Regional Brain Volume Changes in Catholic Nuns: A Cross-Sectional Study Using Deep Learning-Based Brain MRI Segmentation

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Objective   Religious behaviors are considered as complex brain-based phenomena that may be associated with structural brain change. To identify the pattern of regional brain volume change in nuns, we investigated structural alterations in the brains of nuns using a fast processing automated segmentation method based on deep learning algorithms.

Methods   We retrospectively reviewed the medical records of the catholic sisters between the ages of 31 and 80 who are members of the charity of St. Vincent de Paul of Korea. A total of 193 asymptomatic subjects (86 nuns and 107 control subjects) received comprehensive health screening and underwent brain MRI scans. We compared cortical and sub-cortical volume between groups across multiple locations using our in-house U-Net++ deep learning-based automatic segmentation tool.

Results   Compared to the control group, the nun group displayed increased gray matter volume in the right lingual cortex, left isthmus-cingulate, posterior-cingulate, rostral-middle-frontal, superior-frontal, supramarginal, temporal-pole cortices, and bilateral pars-triangularis cortices after correction for multiple comparisons. On the other hand, the nun group showed reduced gray matter volume in the temporal and parietal regions relative to healthy controls.

Conclusion   Our study suggests that spiritual practice may affect brain structure, especially in several frontal regions involved in a higher level of insight function. **Psychiatry Investig 2022;19(9):754-762**

Keywords   Brain volume; MRI; Deep learning; Catholic nuns.

INTRODUCTION

Religiosity has an important role in health promotion and disease prevention, and many studies have provided evidence for the protective effect of religiosity on physical and mental health.[1-6] In particular, epidemiological studies have provided evidence for an inverse relationship between religiosity and

psychiatric disorders; it was reported that the risk of mental disorders such as depression[7] and dementia[8,9] was lower among nuns compared to the general population. Accordingly, religious belief and behavior are considered as complex brain-based phenomena that may be associated with functional and structural brain change. However, there is limited evidence on neurological mechanisms that could explain their relationship, and the mechanisms by which religion affects health status are still unknown.

The recent advance in neuroimaging techniques can help investigators to non-invasively analyze brain structure and function in various conditions. Thus, neuroimaging study may provide a possible explanation for the exact neurological mechanism of religiosity in health. Earlier studies using magnetic resonance imaging (MRI) have shown that religiosity is associated with alterations in brain structure and function in the

general population.[10-12] Several studies have reported that individuals with a higher belief in religion or spirituality had a higher frontal cortical thickness[11,13] and decreased default mode network connectivity.[14] In contrast, other studies found greater atrophy in the hippocampus[15] and decreased temporal lobe blood flow[16] in specific religious groups. This conflicting result might be due to the difficulty of modeling clinical studies of religious intervention.

Most of the previous studies have focused on the acute effects of religious practices, and few studies have evaluated the long-term effects of religion on the differences in brain structure or function. It is not easy to accurately measure religious activity, and there is a subtle difference between participation in religious services and religiosity. Given the uniform religious life of the clergy, studies on clergy such as nuns and priests can overcome these limitations. However, no neuroimaging studies have been reported in nuns compared to the general population so far. A few studies on neurological changes in nuns had been limited to a simple head size measurement[17] or post-mortem brain biopsies.[18,19] Considering head circumference is an unreliable measure of brain volume, more accurate analysis using magnetic resonance scans may lead to stronger associations between religiosity and neuroanatomy.

The present study aimed to identify the pattern of regional brain volume change in nuns. Thus, we investigated structural alterations in the brains of nuns using a fast processing automated segmentation method based on deep learning algorithms.

METHODS

Subjects

This study was conducted by retrospectively checking the medical records of the catholic sisters between the ages of 31 and 80 who are members of the charity of St. Vincent de Paul of Suwon, Korea. The control group was recruited from non-cleric females who underwent a health check-up during the same period. All subjects received comprehensive health screening and underwent brain MRI scans at the health promotion center of St. Vincent's Hospital in South Korea between January 2010 and December 2019. General health check-ups were provided biennially for all subjects by the Korean National Health Insurance Service (NHIS), and additional periodic health examinations were performed at the hospital affiliated with the convent. All subjects were excluded if they had concurrent or a history of diseases that could affect brain structure or function, such as stroke, traumatic brain injury, brain tumor, neurodegenerative disorders, anxiety, and depression, or if they were taking or had previously taken psychotropic medications that could affect the nervous system. Those who with estrogen replacement therapy in the

postmenopausal state were also excluded. To rule out cognitive impairment, the Korean Dementia Screening Questionnaire-Cognition (KDSQ-C) was applied to individuals aged 66 yr. In addition, participants with a history of all-cause dementia (International Classification of Disease, 10th Revision [ICD-10] codes: F00, F01, F02, F03, G23.1, G30, G31) during the follow-up period from 2010 to 2022 were excluded.

After excluding subjects with missing or duplicate values in the observation result that was required for the study, a total of 193 asymptomatic subjects (86 nuns and 107 normal control subjects) were selected for the study. This study was approved by the Research Ethics Committee of the College of Medicine, The Catholic University of Korea (IRB Number: XC20RIDI0058, approved May 2020).

Brain imaging and data processing

MR image acquisition

T1-weighted optimized high-resolution 3D magnetization-prepared rapid acquisitions of gradient echo (3D-MPRAGE) images of the brain were acquired using a 3T scanner equipped with a 32-channel head coil (Verio; Siemens, Erlangen, Germany). Image acquisition parameters were as follows: number of slices, 160; voxel resolution, 0.49×0.49 mm^2; thickness, 1 mm; flip angle (FA), 9°; field of view (FOV), 250×250 mm^2; repetition time (TR), 1,900 ms; inversion time (TI), 900 ms; and echo time (TE), 2.46 ms.

Brain MRI segmentation

This study utilized our in-house deep learning-based automatic segmentation tool. T1-weighted MRIs were preprocessed by the U-Net++ deep learning-based segmentation processing.[20] We used our deep learning method for extracting numerical data into a table format. The set of 97 layer sub-volume-based features used for the training procedure are described in Supplementary Table 1 (in the online-only Data Supplement). Figure 1 shows the segmentation result of brain sub-volumes. The detailed algorithms for our deep learning-based segmentation methods are described in the Supplemental Material (in the online-only Data Supplement).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences version 21 (IBM Co., Armonk, NY, USA) and were presented as means±standard deviation. Independent t-tests were used to compare characteristics at baseline between the nun and control groups. A two-tailed p-value<0.05 was considered statistically significant. In addition, to assess between-group differences in structural measures, the volumes of the 97 automatically segmented brain regions were import-
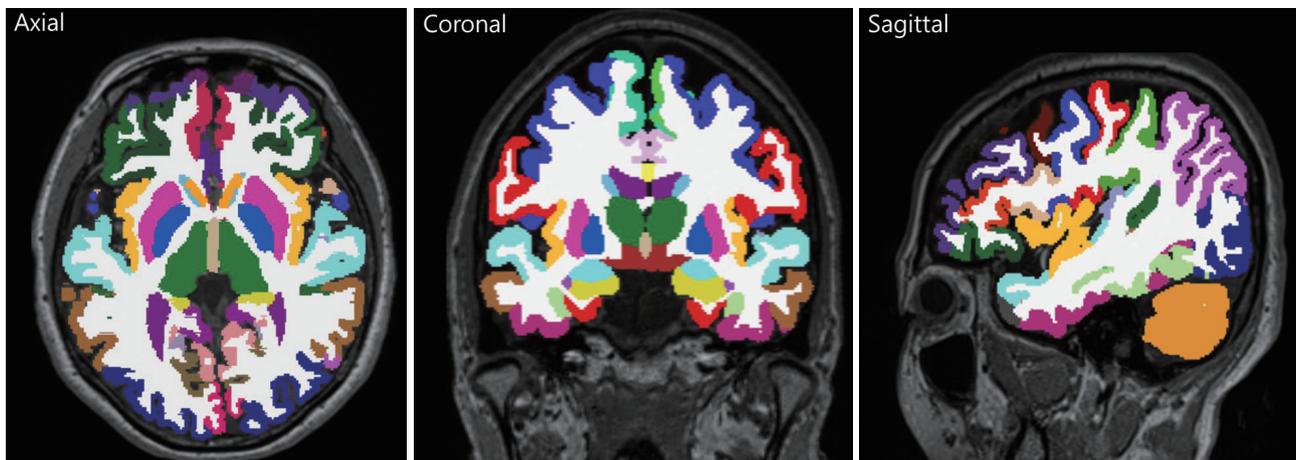
Figure 1. 97-layer brain segmentation results.

ed into the SPSS software for statistical analyses, and analysis of covariance (ANCOVA) was performed in a general linear model of SPSS. Group variables (nun vs. control group) were included as independent variables, with the extracted cortical and subcortical volume (in cm^3) as dependent variables. We included age, educational level, and total intracranial volume (TICV) as covariates to prevent potential confounding effects. For multiple comparisons, Bonferroni corrections were applied to minimize the type I error risk by dividing our p-value by the number of segmented areas: $p<0.05/97$ (number of comparisons in bilateral hemispheres)$=5.15\times10^{-4}$.

RESULTS

Baseline characteristics of the study participants

Table 1 summarizes the baseline characteristics of the study participants. Although there was no significant difference in education level between the two groups, participants in the nun group were older than those in the control group (61.41± 8.48 years vs. 54.56±10.86 years, p<0.001). Body mass index (26.97±4.51 kg/m^2 vs. 23.36±3.47 kg/m^2, p<0.001) and waist circumference (91.60±10.46 cm vs. 79.37±9.74 cm, p<0.001) were significantly higher in the control group than in the nun group. The TICV was also greater in the control group than in the nun group (1,394.95±92.49 cm^3 vs. 1,302.61±235.1 cm^3, p<0.001). The glucose, lipid profile, alanine aminotransferase (ALT), and gamma-glutamyl transferase (γ-GTP) were not significantly different between groups. However, the systolic and diastolic blood pressure was lower in the nun group than in the control group (p<0.05).

Regional brain volume differences between the nun and control groups

For the cortical and subcortical volume analyses, the AN-COVA, adjusted for age, education, and TICV revealed a sig-

Table 1. General characteristics of the study participants (N=193)

Characteristics	Nun (N=86)	Control (N=107)	p-value*
Age (yr)	61.41±8.48	54.56±10.86	<0.001
BMI (kg/m^2)	23.36±3.47	26.97±4.51	<0.001
WC (cm)	79.37±9.74	91.60±10.46	<0.001
SBP (mm Hg)	116.15±14.51	122.58±19.94	0.031
DBP (mm Hg)	68.03±8.71	76.60±8.91	<0.001
Glucose (mg/dL)	101.38±31.52	102.93±17.61	0.666
TC (mg/dL)	195.39±37.67	199.74±35.90	0.420
HDL cholesterol (mg/dL)	55.65±12.56	52.00±11.34	0.038
LDL cholesterol (mg/dL)	120.09±31.00	125.40±34.31	0.278
Triglyceride (mg/dL)	108.51±45.69	117.46±66.70	0.276
ALT (U/L)	26.06±14.20	27.08±12.56	0.595
AST (U/L)	20.13±8.04	28.71±18.55	<0.001
γ-GTP (IU/L)	24.88±17.63	26.12±21.23	0.670
Education (yr)	11.71±1.07	11.41±4.01	0.462
TICV (cm^3)	1,302.61±235.10	1,394.95±92.49	0.001

Data were presented as mean±standard deviation values for continuous variables. *statistical significance was tested using independent t-tests. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, gamma-glutamyl transferase; TICV, total intracranial volume

nificant volume increase in the left isthmus-cingulate, pars-triangularis, posterior-cingulate, rostral-middle-frontal, superior-frontal, supramarginal, temporal-pole, and right lingual, pars-triangularis cortices in the nun group compared with the control group after correction for multiple comparisons (p<0.0005, Figure 2A and Table 2). The volume of the cerebellum-white-

matter, corpus callosum, and right thalamus was also higher in the nun group compared to the control group (p<0.0005).

On the other hand, a group comparison analysis of regional cortical volume between the nun and control groups revealed a significant reduction in the cortical volume of the nun group in the left entorhinal, inferior-temporal, middle-temporal, transverse-temporal cortices, and right bankssts, frontal-pole, inferior-temporal, pars-orbitalis, precentral, superior-parietal cortices compared with those of the control group (p<0.0005; Figure 2B and Table 3). For the subcortical
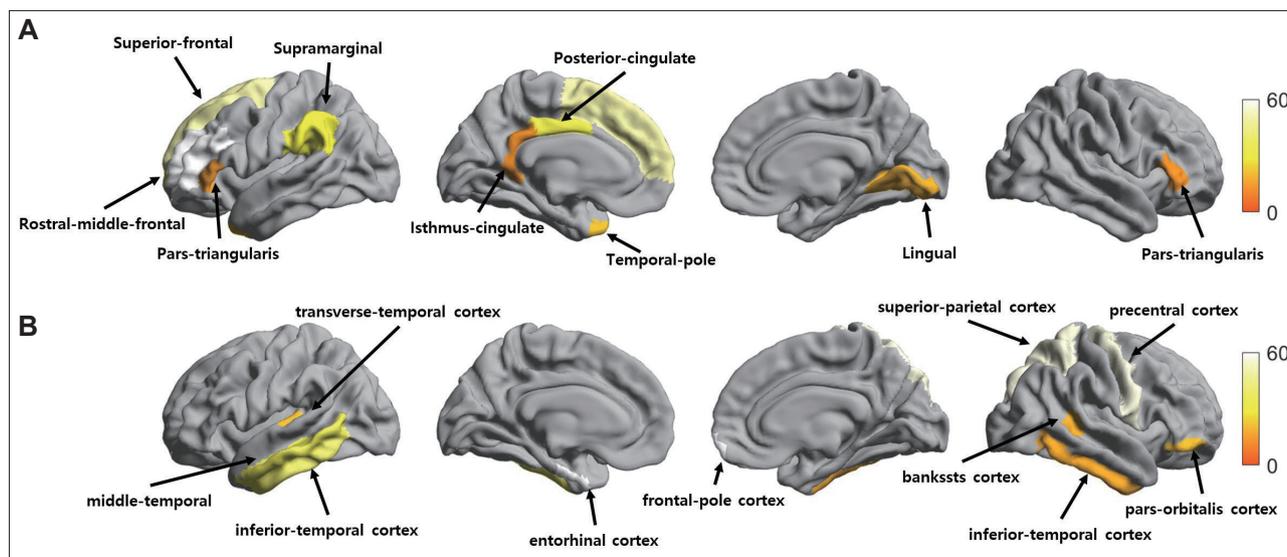
Figure 2. Regional brain volume differences between the nun and control groups. A: Statistical maps showing a significant volume increase in the left isthmus-cingulate, pars-triangularis, posterior-cingulate, rostral-middle-frontal, superior-frontal, supramarginal, temporal-pole, and right lingual, pars-triangularis cortices in the nun group compared with the control group. B: A cortical volume reduction in the nun group relative to the control group in the left entorhinal, inferior-temporal, middle-temporal, transverse-temporal, and right bankssts, frontal-pole, inferior-temporal, pars-orbitalis, precentral, superior-parietal cortices. Statistical significance was tested using analysis of covariance adjusted for age, education, and total intracranial volume. Regions that remained significant after correction for multiple comparisons are displayed (p<0.0005, Bonferroni correction).

Table 2. Regional brain volume where a significant atrophy was observed in the control group compared to nuns

Region	Subcortical and cortical volume (cm³)		F	p-value
	Nun (N=86)	Control (N=107)		
Left				
Isthmus-cingulate	2.30±0.39	2.15±0.28	12.97	<0.0005
Pars-triangularis	3.37±0.54	3.11±0.54	14.32	<0.0005
Posterior-cingulate	2.77±0.36	2.44±0.31	36.01	<0.0005
Rostral-middle-frontal	13.73±1.70	10.70±1.56	144.68	<0.0005
Superior-frontal	19.36±1.85	17.59±1.58	50.18	<0.0005
Supramarginal	10.73±1.69	9.47±1.24	31.67	<0.0005
Temporal-pole	2.74±0.64	2.28±0.36	20.56	<0.0005
Cerebellum-white-matter	12.53±2.05	11.27±1.16	28.79	<0.0005
Right				
Lingual	6.68±0.85	6.22±0.79	18.56	<0.0005
Pars-triangularis	3.89±0.54	3.58±0.71	12.71	<0.0005
Thalamus	7.08±0.57	6.84±0.71	16.70	<0.0005
Cerebellum-white-matter	12.22±1.96	10.82±1.22	35.01	<0.0005
Corpus callosum	3.83±0.65	3.50±0.39	15.14	<0.0005

Statistical significance was tested using analysis of covariance adjusted for age at magnetic resonance imaging (MRI) scan, education, and total intracranial volume (TICV). Values are mean±standard deviation. Regions that remained significant after Bonferroni correction are listed; 97 regions of the brain, corrected p<0.05/97=5.15×10⁻⁴

Table 3. Adjusted mean atrophy in regional brain volume for nuns when compared to the control group

Region	Brain volume (cm³)		F	p-value
	Nun (N=86)	Control (N=107)		
Left				
Entorhinal cortex	1.58±0.31	2.05±0.30	79.79	<0.0005
Inferior-temporal cortex	9.99±1.59	11.36±1.22	41.96	<0.0005
Middle-temporal	10.89±1.38	12.53±1.51	38.72	<0.0005
Transverse-temporal cortex	0.97±0.20	1.13±0.28	19.93	<0.0005
Accumbens-area	0.38±0.06	0.42±0.05	15.74	<0.0005
Caudate	3.20±0.40	3.56±0.40	48.58	<0.0005
Putamen	4.10±0.41	4.91±0.52	111.03	<0.0005
Right				
Caudate	3.25±0.38	3.48±0.41	14.99	<0.0005
Pallidum	1.82±0.22	2.23±0.24	136.37	<0.0005
Putamen	4.31±0.41	4.75±0.60	24.71	<0.0005
Ventraldc	3.79±0.30	4.10±0.31	45.48	<0.0005
Bankssts cortex	1.07±0.27	1.96±0.38	17.56	<0.0005
Frontal-pole cortex	0.98±0.14	1.45±0.25	133.09	<0.0005
Inferior-temporal cortex	10.26±1.25	11.05±1.39	16.36	<0.0005
Pars-orbitalis cortex	2.50±0.33	2.80±0.36	20.03	<0.0005
Precentral cortex	12.47±1.06	13.46±1.06	55.89	<0.0005
Superior-parietal cortex	11.59±1.35	12.87±1.41	37.41	<0.0005

Values are mean±standard deviation. The general linear model (GLM) was adjusted for age, sex, educational level, and total intracranial volume as covariates. Regions that remained significant after Bonferroni correction are listed; 97 regions of the brain, corrected $p<0.05/97=5.15×10^{-4}$

structures, the nun group showed a significant reduction in the left accumbens, caudate, putamen, and right caudate, pallidum, putamen compared to the control group ($p<0.0005$).

DISCUSSION

In the present study, we investigated regional brain volume differences between the nun and control groups using a deep learning algorithm. We found group differences in several brain regions; compared to the control group, the nun group displayed increased gray matter volume in the right lingual cortex, left isthmus-cingulate, posterior-cingulate, rostral-middle-frontal, superior-frontal, supramarginal, temporal-pole cortices and bilateral pars-triangularis cortices. On the other hand, the nun group showed reduced gray matter volume in the temporal, and parietal regions relative to healthy controls. To the best of our knowledge, this is the first study evaluating the brain structural changes in nuns using MRI.

Although religious activity appears to improve physical and mental health, the exact neurological mechanisms by which religiosity affects health are uncertain. Identifying differences in regional brain volume between nuns and controls might explain the role of religion in brain structural changes. How-

ever, few studies had examined the relationship between religiosity and structural neuroanatomy so far. A small number of studies have found that religious activity is correlated with the volume of specific brain regions, but the focus has been limited to epilepsy patients[21] and short-term religious practices.[22] The current study extends these researches by examining whole-brain segmentation in nuns. The result of our study might help to understand the relationship between brain structure and religion.

In this study, the nun group exhibited significantly increased cortical volume for a number of frontal regions, namely the left rostral-middle-frontal, superior-frontal, and bilateral pars-triangularis cortices. Of these regions, the dorsolateral prefrontal cortex (DLPFC), corresponding to the rostral middle frontal region, plays a key role in the processing of cognition[23] and emotions.[24] Thus, the DLPFC is associated with many neuropsychiatric diseases, and the hypertrophy of this region might explain the low prevalence of dementia[8,9] and mood disorders[7] in the nuns. Furthermore, the DLPFC plays a crucial role in a higher level of insight functions such as executive functioning, concept flexibility, and self-monitoring.[25] Interestingly, a previous fMRI study investigating brain activity engaged during self-reflective introspection showed activated

regions in the superior frontal gyrus as well as bilateral activation of the inferior frontal gyrus, which includes the pars triangularis. This is in keeping with the findings of our study and supports the hypothesis that the increased frontal lobe volume of nuns in this study might be the result of lifelong introspection and self-reflection in the nuns. In fact, previous studies showed that reduced frontal lobe volume caused a failure of metacognition[26] and a lack of insight in neuropsychiatric disorders.[27] A combination of structural, functional imaging data and clinical information gathered longitudinally will be required to understand the mechanisms by which the frontal lobe hypertrophy in nuns influences the neuropsychiatric outcome.

The control group showed smaller cortical volume in frontal regions, as well as less cerebellar white matter volume, when compared to nuns. Considering that the frontal regions found in this study (i.e., rostral-middle-frontal, superior-frontal, and pars-triangularis) are important brain regions for executive control, including inhibitory functioning, attention, impulsivity, and self-regulation,[28] these structural brain differences might explain less substance abuse and better self-control in nuns. Consistent with our findings, Squeglia et al.[29] had reported volume reduction of the frontal cortex and cerebellar white matter in adolescent heavy drinkers.

The pars triangularis (BA45), which is part of Broca's area is a speech and language-related area, and is known to be an overlapping region for semantic and syntactic functions.[30] In particular, the pars triangularis has been shown to be involved in understanding God's intent and resolving the negative emotional significance of his lack of involvement. The hypertrophy of this area in the present study might be due to the religious lifestyle of the nuns, who spend a lot of time reading the Bible and praying. Another possible explanation is the prosocial characteristics of nuns. In general, nuns try to overcome various social prejudices and stereotyping through pro-social dialogue with heterogeneous subjects.

In this study, the left DLPFC as well as pars triangularis displayed increased cortical volume in the nun group compared with controls. Experimental evidence shows that prosocial choices positively relate to local gray matter volume and the activation of brain areas that control selfish impulsive drives, such as the DLPFC.[31] Thus, human prosociality is a consequence of cognitive control of selfish impulses. Pro-social communications between heterogeneous dyads are also relevant to recently proposed coding models relating the theory of mind with predictive processes.[32] The theory of mind refers to the capacity to understand other people by ascribing mental states to them. Possessing a functional theory of mind is considered crucial for success in everyday human social interactions and is used when analyzing, judging, and infer-

ring others' behaviors. The increased brain regions in the nuns in this study (i.e., DLPFC and pars-triangularis) overlapped with areas commonly activated in the theory of mind. The results of our study explain the altruistic and prosocial tendencies of the nuns. Similar to our findings, Descorbeth et al.[33] reported that left DLPFC and pars triangularis were more active during speech dialogue in high than in low-disparity groups.

Our results showed significant decreases in gray matter volume located within the temporal brain region. To date, a few neurotheological studies have reported the functional and structural brain changes according to religious and spiritual performance.[16,34] Previous data have shown that the temporal lobe, which is involved in the activation of associative and spatial orientations, is inactivated during religious experiences, whereas the area of attention is activated, particularly around the prefrontal cortex.[35] Newberg et al.[16] studied changes in blood flow rate in the brain during prayers of three Franciscan nuns. In this study, the authors found a lack of self-awareness during prayer due to decreased temporal lobe blood flow. Other researchers have also cited the temporal lobe as a potential site for mystical experiences and religious feelings.[36,37] In line with these findings, the results of our study showed the increased frontal gray matter volume and reduced temporal cortical volume in nuns compared to the control group. Together with the previous findings, our present results may indicate the greater difficulty and procedural demands for imagining and processing the intent of supernatural agents in non-clerics. Consistent with our findings, Owen et al.[15] reported hippocampal atrophy in participants reporting a life-changing religious experience, particularly among Catholics. Kapogiannis et al.[22] also reported that the temporal to occipital pathway was more active in non-religious compared to religious subjects, suggesting a lack of religious beliefs related to supernatural agents or God. Another possible explanation for temporal atrophy in nun groups might be related to stress. Those who struggle with their beliefs tend to experience higher levels of stress. From a psychoneuroendocrine perspective, this might cause a release of stress hormones that are known to reduce the regional brain volume over time. However, limited medical record data may not accurately reflect patient's future risk of dementia; although we examined changes in mood and cognition at health screenings annually, follow-up was relatively short, considering the time for dementia occurrence. Furthermore, considering causes of dementia are multifactorial and heterogeneous, the current study did not include some risk factors of dementia, such as various genetic (i.e., apolipoprotein E ε4 allele) and familial factors. However, periodic health examinations were continuously implemented for all participants, and thus, minimized the possibility of misclassification. Further prospective studies

with longer follow-up periods are required to strengthen the evidence for this conclusion and to determine the underlying mechanism of association between religiosity and brain structure.

The positive effects of religion on health were known as psychological optimism, healthy lifestyles such as low rates of smoking and alcohol drinking,[38] sufficient social networks through religious activity, and reduced exposure to risky sexual behavior.[39] However, the mechanisms by which religiosity influences brain structure are unknown. One possible mechanism of the cortical changes in nuns is the altered activity of brain-derived neurotrophic factor (BDNF) by spiritual practices. A previous study reported that depressed patients with high religiosity had significantly higher serum BDNF than do low religiosity.[40] BDNF affects synaptic plasticity and dendritic and neuronal fiber growth, promotes neuronal survival, and has been considered a biological marker of brain neuroplasticity.[41,42] Indeed, it was reported that a higher religiosity or spirituality was associated with greater cortical thickness.[11] Moreover, another study had shown that the BDNF level was inversely correlated with cortical atrophy in depressed individuals.[43] Thus, religiosity might affect brain structure in catholic nuns by increasing cortical neuroplasticity and neuroprotection through BDNF. However, we did not measure BDNF in this study; thus, we could not identify the potential mechanisms through which BDNF affects brain plasticity.

There are some limitations in this study. First, we performed a cross-sectional study of healthy subjects without psychiatric problems, and we could not determine the causality between structural brain changes and mental disorders. Second, given the small sample size in this preliminary study, the generalizability of our findings should be approached with caution. However, our study design has strength in allowing for minimizing potential confounding factors. A wide variety of factors that confound most epidemiologic studies were eliminated or minimized in the present study because of the relative homogeneity of the sisters' environments and lifestyles. Participants in this study had the same reproductive and marital histories; had same religious backgrounds; had similar social networks, activities, and support; did not smoke or consume excessive alcohol; had similar education level, occupations, income, and socioeconomic status; all lived in the same houses; ate food prepared in the same kitchens; and had comparable access to similar preventive and medical care services. Moreover, we analyzed changes in brain structure and intrinsic religious beliefs, which was different from earlier studies in which only attendance at religious services and religious denominations were investigated. It was known that intrinsic religious beliefs, rather than externalized components of religiosity, were associated with psychiatric disease.[11] Thus, the results of our study provide robust evidence for the association between religiosity and neuroanatomy. Considering brain atrophy may be associated with many neuropsychiatric disorders, further prospective studies are required to evaluate the relationship between brain structural changes in nuns and clinical outcomes over time.

In conclusion, our study provides a unique model to explain the role of religion in brain structure. The results of our study suggest that spiritual practice may affect cortical and subcortical brain volume, especially in a number of frontal regions involved in a higher level of insight function. Although these results require further study, locating changes in brain regions related to spiritual practice may provide researchers with another clue into how spirituality and religiosity contribute to mental health benefits. The results of the present study should be considered preliminary, and further studies are needed to reveal that the changes in specific brain areas in nuns may serve as a compensatory or protective mechanism against certain psychiatric diseases. Nevertheless, the unique characteristics of our study, which minimizes potential confounding factors, strengthen the relationship between religiosity and brain structural change. The combination of brain imaging and clinical information collected longitudinally will be required to understand the dynamic mechanisms by which the structural brain changes in nuns influences health outcome.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.30773/pi.2022.0165.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

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REFERENCES

1. Abdel-Khalek AM, Lester D. A significant association between religiosity and happiness in a sample of Kuwaiti students. Psychol Rep 2009; 105:381-382.

2. Haslam SA, Jetten J, Postmes T, Haslam C. Social identity, health and well-being: an emerging agenda for applied psychology. Appl Psychol 2009;58:1-23.

3. Koenig HG, Cohen HJ, George LK, Hays JC, Larson DB, Blazer DG. Attendance at religious services, interleukin-6, and other biological parameters of immune function in older adults. Int J Psychiatry Med 1997; 27:233-250.

4. Lutgendorf SK, Russell D, Ullrich P, Harris TB, Wallace R. Religious participation, interleukin-6, and mortality in older adults. Health Psychol 2004;23:465-475.

5. Zullig KJ, Ward RM, Horn T. The association between perceived spirituality, religiosity, and life satisfaction: the mediating role of self-rated health. Soc Indic Res 2006;79:255-274.

6. Coruh B, Ayele H, Pugh M, Mulligan T. Does religious activity improve health outcomes? A critical review of the recent literature. Explore (NY) 2005;1:186-191.

7. Koenig H, Koenig HG, King D, Carson VB. Handbook of religion and health. New York: Oxford University Press; 2012.

8. Keohane K, Balfe M. The Nun Study and Alzheimer's disease: quality of vocation as a potential protective factor? Dementia (London) 2019;18: 1651-1662.

9. White LR, Edland SD, Hemmy LS, Montine KS, Zarow C, Sonnen JA, et al. Neuropathologic comorbidity and cognitive impairment in the Nun and Honolulu-Asia Aging Studies. Neurology 2016;86:1000-1008.

10. Li X, Weissman M, Talati A, Svob C, Wickramaratne P, Posner J, et al. A diffusion tensor imaging study of brain microstructural changes related to religion and spirituality in families at high risk for depression. Brain Behav 2019;9:e01209.

11. Miller L, Bansal R, Wickramaratne P, Hao X, Tenke CE, Weissman MM, et al. Neuroanatomical correlates of religiosity and spirituality: a study in adults at high and low familial risk for depression. JAMA Psychiatry 2014;71:128-135.

12. Kapogiannis D, Deshpande G, Krueger F, Thornburg MP, Grafman JH. Brain networks shaping religious belief. Brain Connect 2014;4:70-79.

13. Svob C, Liu J, Wickramaratne P, Hao X, Talati A, Kayser J, et al. Neuroanatomical correlates of familial risk-for-depression and religiosity/spirituality. Spiritual Clin Pract (Wash D C) 2017;4:32-42.

14. Svob C, Wang Z, Weissman MM, Wickramaratne P, Posner J. Religious and spiritual importance moderate relation between default mode network connectivity and familial risk for depression. Neurosci Lett 2016; 634:94-97.

15. Owen AD, Hayward RD, Koenig HG, Steffens DC, Payne ME. Religious factors and hippocampal atrophy in late life. PLoS One 2011;6:e17006.

16. Newberg A, Pourdehnad M, Alavi A, d'Aquili EG. Cerebral blood flow during meditative prayer: preliminary findings and methodological issues. Percept Mot Skills 2003;97:625-630.

17. Mortimer JA, Snowdon DA, Markesbery WR. Head circumference, education and risk of dementia: findings from the Nun study. J Clin Exp Neuropsychol 2003;25:671-679.

18. Mortimer JA. The Nun Study: risk factors for pathology and clinical-

19. Mortimer JA, Gosche KM, Riley KP, Markesbery WR, Snowdon DA. Delayed recall, hippocampal volume and Alzheimer neuropathology: findings from the Nun Study. Neurology 2004;62:428-432.

20. Lee M, Kim J, Ey Kim R, Kim HG, Oh SW, Lee MK, et al. Split-attention U-Net: a fully convolutional network for robust multi-label segmentation from brain MRI. Brain Sci 2020;10:974.

21. Wuerfel J, Krishnamoorthy ES, Brown RJ, Lemieux L, Koepp M, Tebartz van Elst L, et al. Religiosity is associated with hippocampal but not amygdala volumes in patients with refractory epilepsy. J Neurol Neurosurg Psychiatry 2004;75:640-642.

22. Kapogiannis D, Barbey AK, Su M, Krueger F, Grafman J. Neuroanatomical variability of religiosity. PLoS One 2009;4:e7180.

23. Nathaniel-James DA, Frith CD. The role of the dorsolateral prefrontal cortex: evidence from the effects of contextual constraint in a sentence completion task. Neuroimage 2002;16:1094-1102.

24. Tekin S, Cummings JL. Frontal-subcortical neuronal circuits and clinical neuropsychiatry: an update. J Psychosom Res 2002;53:647-654.

25. Morgan KD, Dazzan P, Morgan C, Lappin J, Hutchinson G, Suckling J, et al. Insight, grey matter and cognitive function in first-onset psychosis. Br J Psychiatry 2010;197:141-148.

26. David AS, Bedford N, Wiffen B, Gilleen J. Failures of metacognition and lack of insight in neuropsychiatric disorders. Philos Trans R Soc Lond B Biol Sci 2012;367:1379-1390.

27. Asmal L, du Plessis S, Vink M, Chiliza B, Kilian S, Emsley R. Symptom attribution and frontal cortical thickness in first-episode schizophrenia. Early Interv Psychiatry 2018;12:652-659.

28. Fjell AM, Walhovd KB, Brown TT, Kuperman JM, Chung Y, Hagler DJ Jr, et al. Multimodal imaging of the self-regulating developing brain. Proc Natl Acad Sci U S A 2012;109:19620-19625.

29. Squeglia LM, Rinker DA, Bartsch H, Castro N, Chung Y, Dale AM, et al. Brain volume reductions in adolescent heavy drinkers. Dev Cogn Neurosci 2014;9:117-125.

30. Vigneau M, Beaucousin V, Hervé PY, Duffau H, Crivello F, Houdé O, et al. Meta-analyzing left hemisphere language areas: phonology, semantics, and sentence processing. Neuroimage 2006;30:1414-1432.

31. Steinbeis N, Bernhardt BC, Singer T. Impulse control and underlying functions of the left DLPFC mediate age-related and age-independent individual differences in strategic social behavior. Neuron 2012;73:1040-1051.

32. Koster-Hale J, Saxe R. Theory of mind: a neural prediction problem. Neuron 2013;79:836-848.

33. Descorbeth O, Zhang X, Noah JA, Hirsch J. Neural processes for live pro-social dialogue between dyads with socioeconomic disparity. Soc Cogn Affect Neurosci 2020;15:875-887.

34. Newberg AB. The neuroscientific study of spiritual practices. Front Psychol 2014;5:215.

35. Beauregard M. The emerging field of spiritual neuroscience: an interview with Mario Beauregard, PhD. Interview by Sheldon Lewis. Adv Mind Body Med 2008;23:20-23.

36. Mishra SK, Togneri E, Tripathi B, Trikamji B. Spirituality and religiosity and its role in health and diseases. J Relig Health 2017;56:1282-1301.

37. Aaen-Stockdale C. Neuroscience for the soul. Psychologist 2012;25: 520-523.

38. Michalak L, Trocki K, Bond J. Religion and alcohol in the U.S. national alcohol survey: how important is religion for abstention and drinking? Drug Alcohol Depend 2007;87:268-280.

39. McCree DH, Wingood GM, DiClemente R, Davies S, Harrington KF. Religiosity and risky sexual behavior in African-American adolescent females. J Adolesc Health 2003;33:2-8.

40. Mosqueiro BP, Fleck MP, da Rocha NS. Increased levels of brain-derived neurotrophic factor are associated with high intrinsic religiosity among depressed inpatients. Front Psychiatry 2019;10:671.

41. Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML. BDNF as a biomarker for successful treatment of mood

18. pathologic correlations. Curr Alzheimer Res 2012;9:621-627.

disorders: a systematic & quantitative meta-analysis. J Affect Disord 2015;174:432-440.

42. Legge RM, Sendi S, Cole JH, Cohen-Woods S, Costafreda SG, Simmons A, et al. Modulatory effects of brain-derived neurotrophic factor Val66Met polymorphism on prefrontal regions in major depressive disorder. Br J Psychiatry 2015;206:379-384.

43. Na KS, Won E, Kang J, Chang HS, Yoon HK, Tae WS, et al. Brain-derived neurotrophic factor promoter methylation and cortical thickness in recurrent major depressive disorder. Sci Rep 2016;6:21089.

Supplementary Table 1. Summary of brain sub-volumes

Left	Right	Center
Left-cerebral-white-matter	Right-cerebral-white-matter	Corpus-callosum
Left-lateral-ventricle	Right-lateral-ventricle	3rd-ventricle
Left-inferior-lateral-ventricle	Right-inferior-lateral-ventricle	4th-ventricle
Left-cerebellum-white-matter	Right-cerebellum-white-matter	
Left-cerebellum-cortex	Right-cerebellum-cortex	
Left-thalamus	Right-thalamus	
Left-caudate	Right-caudate	
Left-putamen	Right-putamen	
Left-pallidum	Right-pallidum	
Left-hippocampus	Right-hippocampus	
Left-amygdala	Right-amygdala	
Left-accumbens-area	Right-accumbens-area	
Left-ventralDC	Right-ventralDC	
Left-choroid-plexus	Right-choroid-plexus	
Ctx-left-bankssts	Ctx-right-bankssts	
Ctx-left-caudal-anterior-cingulate	Ctx-right-caudal-anterior-cingulate	
Ctx-left-caudal-middle-frontal	Ctx-right-caudal-middle-frontal	
Ctx-left-cuneus	Ctx-right-cuneus	
Ctx-left-entorhinal	Ctx-right-entorhinal	
Ctx-left-fusiform	Ctx-right-fusiform	
Ctx-left-inferior-parietal	Ctx-right-inferior-parietal	
Ctx-left-inferior-temporal	Ctx-right-inferior-temporal	
Ctx-left-isthmus-cingulate	Ctx-right-isthmus-cingulate	
Ctx-left-lateral-occipital	Ctx-right-lateral-occipital	
Ctx-left-lateral-orbito-frontal	Ctx-right-lateral-orbito-frontal	
Ctx-left-lingual	Ctx-right-lingual	
Ctx-left-medial-orbito-frontal	Ctx-right-medial-orbito-frontal	
Ctx-left-middle-temporal	Ctx-right-middle-temporal	
Ctx-left-parahippocampal	Ctx-right-parahippocampal	
Ctx-left-paracentral	Ctx-right-paracentral	
Ctx-left-pars-opercularis	Ctx-right-pars-opercularis	
Ctx-left-pars-orbitalis	Ctx-right-pars-orbitalis	
Ctx-left-pars-triangularis	Ctx-right-pars-triangularis	
Ctx-left-pericalcarine	Ctx-right-pericalcarine	
Ctx-left-postcentral	Ctx-right-postcentral	
Ctx-left-posterior-cingulate	Ctx-right-posterior-cingulate	
Ctx-left-precentral	Ctx-right-precentral	
Ctx-left-precuneus	Ctx-right-precuneus	
Ctx-left-rostral-anterior-cingulate	Ctx-right-rostral-anterior-cingulate	
Ctx-left-rostral-middle-frontal	Ctx-right-rostral-middle-frontal	
Ctx-left-superior-frontal	Ctx-right-superior-frontal	
Ctx-left-superior-parietal	Ctx-right-superior-parietal	
Ctx-left-superior-temporal	Ctx-right-superior-temporal	
Ctx-left-supramarginal	Ctx-right-supramarginal	
Ctx-left-frontal-pole	Ctx-right-frontal-pole	
Ctx-left-temporal-pole	Ctx-right-temporal-pole	
Ctx-left-transverse-temporal	Ctx-right-transverse-temporal	
Ctx-left-insula	Ctx-right-insula	

ctx, cortex

SUPPLEMENTARY MATERIALS

Deep Learning-Based Segmentation Method

Deep learning-based Brain MRI segmentation method

We used Split-Attention U-Net (SAU-Net), a convolutional neural network with skip pathways and a split-attention module that segments brain MRI scans. The entire image processing pipeline was implemented using Python 3.7 with TensorFlow version 1.5. Previously, we demonstrated that SAU-Net achieved better segmentation accuracy with better reliability that surpasses those of state-of-the-art methods.[1] We believe that SAU-Net has excellent potential due to its robustness to neuroanatomical variability that would enable almost instantaneous access to accurate neuroimaging biomarkers and its swift processing runtime compared to other methods investigated.

Preprocessing

We applied an identical pipeline from the pre-processing pipeline for all the data utilized in this study. Our preprocessing includes resampling, zero-padding, and intensity normalization using histogram matching. We first resampled the image in an isotropic voxel (1 mm^3), then padded it with zero using a filter size of 16×16×16 and 24×24×24 for training and testing, respectively. Finally, we normalized the MRI intensity by applying a histogram matching algorithm as described elsewhere.[2] The entire preprocessing pipeline was implemented using the NiftiNet library.[3]

Deep learning segmentation

Our in-house segmentation tool was developed from the existing UNet++ deep learning architecture with a three-dimensional methodology to train 97 labels. Our deep learning design has a convolutional layer in the skip path, which bridges the semantic gap between the encoder and decoder characteristic maps. A dense skip connection in the skip path, which improves the gradient flow, has deep supervision, which enables model pruning, improves performance, or, at worst, compares using only one lossy layer (Supplementary Figure 1). The cross-entropy loss function was used for the voxel-by-voxel segmentation learning and the learning rate for the Adam optimizer was 0.0001.

For the training dataset, we performed Desikan–Killiany atlas-based FreeSurfer segmentation on 388 patients from public datasets including HCP, ADNI, PPMI, AIBL, and IXI, and two experts performed manual correction to produce a fine-tuned gold standard. Among the entire data set, we first randomly shuffled and set aside 49 datasets for testing. The remaining data were then categorized for training and validation (9.5:0.5). The training data were constructed by extracting the three-dimensional patch image using uniform sampling (96×96×96) for the individual ground truth data (Supplementary Figure 2). With the aforementioned training parameters, the model was iteratively trained 500,000 times. The batch size was set to 1, which was the limit that could be handled by the 11 GB RAM of one RTX 2080Ti GPU. For comparison, FreeSurfer software (version 7.0.0, https://surfer.nmr.mgh.harvard.edu) was used to identify sub-regional brain volumes.

Postprocessing

To increase the validity of the structures, we further applied connected-component labeling. No other post-processing was applied and volumes with regard to the original MRI were computed for the analysis. Segmentation results are obtained by merging inference data using a three-dimension patch sliding aggregator. Supplementary Figure 3 shows the segmentation result of brain sub-volumes.

Trial registration

We used MR images from the Carnus (cardiovascular risk of Korean nun study) sub-dataset. Clinical trial was registered with the Clinical Trials Registry at https://cris.nih.go.kr (KCT0006054).

REFERENCES

1. Lee M, Kim J, Ey Kim R, Kim HG, Oh SW, Lee MK, et al. Split-attention U-Net: a fully convolutional network for robust multi-label segmentation from brain MRI. Brain Sci 2020;10:974.
2. Nyúl LG, Udupa JK, Zhang X. New variants of a method of MRI scale standardization. IEEE Trans Med Imaging 2000;19:143-150.
3. Gibson E, Li W, Sudre C, Fidon L, Shakir DI, Wang G, et al. NiftyNet: a deep-learning platform for medical imaging. Comput Methods Programs Biomed 2018;158:113-122.
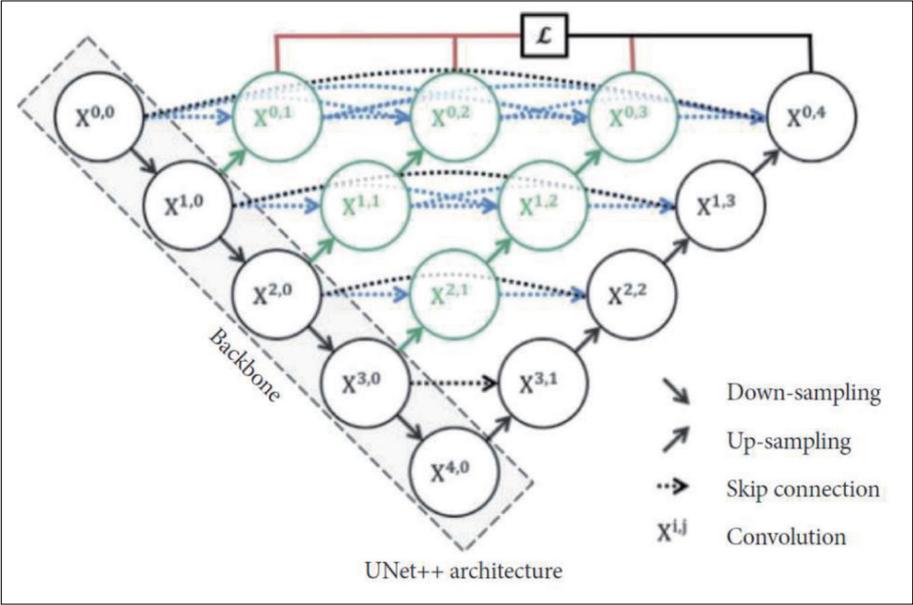
Supplementary Figure 1. U-Net++ deep learning architecture.
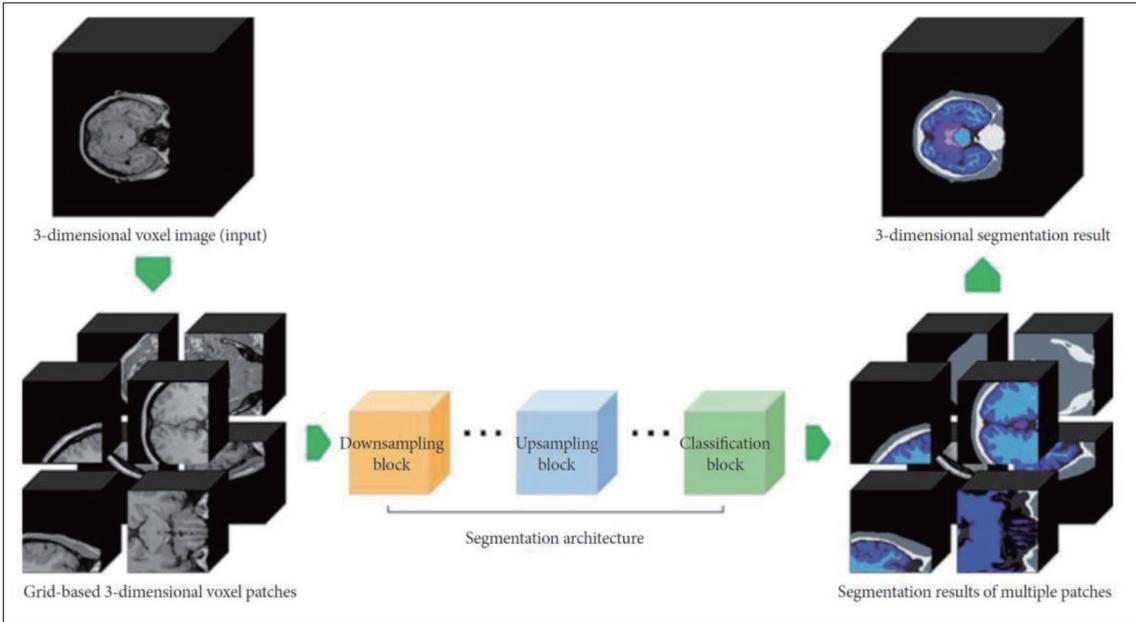
Supplementary Figure 2. Three-dimensional patch-based training scheme explanation.
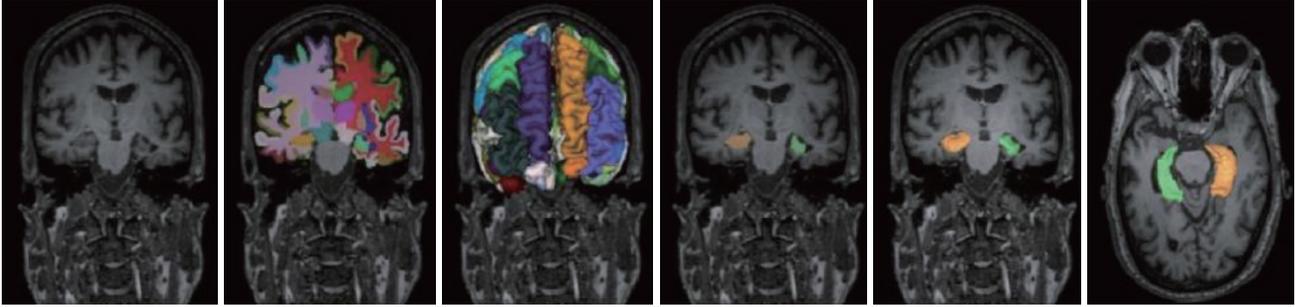
Supplementary Figure 3. Deep learning-based segmentation result.