD values of the cortex and white matter, determined from two-dimensional images, which could be utilized as control values.

At the same time, most studies have focused either on the cortex or white matter, with only a few papers investigating both components simultaneously [22–25]. However, there is limited data regarding the anatomical correlations between the FD of the cortex and white matter.

Considering the aforementioned issues, in the present study, we **aimed** to determine the FD values of the cortex and white matter of the cerebral hemispheres using fractal analysis of two-dimensional magnetic resonance images, explore the anatomical correlations of the cortex and white matter FD, and study age-associated changes in the cortex and white matter. The present study extends our previous research conducted on the same sample but applying different methodologies including Euclidean geometry-based morphometry [29], fractal analysis of the overall cerebral tissue using the box-counting method [30], and fractal analysis of the cerebral pial surface employing a novel contour smoothing method [31].

Materials and Methods

Study Participants

Two-dimensional MR brain images of 100 individuals of both genders (44 males and 56 females) aged 18 to 86 years were reused [29]. The age distribution of the studied sample is presented in Table 1. The participants underwent brain MRI for diagnostic purposes. The reasons for undergoing MRI scanning differed among participants, with the most frequent reasons being headache, dizziness, and mild injuries to the soft tissues of the head. Furthermore, some participants chose to undergo the examination without specific complaints. Patients with severe neurological symptoms were not eligible for inclusion in the study. A radiology expert primarily assessed the MR images to confirm the absence of pathological changes in the brain and surrounding structures. The sample included individuals without MRI-confirmed pathological changes in the structures of the brain and surrounding areas; thus, their brain structure was considered conditionally normal.

Table 1. Age and sex distribution of the study participants.

Age range, years	Males		Females		Total	
	N	Average age, years	N	Average age, years	N	Average age, years
18-30	14	23.93	17	24.41	31	24.19
31-45	14	37.57	15	37.87	29	37.72
46-60	8	51.88	16	52.56	24	52.33
61-86	8	68.38	8	65.63	16	67.00
Total	44	41.43	56	41.95	100	41.72

MRI Protocol

Brain imaging was conducted using a Siemens Magnetom Symphony MRI scanner with a magnetic induction value of 1.5 Tesla. Images obtained through the following sequences were selected: T2, PD (proton density), and FLAIR (fluid-attenuated inversion recovery). The MRI scanning parameters were as follows: T2 sequence - echo time (TE) 130 ms, repetition time (TR) 4440 ms; PD sequence - TE 26 ms, TR 4440 ms; FLAIR sequence - TE 114 ms, TR 9000 ms, inversion time (TI) 2500 ms; the section thickness (the distance between the sections) for all sequences was 5 mm. The digital MR images had a resolution of 72 pixels per inch, and the absolute scale was 3 pixels = 1 mm.

Brain Section Selection

For the investigation of each individual's brain, five sections were selected, including four sections in the coronal (frontal) plane and one section in the axial (horizontal) plane (Fig. 1). Sections corresponding to different regions of the cerebral hemispheres were chosen, easily identified by anatomical landmarks, and corresponding to the brain regions where pathological lesions are most commonly detected in certain neurodegenerative diseases, including Alzheimer's disease [17]. The first coronal section (Coronal 1) was located at the level of the most anterior points of the temporal poles, the second (Coronal 2) at the level of the mammillary bodies (*corpus mamillare*), the third (Coronal 3) at the level of the quadrigeminal plate (*lamina quadrigemina*), the fourth (Coronal 4) at the level of the splenium of the corpus callosum. The axial section was located at the level of the thalamus (the uppermost section intercepting thalamus). Coronal sections were obtained in the T2 and PD sequences, while axial sections were obtained in the FLAIR or T2 sequences.

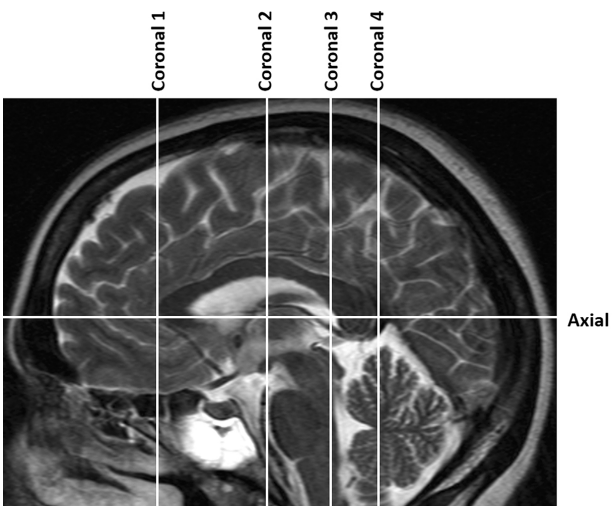

Figure 1. Localization of the studied sections: the coronal and axial planes are shown on the midsagittal magnetic resonance brain section.

MRI Preprocessing

After obtaining the images, their preprocessing was carried out using the graphic editor Adobe Photoshop CS5 to obtain binary silhouette images of the cerebral cortex and white matter (Fig. 2). For the detailed image preprocessing algorithm, refer to Supplement 1. As our goal was to investigate the features of the configuration of the white matter as a whole, the gray matter of subcortical nuclei within the boundaries of the white matter silhouettes was included in the obtained silhouettes (Fig. 2B). Excluding deep nuclei from the white matter silhouettes would result in the creation of "negative space" within them, corresponding to the nuclei silhouettes. Consequently, in such instances, the configuration of nuclei would exert influence on the configuration of the white matter silhouette. To counteract this potential influence, the decision was made not to partition the entire white matter silhouette into separate silhouettes delineating deep nuclei and white matter, thereby averting the introduction of negative space.

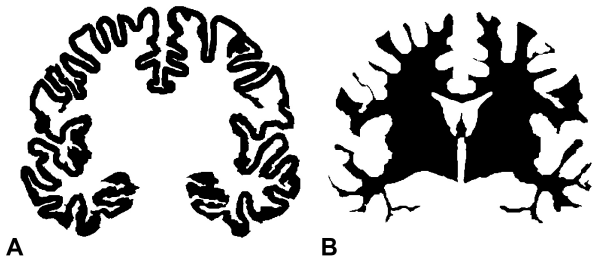

Figure 2. Magnetic resonance imaging preprocessing: binary silhouette images of the cerebral cortex (A) and white matter (B), obtained from Coronal 2.

Morphometric Assessment of the Cerebral Cortex and White Matter

The obtained silhouette images of the cortex (Fig. 2A) and white matter (Fig. 2B) were used for further morphometric analysis in the Image J software (Image J2, Fiji package) [32].

Fractal analysis was performed using the box-counting method, employing the "fractal box count" tool of the Im-

age J software. For each study participant, the FD values for the cortex and white matter were determined in five brain sections. To obtain the cortex FD, we selected the following box size values: 1, 2, 3, 4, 6, 8, 12, 16, 32, 64, 128, and 256 pixels. For the white matter, the box size values chosen were as follows: 4, 6, 8, 12, 16, 32, 64, 128, and 256 pixels. These specific box size ranges were determined based on the linear segments of the log-log plots representing the scaling factor (1/box size) versus the number of boxes intercepting the analyzed structure. Additionally, the average FD values for 1-4 coronal sections were calculated.

In addition to FD, the sectional area values of the cortex and white matter were determined, and the average sectional area values for 1-4 coronal sections were calculated.

Additionally, we conducted a correlation analysis with additional morphometric parameters of the cerebral hemispheres deriving from Euclidean geometry (Supplement 3) investigated in our previous work [29].

Statistical Analysis

The statistical data analysis was performed using Microsoft Excel 2016. The normality of distribution was verified using the Shapiro-Wilk SW test; since some parameters showed non-normal distributions (Table 2), the non-parametric statistics was chosen. Descriptive data are presented as median (Me), 25th percentile (Q1) and 75th percentile (Q3), minimum (min) and maximum (max) values. The differences between values (FD and sectional area) determined at brain sections were assessed using the Kruskal-Wallis H test, followed by post-hoc Dunn's test with Bonferroni adjustment (Supplement 2). Gender-dependent comparisons of the FD and sectional area were conducted using the Mann-Whitney U test. Associations with age were investigated through linear regression equations, followed by comparisons using the F test. The Spearman's rank correlation coefficient (R) was used to determine the relationships between the obtained values. The significance level for all results was set at α=0.05.

Results

Cortex and White Matter FD and Sectional Area Values

Descriptive statistical parameters of the FD and sectional area values of the cerebral cortex and white matter are presented in Table 2.

The studied parameters in five brain sections significantly differed from each other except for the following section pairs: cortical FD - Coronal 3 and 4; white matter FD - Coronal 2 and 3; cortical and white matter sectional area - Coronal 2 and 3, 3 and 4, 2 and 4 (Supplement 2). The highest FD and sectional area values were observed in the axial section. Among the coronal sections, the highest cortical FD value was in the section Coronal 1, with each subsequent section having a lower value than the previous one (Coronal 1 > Coronal 2 > Coronal 3 > Coronal 4). However, when comparing the white matter FD values, there was not a decrease but an increase in values in the rostro-caudal direction (Coronal 1 < Coronal 2 < Coronal 3 < Coronal 4).

The FD and sectional area values of the cortex and white matter were also assessed in terms of sex (Table 3). The FD values for both the cortex and white matter in males and females were found not to differ significantly ($p > 0.05$). However, the gender-dependent differences in the sectional areas of the cortex (Coronal 3 and 4, and average values of the coronal sections) and white matter (Coronal 4) were revealed.

Table 2. Descriptive statistical parameters of the fractal dimension and sectional area values of the cerebral cortex and white matter.

Section	Parameter	FD		Sectional Area, cm^2	
		Cortex	White matter	Cortex	White matter
Coronal 1	Me (Q1-Q3)	1.699 (1.688-1.723)	1.547 (1.505-1.582)	28.25 (26.34-31.47)	18.27 (15.05-21.82)
	min - max	1.659-1.765	1.446-1.685	22.39-39.84	10.55-34.32
	p (SW test)	0.02	0.28	<0.01	<0.01
Coronal 2	Me (Q1-Q3)	1.643 (1.628-1.656)	1.616 (1.597-1.641)	39.78 (37.12-42.58)	43.08 (40.16-48.58)
	min - max	1.598-1.702	1.508-1.731	30.04-52.53	29.12-59.62
	p (SW test)	0.73	0.12	0.24	0.98
Coronal 3	Me (Q1-Q3)	1.625 (1.614-1.641)	1.623 (1.602-1.636)	38.25 (35.54-40.80)	45.66 (41.82-50.43)
	min - max	1.593-1.691	1.512-1.756	32.77-52.56	30.27-59.84
	p (SW test)	0.05	<0.001	<0.001	0.63
Coronal 4	Me (Q1-Q3)	1.624 (1.612-1.641)	1.637 (1.624-1.664)	37.01 (34.88-39.70)	45.93 (42.43-50.00)
	min - max	1.583-1.698	1.541-1.776	30.53-55.14	30.57-61.56
	p (SW test)	0.03	<0.001	<0.001	0.77
Average (Coronal 1-4)	Me (Q1-Q3)	1.650 (1.636-1.661)	1.607 (1.591-1.625)	35.78 (33.77-38.26)	38.45 (35.83-41.59)
	min - max	1.617-1.704	1.514-1.671	30.13-48.92	25.69-51.32
	p (SW test)	<0.01	0.26	<0.01	0.88
Axial	Me (Q1-Q3)	1.734 (1.712-1.760)	1.760 (1.730-1.782)	60.79 (55.96-65.84)	86.90 (82.39-99.02)
	min - max	1.659-1.826	1.649-1.821	42.95-91.30	63.87-118.57
	p (SW test)	0.87	<0.01	<0.01	0.15

Note: SW test – Shapiro-Wilk SW test.

Table 3. Descriptive statistical parameters of the fractal dimension and sectional area values of the cerebral cortex and white matter in males and females.

Section	Parameter	Sex	FD		Sectional Area, cm^2	
			Cortex	White matter	Cortex	White matter
Coronal 1	Me (Q1-Q3)	Males	1.705 (1.688-1.724)	1.554 (1.521-1.581)	28.96 (26.59-32.48)	19.09 (15.43-21.12)
		Females	1.695 (1.688-1.718)	1.537 (1.504-1.584)	27.60 (26.20-31.12)	17.45 (14.85-21.84)
	p (U test)		0.24	0.55	0.22	0.40
Coronal 2	Me (Q1-Q3)	Males	1.642 (1.630-1.656)	1.621 (1.604-1.645)	40.16 (38.16-43.99)	44.55 (41.05-49.81)
		Females	1.646 (1.626-1.657)	1.613 (1.593-1.635)	39.43 (36.46-42.30)	42.49 (40.07-46.46)
	p (U test)		0.84	0.22	0.11	0.08
Coronal 3	Me (Q1-Q3)	Males	1.627 (1.616-1.642)	1.623 (1.606-1.634)	38.96 (36.34-41.71)	46.27 (43.23-51.63)
		Females	1.624 (1.609-1.641)	1.623 (1.601-1.639)	37.12 (35.06-39.88)	43.97 (40.30-49.57)
	p (U test)		0.25	0.97	0.04	0.08
Coronal 4	Me (Q1-Q3)	Males	1.625 (1.613-1.641)	1.633 (1.622-1.658)	38.70 (35.22-41.27)	47.12 (43.33-51.26)
		Females	1.621 (1.611-1.642)	1.640 (1.625-1.667)	36.63 (34.49-38.63)	45.47 (41.55-48.17)
	p (U test)		0.70	0.24	0.05	0.05
Average (Coronal 1-4)	Me (Q1-Q3)	Males	1.650 (1.639-1.662)	1.607 (1.591-1.624)	36.85 (34.58-39.91)	39.42 (36.17-43.46)
		Females	1.648 (1.635-1.661)	1.608 (1.593-1.627)	35.26 (33.27-37.26)	37.41 (35.09-40.63)
	p (U test)		0.35	0.97	0.05	0.07
Axial	Me (Q1-Q3)	Males	1.733 (1.712-1.762)	1.755 (1.729-1.782)	62.24 (58.89-67.68)	91.81 (84.52-101.20)
		Females	1.734 (1.712-1.751)	1.760 (1.733-1.782)	60.00 (55.25-64.91)	86.09 (80.34-92.88)
	p (U test)		0.89	0.87	0.07	0.06

Note: U test – Mann-Whitney U test.

Anatomical Correlations Between Cortex and White Matter Parameters

During the correlation analysis, the studied parameters from different brain sections were found to be positively correlated (Fig. 3). The adjacent coronal sections exhibited stronger correlations compared to other section pairs. In contrast, the axial section weakly correlated with the coronal section values. The FD values and the sectional area of the cortex exhibited stronger correlations between different brain sections compared to the corresponding values of the white matter. Significantly, the sectional area values of different brain sections exhibited stronger correlation relationships compared to their corresponding FD values, which demonstrated weak to moderate correlations.

It was also found that the FD values of the cortex and white matter were either weakly negatively correlated or the correlation was not statistically significant (Table 4). Age-normalized correlation coefficients were calculated to eliminate the influence of age-related changes, resulting in stronger correlation trends. The sectional area values of the cortex and white matter were weakly correlated with no statistical significance, except for the axial section. The FD and sectional area values of both the cortex and white matter were significantly and positively correlated, except for the white matter parameters of the Coronal 4 section (Table 4).

The relationships between the FD and sectional area values of the cortex and white matter, along with additional morphometric parameters determined in [29] (Supplement 3), were further explored. Most additional parameters exhibited similar relationships with the FD and sectional area of the cortex and white matter, while the notable difference was observed in the correlation patterns with the gyrification index which showed positive correlations with the FD of the cortex but negative correlations

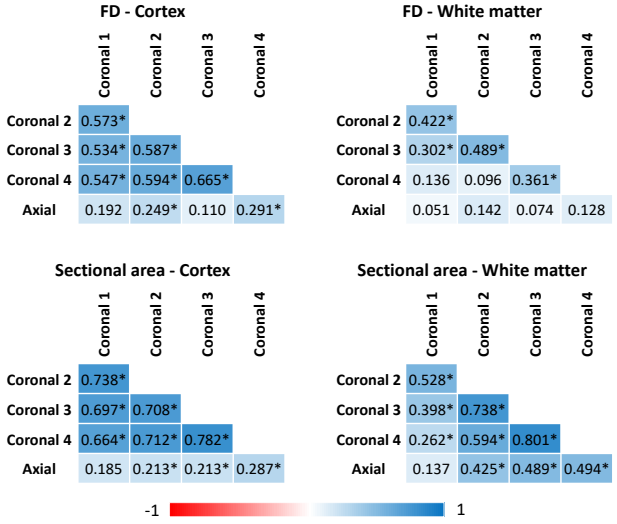

Figure 3. Correlation matrices of the fractal dimension and sectional area of the cerebral cortex and white matter from different brain sections; Spearman's correlation coefficient (R) values are provided; * – $p<0.05$.

with the FD of the white matter.

Thus, it was hypothesized that variations in the number of gyri on the section and, consequently, the gyrification index may influence the interrelationships between the cortex and the degree of white matter space filling. To test this hypothesis, brain anatomy was modeled with varying degrees of gyrification, or frequency of folding (Fig. 4). The cortex and white matter silhouettes were derived from MR brain sections of an 18-year-old individual from the studied dataset (Fig. 4A), with adjustments made by removing certain sulci. This modification led to the fusion of some neighboring gyri, resulting in two additional models of

Table 4. Correlation relationships between the fractal dimension and sectional area values of the cerebral cortex and white matter.

Section	Correlating Parameters							
	FDs Cortex and white matter		Sectional areas Cortex and white matter		Cortical parameters FD and sectional area		White matter parameters FD and sectional area	
	R	Rn	R	Rn	R	Rn	R	Rn
Coronal 1	-0.286*	-0.337*	-0.045	-0.038	0.908*	0.842*	0.963*	0.963*
Coronal 2	-0.286*	-0.371*	0.034	-0.018	0.831*	0.694*	0.759*	0.762*
Coronal 3	-0.232*	-0.379*	-0.006	-0.055	0.884*	0.811*	0.630*	0.629*
Coronal 4	-0.133	-0.248*	-0.036	0.041	0.819*	0.671*	0.159	0.167
Average (Coronal 1-4)	-0.151	-0.284*	0.079	0.144	0.888*	0.745*	0.611*	0.613*
Axial	-0.269*	-0.329*	-0.260*	-0.319*	0.588*	0.558*	0.649*	0.644*

Notes: R – regular Spearman's correlation coefficients (R); Rn – correlation coefficients normalized by age; * – $p<0.05$.

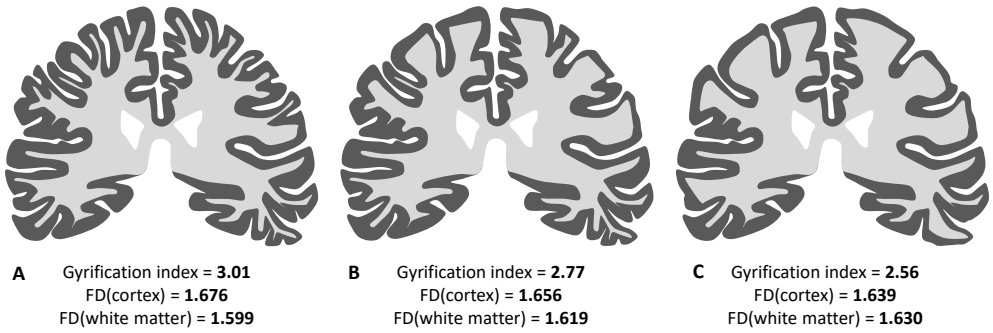

Figure 4. Modeling of cortical and white matter space-filling patterns at varying degrees of gyrification (Coronal 3). A – initial image (derived from the brain magnetic resonance image of an 18-year-old individual) with a relatively high gyrification index; B, C – sulci were manually removed to artificially decrease the degree of gyrification, resulting in average (B) and low (C) gyrification index values. Fractal dimension values for the cortex and white matter models are provided.

brain sections with lower degrees of gyrification (Fig. 4B, C). In the initial image with the highest degree of gyrification (Fig. 4A), the gyri appeared narrower, while in the model with the lowest gyrification, the gyri were noticeably broader (Fig. 4C). All other characteristics of the modeled sections remained unchanged. As depicted in Fig. 4, the cortex FD decreased with a decrease in the degree of gyrification, whereas the white matter FD increased.

Age-Related Changes in the Cortex and White Matter

The age dynamics of the FD and sectional area values of the cortex and white matter were also investigated (Table 5, Fig. 5, 6). The FD and sectional area values of the cerebral cortex across all brain sections decreased with age, showing moderate to strong statistically significant correlations. In contrast, the white matter parameters exhibited weak negative correlations with age.

Across the majority of sections, males exhibited slightly stronger correlations of the cortical parameters with age as compared to females. Similarly, in most brain sections of males, there were weak to moderate correlation relationships between the white matter parameters and age, while in females, these relationships tended to be weak and non-significant. No statistically significant differences were found in the regression equations characterizing the age dynamics of the studied parameters in males and females

Table 5. Correlation between age and the fractal dimension and sectional area values of the cerebral cortex and white matter.

Section	Sex	FD		Sectional Area	
		Cortex	White matter	Cortex	White matter
Coronal 1	Both	-0.636*	0.041	-0.667*	0.025
	Males	-0.772*	0.125	-0.748*	0.103
	Females	-0.514*	0.009	-0.615*	-0.021
Coronal 2	Both	-0.664*	0.013	-0.677*	-0.070
	Males	-0.670*	-0.138	-0.766*	-0.172
	Females	-0.656*	0.162	-0.601*	0.051
Coronal 3	Both	-0.628*	-0.099	-0.613*	-0.060
	Males	-0.630*	-0.282	-0.644*	-0.272
	Females	-0.627*	0.044	-0.587*	0.157
Coronal 4	Both	-0.694*	-0.064	-0.656*	0.101
	Males	-0.739*	-0.042	-0.730*	-0.002
	Females	-0.622*	-0.111	-0.625*	0.234
Average (Coronal 1-4)	Both	-0.787*	-0.031	-0.728*	0.028
	Males	-0.835*	-0.065	-0.795*	-0.118
	Females	-0.752*	0.015	-0.689*	0.205
Axial	Both	-0.354*	-0.103	-0.224*	-0.199*
	Males	-0.323*	-0.160	-0.126	-0.345*
	Females	-0.372*	-0.052	-0.290*	-0.082

Notes: table presents Spearman's correlation coefficient values (R); independent variable - age; * - $p<0.05$.

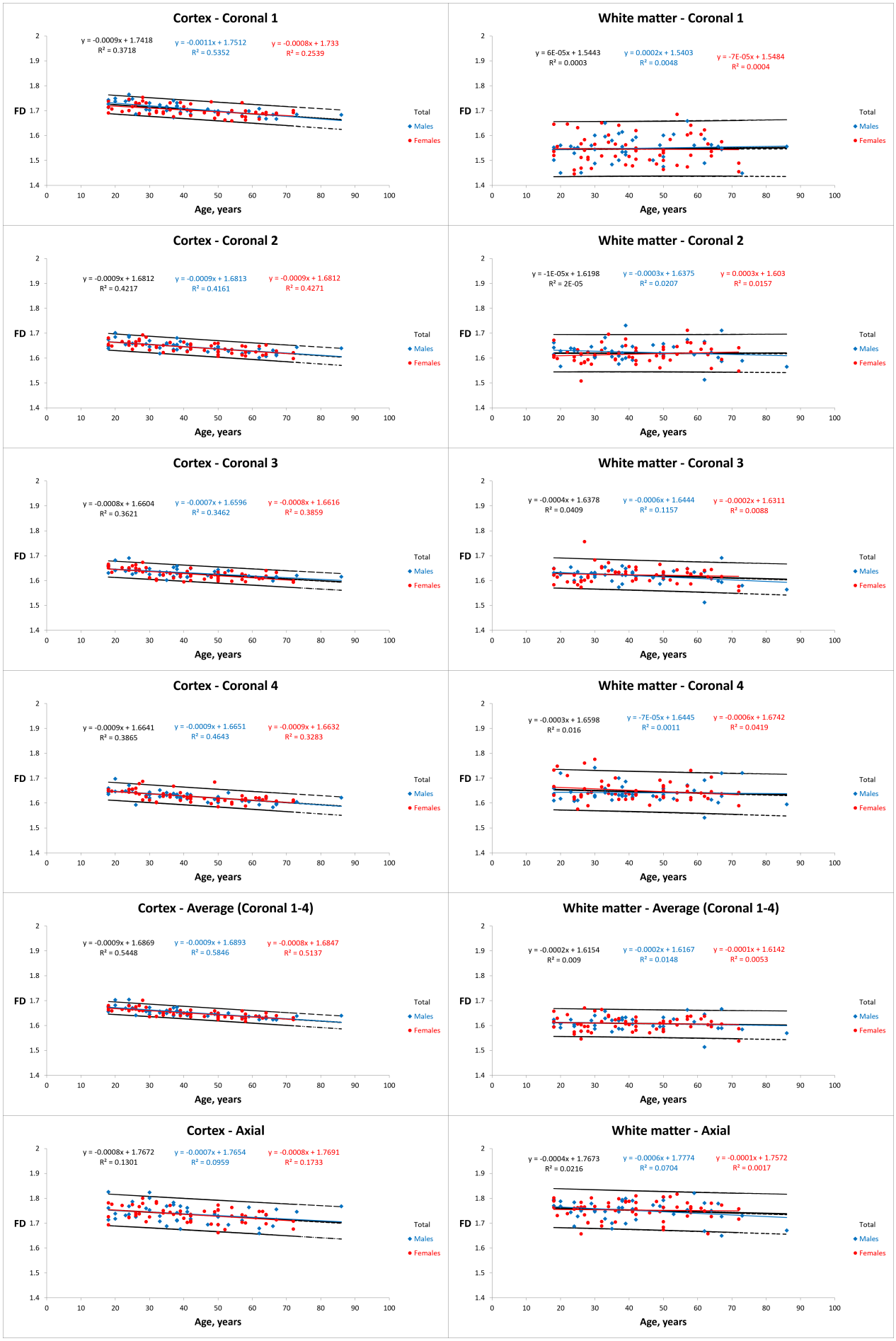

Figure 5. Dynamics of fractal dimension values and their 95% confidence intervals in terms of age.

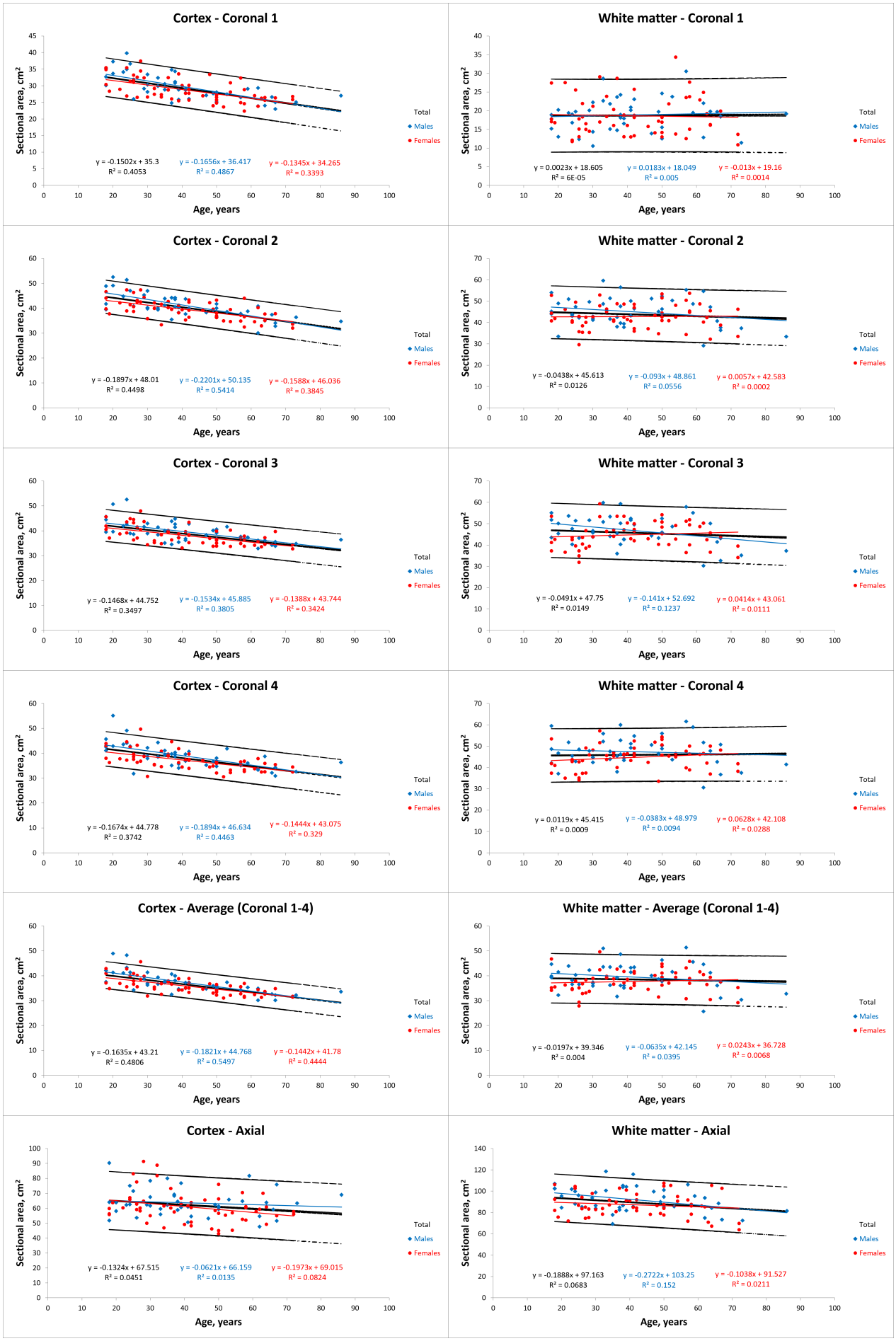

Figure 6. Dynamics of sectional area values and their 95% confidence intervals in terms of age.

(independent variable - age, dependent variables - the FD and sectional area values of the cortex and white matter) ($p > 0.05$).

Discussion

The present study was dedicated to exploring the FD of the cortex and white matter using a two-dimensional variant of fractal analysis. Previous studies employing fractal analysis of the cerebral hemispheres have used different approaches and investigated various aspects of brain structure [9, 10, 12–28]. Among these works, a study by King *et al.* [17] is the most methodologically close to our study. In their research, they identified the FD values of the cortical ribbon in both healthy individuals and patients with Alzheimer's disease (N=15 for both groups). The cortex FD values obtained by the researchers in healthy individuals were slightly lower than those obtained in our study, which may be attributed to variations in the age range of the studied samples.

Considering the lack of information regarding the anatomical basis for the relationships between the FD of the cortex and white matter, we analyzed the correlations between these parameters. According to our obtained data, the FD values of the cortex and white matter either exhibited no significant correlation relationships, or these relationships were weakly negative. The age-normalized correlation coefficients indicated weak to moderate negative correlations. Notably, there were no significant correlations between the cortical sectional area and the white matter sectional area. It would be logical to expect positive correlations between the two FD values, as an increase in the spatial complexity of the white matter configuration anticipate a more intricate cortical configuration. To understand the factors contributing to the negative correlation between the FD of the cortex and white matter, we analyzed the relationships between the FD and parameters derived from Euclidean geometry (Supplement 3). If most of these parameters exhibited similar relationships with the FD of the cortex and white matter, the difference in the correlation patterns with the gyrification index stood out: this parameter had positive correlations with the FD of the cortex but negative correlations with the FD of the white matter. The studies by Im *et al.* [10] and King *et al.* [17] have shown a close association between the cortical FD and the degree of gyrification, as characterized by the gyrification index, frequency of folds, and folding area. This indicates a significant impact of the degree of gyrification on the cortical FD. However, it raises the question of whether the degree of gyrification also affects the simultaneous decrease in the white matter FD. In our analysis, we propose that the frequency of folds serves as the primary factor influencing the negative correlation between the cortex and white matter FD. As the number of gyri increases (and consequently, the gyrification index), gyri tend to become narrower. All else being equal (such as brain size and cortical thickness), brain sections with a high frequency of folds exhibit numerous but narrow gyri, resulting in the white matter appearing as thin rods (Fig. 4). Conversely, in sections with less frequent folds, gyri are fewer but broader, leading to the formation of wide and well-defined white matter gyral bases. We suggest that the expansion of the cortex, accompanied by an increase in gyri frequency, cortical space-filling capacity (quantified by the cortical FD), and gyrification index, contributes to a decrease in the space-filling capacity of the white matter, consequently leading to a decrease in the white matter FD. However, we acknowledge that these anatomical patterns may not be the sole factors influencing the white-gray matter interactions in the cerebral gyri. Therefore, the relationships between the cortex and white matter FD are generally weak to moderate rather than strong.

Another significant finding of the present study was the gradual increase in the average white matter FD along the brain, accompanied by a corresponding decrease in the cortical FD. This observation can be attributed to the unique brain configuration in different sections, reflecting the anatomical features and spatial distribution of the cortex and white matter. In the anterior brain regions, where gyri are closely positioned, there is less space inside the cortical silhouette occupied by the white matter. Conversely, in subsequent sections (intersecting parietal and temporal lobes), where the cortex occupies less space compared to the frontal lobes, an increase in the space occupied by the white matter inside the cortical silhouette is observed. Therefore, this quantitative finding underscores the general trends in cross-sectional brain anatomy and the spatial distribution features of the cortex and white matter. Furthermore, it is important to note that the FD values obtained from different sections exhibited weak to moderate correlation relationships. Therefore, the correlations obtained were not strong enough to justify the assumption that the FD values can be reliably and predictably extrapolated from one section to another. In our view, it is essential to consider the identified anatomical features and variations in the FD values across different brain regions for accurate data interpretation. Among the investigated FD values, the highest correlation with age was demonstrated by the average cortex FD values of 1-4 coronal sections. Using the average FD values helps to mitigate the influence of potential outliers and variability in values, which is due to regional anatomical variability of the brain. Therefore, it is advisable to determine and verify not only the FD of individual sections but also their average values.

Concerning age-related changes, the present study revealed a significant reduction in the cortex FD values, while the white matter demonstrated weak correlations with age. Given that our study used a two-dimensional variant of fractal analysis, we attempted to compare the correlations between the cortex and white matter FD and age, as observed in our findings, with those reported by other researchers who used three-dimensional analyses [16, 26]. The mentioned studies revealed significant decrease in the cortical FD (the overall cerebral cortex FD – R = -0.732; the FD of the cortex surface – R = -0.719) [16] and weak to moderate decrease in the white matter FD (the overall white matter FD – $R^2 = 0.116$; the FD of the white matter skeleton – $R^2 = 0.202$; the FD of the white matter surface – $R^2 = 0.040$) [26]. The correlations between the FD and age obtained through three-dimensional analyses closely

matched those determined in the present study. Therefore, two-dimensional fractal analysis can be applied to uncover age-associated changes in a manner similar to its three-dimensional counterpart. Two-dimensional studies on FD changes in normal and pathological brain aging are not plentiful [15]. Specifically, a study by Kalmanti and Maris [15] reported weak to moderate correlations between the FD of the cortical contour and age (R ranging from -0.08 to -0.497). In addition, we compared our results to our previous two-dimensional studies, which were conducted on the same sample but employed different fractal analysis methods [30, 31]. Thus, the FD values of the cerebral silhouettes without segmentation into the cortex and white matter showed moderate negative correlation relationships with age (the average FD values of the coronal sections – R = -0.491) [30]. Additionally, the FD values of the outer cortical surface contour also displayed negative correlation relationships with age (the average FD values of the coronal sections – R = -0.709) [31]. Therefore, we can conclude that the FD of the overall cortex (as determined in the present study) and cortical surface [31] exhibited the strongest correlations with age among all two-dimensional FD measurements. Therefore, both the data from the present study and findings from previous studies [15, 16, 26, 31] indicate that the cortex FD significantly decreases with age, unlike the FD values of the white matter, whose correlation relationships with age were much weaker. This may suggest more pronounced atrophic changes in the cortex of the cerebral hemispheres compared to the white matter [16, 26]. Age-related changes in the cortex lead to a reduction in its thickness, smoothing of gyri surfaces, and widening of sulci, resulting in a simplification of the cortical spatial configuration and space-filling degree [9, 16]. In our opinion, these alterations contribute to the age-related decrease in the sectional area of the cortex. However, the average cortex FD value of the coronal sections, as well as the cortex FD value of the axial section, showed slightly stronger correlation relationships with age compared to the corresponding sectional area values. This distinction may be attributed not only to the reduction in space filling due to cortical thinning but also to the simplification of the spatial configuration of the cortex, amplifying the correlation of the cortex FD with age [16]. Conversely, age-related changes in the white matter of the cerebral hemispheres predominantly involve a decrease in volume (and consequently, sectional area in two-dimensional images). However, the changes in the spatial configuration of the white matter are minor, resulting in weak correlation relationships between the white matter FD and age.

Limitations

The present study encountered several limitations. Firstly, the sample studied encompassed participants from diverse age groups, although the age distribution skewed slightly towards younger ages, potentially limiting the generalizability of the findings to larger populations. Secondly, a relatively small number of brain sections were chosen for analysis. Additionally, all MR images were acquired using the same MRI scanner and protocol, necessitating potential adjustments when analyzing MR brain images obtained using different MRI scanners and protocols during segmentation of the cerebral cortex and white matter.

Conclusions

In this study, we provided fractal analysis of two-dimensional MR brain images and obtained the FD values of the cerebral cortex and white matter. The study demonstrated that the FD values of the cerebral cortex and white matter were negatively correlated, influenced by anatomical factors such as the number of gyri and cortex configuration in two-dimensional MR brain images. Specifically, brain sections with a higher degree of gyrification exhibited higher cortex FD values and lower white matter FD values. The cortex and white matter FD values showed stronger correlation relationships between the adjacent sections, although they were not sufficiently high to support the assumption that the FD values can be predictably extrapolated from one section to another. The study found that the cortex FD values obtained from two-dimensional images significantly decreased with age, while age-related changes in the white matter FD values were relatively weak. The FD approach showed more pronounced distinctions in the context of age-related changes compared to the sectional areas.

Ethical Statement

The study was conducted in compliance with the World Medical Association Declaration of Helsinki “Ethical Principles for Conducting Medical Research Involving Human Subjects”. The study received approval from the Ethics and Bioethics Committee of Kharkiv National Medical University (Minutes No. 10 dated November 7, 2018).

Informed Consent

Written informed consent was obtained from all study participants.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Financial Disclosure

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Supplement 1. Preprocessing Algorithm for Brain Magnetic Resonance Images: Cerebral Cortex and White Matter Segmentation.

The image segmentation procedure was conducted using Adobe Photoshop CS5 software to reveal the silhouettes of the cerebral cortex and white matter.

MR image transferring to the graphics editor

In the graphic editor Adobe Photoshop CS5, blank frame images were created for further work with the digital MR image. A fragment of the digital MR image corresponding to the investigated area was inserted into the pre-created frame image. During this process, the fragment was positioned so that the area of the cerebral hemispheres was entirely accommodated within the created image and did not extend beyond its frame (Fig. 1S). Image scaling was not performed, and the pixels of the copied MR image fragments were pasted as they were. The dimensions of the frame images for the investigation of the coronal sections were 512 $\times$ 400 pixels, and for the axial sections, they were 512 $\times$ 800 pixels. These dimensions were chosen to ensure that even MR brain sections with the largest dimensions could be fully accommodated within the created frame. The resolution was 128 pixels per inch.

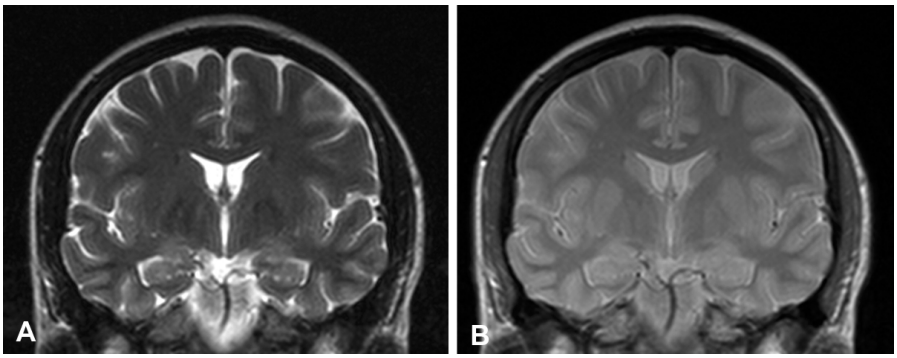

Figure 1S. Initial brain magnetic resonance images (section Coronal 2) used for segmentation: A – T2-weighted image, B – PD-weighted image.

Image Segmentation

Background removal

The first step in image segmentation involved removing background structures from the examined images (Fig. 2S). To achieve this, areas not corresponding to the assessed structure (section of the cerebral hemispheres) were manually selected and filled with white (for T2-weighted and PD-weighted images) or black color (for images obtained in the FLAIR sequence). Subsequently, image segmentation was performed, aiming to obtain binary images - those containing pixels of black and white colors: pixels of black color corresponded to the structure under investigation (the cortex or white matter), while background pixels were of white color.

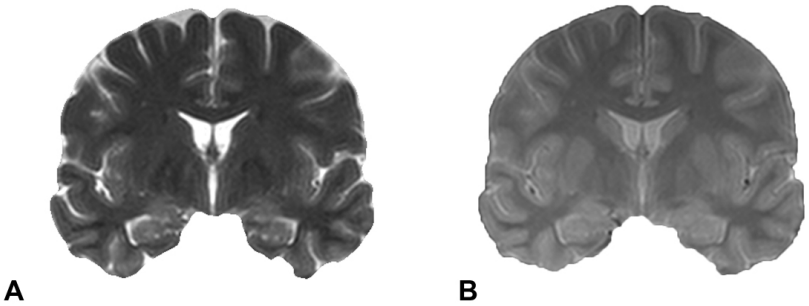

Figure 2S. Removal of the background from the T2-weighted image (A) and PD-weighted image (B).

Obtaining the silhouettes of the brain tissue as a whole

At the second stage of segmentation, silhouette images corresponding to the brain tissue as a whole were obtained (Fig. 3S). Initially, a preliminary ("rough") segmentation was conducted. For this purpose, the image with the removed background was processed using the "threshold" tool of the Adobe Photoshop CS5 software. This tool converted the image into binary according to the specified threshold brightness value for pixels: all pixels with brightness values less than

the specified threshold were colored black, while the rest were colored white. At this stage, T2-weighted images were used for segmentation of the coronal sections, which, compared to PD-weighted images, are characterized by better contrast, making it easier to reveal brain tissue as a whole and separate it from the surrounding structures. For segmentation of the axial sections, images obtained in the FLAIR sequence or T2 sequence were used. A brightness threshold value of 128 was empirically determined for T2-weighted images, while a value of 65 was used for images obtained from the FLAIR sequence. Subsequently, the obtained image was inverted. Afterwards, precise segmentation was performed through manual correction to improve the anatomical accuracy of the obtained images.

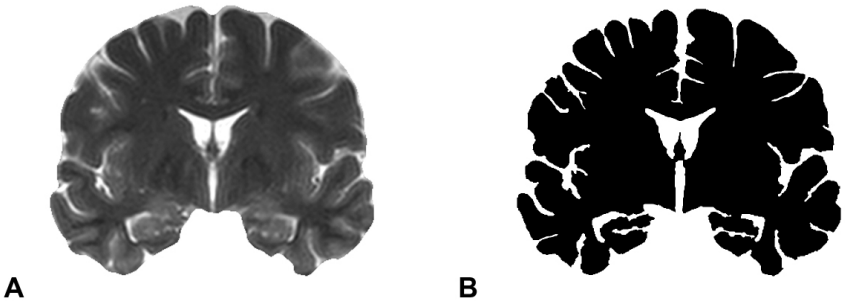

Figure 3S. Segmentation of the T2-weighted image after background removal (A) to obtain a silhouette image of the cerebral hemispheres as a whole (B).

Obtaining the silhouettes of the cerebral white matter

At the third stage of segmentation, silhouette images corresponding to the cerebral white matter were obtained (Fig. 4S). Similarly, an initial ("rough") segmentation was performed using the "threshold" tool of the Adobe Photoshop CS5 software. The brightness threshold values for pixels were set to 80 for T2-weighted images, 128 for PD-weighted images, and 105 for images obtained in the FLAIR sequence. T2-weighted and PD-weighted images were used for segmentation of the coronal sections. Since the boundary between the cortex and white matter is less distinct on T2-weighted images compared to PD-weighted images, PD-weighted images were additionally used at this stage. After "rough" segmentation, the silhouettes obtained from T2- and PD-weighted images were compared and overlaid, and manual correction of the obtained silhouettes was carried out. As our goal was to investigate the features of the configuration of the white matter as a whole, the gray matter of subcortical nuclei within the boundaries of the white matter silhouettes was included in the obtained silhouettes (Fig. 4S B).

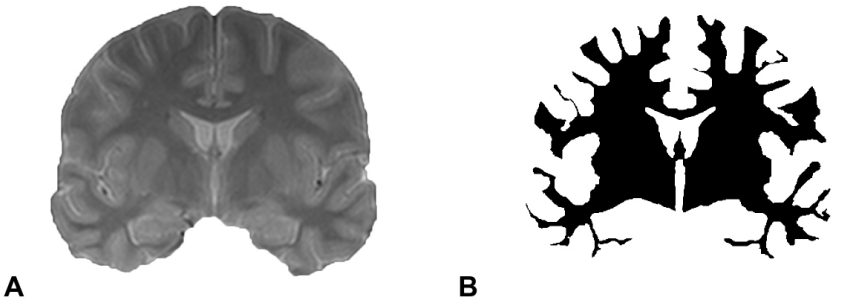

Figure 4S. Segmentation of the PD-weighted image after background removal (A) to obtain a silhouette image of the white matter (B).

Obtaining the silhouettes of the cerebral cortex

At the fourth stage of segmentation, silhouette images corresponding to the cerebral cortex were obtained (Fig. 5S). This involved overlaying silhouettes corresponding to the tissue of the cerebral hemispheres as a whole (Fig. 3S B) and the white matter (Fig. 4S B), obtained during the previous two stages. Each silhouette was placed on a separate layer, overlaying on one another (Fig. 5S A). Subsequently, for one of the layers, the blending mode was changed to "difference" (Fig. 5S B). After that, the layers were merged into one, followed by color inversion and manual correction of the obtained cortical silhouette (Fig. 5S C).

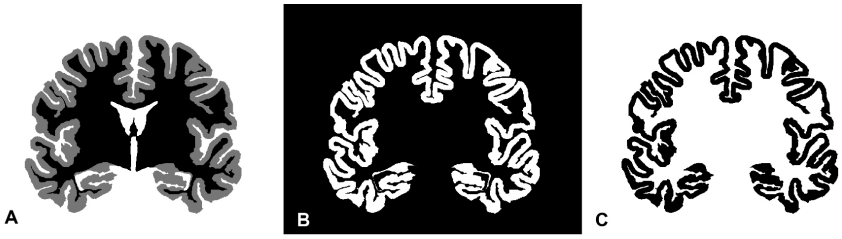

Figure 5S. Segmentation of the image to obtain a silhouette image of the cortex: A - overlaying the silhouette images of the cerebral hemispheres as a whole and the white matter; B - excluding the white matter silhouette from the cerebral hemisphere silhouette in the "difference" mode; C - inverting image B followed by manual correction to obtain a silhouette image of the cortex.

Supplement 2. Comparison of the Cortex and White Matter Fractal Dimension and Sectional Area Values Measured in Different Tomographic Sections.

Table S1. Comparison of the cortex and white matter fractal dimension and sectional area values measured in different tomographic sections.

Parameter		FD		Sectional Area	
		Cortex	White matter	Cortex	White matter
		Kruskal-Wallis test			
p values (α=0.05)		<0.001	<0.001	<0.001	<0.001
Multiple comparisons, post-hoc Dunn test					
Pair of the sections		p-values (Bonferroni corrected α=0.05/10(section pairs)=0.005)			
Coronal 1	Coronal 2	<0.001	<0.001	<0.001	<0.001
Coronal 1	Coronal 3	<0.001	<0.001	<0.001	<0.001
Coronal 1	Coronal 4	<0.001	<0.001	<0.001	<0.001
Coronal 1	Axial	<0.005	<0.001	<0.001	<0.001
Coronal 2	Coronal 3	<0.005	0.71	0.12	0.18
Coronal 2	Coronal 4	<0.001	<0.001	0.01	0.15
Coronal 2	Axial	<0.001	<0.001	<0.001	<0.001
Coronal 3	Coronal 4	0.81	<0.005	0.32	0.92
Coronal 3	Axial	<0.001	<0.001	<0.001	<0.001
Coronal 4	Axial	<0.001	<0.001	<0.001	<0.001

Supplement 3. Correlation Relationships of the Cortex and White Matter Fractal Dimension and Sectional Area Values with Additional Morphometric Parameter Derived from Euclidean Geometry.

Fig. 6S displays the correlation relationships of the parameters studied in the present work (the cortex and white matter FD and sectional area) with additional morphometric parameters derived from Euclidean geometry that were studied in the previous study [29].

The additional morphometric parameters included perimeter (P) and area (A), measured in two approaches: A: brain sections were outlined excluding sulci, yielding perimeter (P_A) and area (A_A) of the entire brain tissue; B: the entire pial surface, including sulci, was outlined, giving perimeter (P_B) and area (A_B), excluding sulci but including ventricles. From these measurements, perimeter-to-area ratios (P_A/A_A, P_B/A_B), shape factors (circularity) (SF_A, SF_B), gyrification index (P_B/P_A), and area ratio (A_B/A_A) were calculated. For a detailed analysis and descriptive data of these parameters, please refer to the cited study [29].

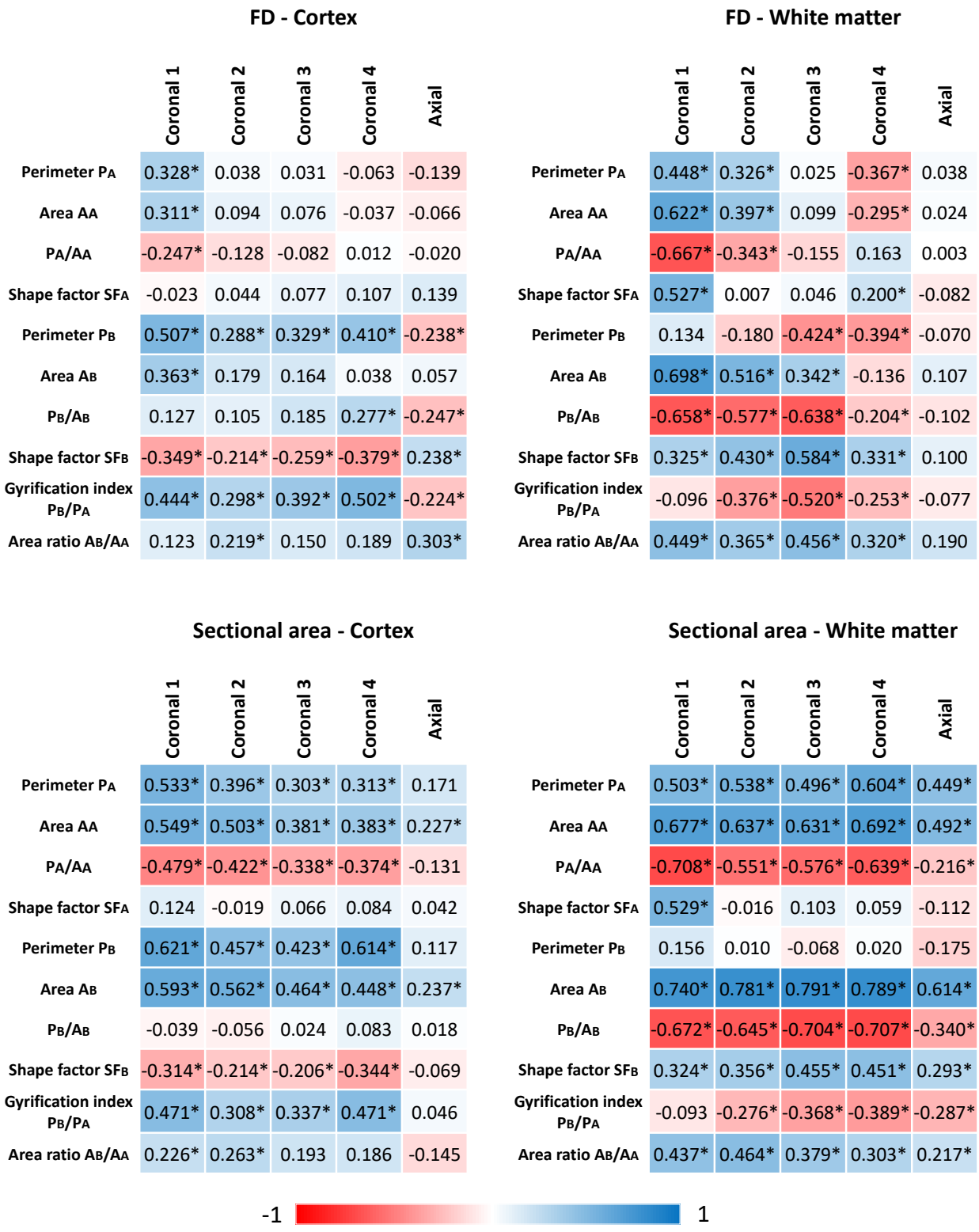

Figure 6S. Correlation matrices displaying relationships between the fractal dimension and sectional area values of the cerebral cortex and white matter and additional morphometric parameters of the cerebral hemispheres; Spearman's correlation coefficient (R) values are provided; * - $p<0.05$.