

# Clinical Significance of Changes in the Subendocortical Volume of Wilson Disease

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## Research Article

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# **Abstract**

Wilson disease (WD) is a rare neurogenetic disease with a variety of clinical manifestations. The disorder of copper metabolism can lead to cell necrosis, while nerve cell necrosis can reduce the volume of the corresponding parts. This study quantifies the degree of nerve cell injury by studying the subcortical volume changes in WD patients, and discusses the correlation between nerve cell injury in different parts and clinical manifestations. The results showed that compared with the healthy control group, the subcortical volume of WD decreased significantly, and the decrease in different parts was related to clinical symptoms. This study shows that we can quantify the degree of nerve cell injury by measuring the change of subcortical volume. Predict possible clinical symptoms.

## **Introduction**

Wilson disease is an autosomal recessive disorder of copper metabolism caused by ATP7B gene mutation. Copper is the most abundant alkaline metal in human body<sup>[1]</sup>. It is involved in iron homeostasis, antioxidant defense, neurotransmitter synthesis and many cellular pathways. In the nervous system, copper participates in the formation of myelin sheaths. It regulates synaptic activity and excitatory cell death induced by neurotrophic factors. When copper metabolism in the liver is disordered, it will be excessively deposited in the liver, causing liver cell damage. After copper enters the blood, it will enter the brain and other organs with the blood, causing corresponding cell damage and death. In the brain, copper will be selectively deposited in the basal nucleus, resulting in damage to the nerve cells of the nucleus<sup>[2]</sup>. The degree of damage in different parts is different, which will cause different clinical symptoms. Therefore, measuring the size of the subcortical volume may reflect the degree of cell damage. Freesurfer is an open source software for Magnetic Resonance Imaging(MRI) processing and analysis. Through cortical reconstruction and thickness analysis of T1 data, the corresponding volume of interest can be obtained. In this study, we used Freesurfer software to analyze the T1 data of 45 WD patients and 21 normal controls, and obtained volume data of the corresponding regions of interest to understand the damage characteristics of the subcortical volume of neurotype WD patients.

## **Patients And Methods**

### **Research subjects**

collected 45 genetically and clinically diagnosed patients in the Neurology Department of the East Hospital of the First Affiliated Hospital of Sun Yat-sen University from 2019 to 2021[Allenbach, 2016 #87]<sup>[3]</sup>. After admission, the patients were asked in detail about the medical history, first symptoms, process of the disease, etc., and used the Hamilton Depression Scale, Hamilton Anxiety Scale, MMSE Scale, and Modified Young Scale to score the parameters of the patients. Each patient has 2 seniors. Neurologists scored and averaged the results. The modified YOUNG scale mainly includes eight items such as language, throat muscle tension, limb muscle tension, gait, ataxia, tremor, choreography, and advanced neurological functions, According to the modified YOUNG score, WD patients were then divided into 4

types, mainly including dyskinesia, Parkinson's symptom, oral-mandibular dystonia, and mental disorder. See the table 1 for the scoring details. Twenty-one healthy controls matching the age and education level of WD patients were selected. All subjects were right-handed, and no MR examination contraindications. This study was approved by the Ethical Review Committee of the First Affiliated Hospital of Sun Yat-sen University, and all subjects signed an informed consent form before the experiment. Exclude previous mental illness, drug abuse, and history of head trauma, and exclude normal controls with lesions in the skull, such as cerebral hemorrhage, cerebral infarction, aneurysm, arteriovenous malformations, etc.

### **MRI data collection**

All MRI scans were obtained from a 3.0 T MRI scanner (Magnetom Trio; Siemens Healthcare, Erlangen, Germany) using a 12-channel head coil. For each subject, a three-dimensional (3D) magnetization preparation-rapid acquisition gradient echo sequence was used to acquire T1-weighted images of the whole brain. The scanning parameters were as follows: 256 sagittal slices, flip angle = 9 degrees, repetition time = 1750 ms, echo time = 2.88 ms, reversal time = 900 ms, matrix = 384 × 384, field of view = 260 mm, voxel size = 0.7 mm 3, 0.7 mm slice thickness, no slice gap, acquisition time = 4.21 minutes. During the scan, each subject used foam padding and earplugs to reduce head movement and scanner noise. All MRI images are monitored and checked by two experienced neurologists and a neuroradiologist at the same time to facilitate the control of data quality.

### **Volume segmentation**

FreeSurfer 7.0 software was used for cortical reconstruction and thickness analysis of 3D T1 data. The specific treatment method has been described in the previous study<sup>[4][5]</sup>. The analysis process mainly includes non-uniform intensity correction, Talairach conversion, removal of non-brain tissue, segmentation of the image into gray matter, white matter, and cerebrospinal fluid, and analysis of the structures of interest (caudate nucleus, putamen, globus pallidus, thalamus, amygdala, hippocampus, Nucleus accumbens, brain stem, corpus callosum) for volume segmentation. In this segmentation process, we edit the segmentation results by adding control points and manually review the final segmentation results. After that, the volume information of the relevant area is obtained through processes such as spherical expansion, registration, and smoothing.

### **Group comparative analysis**

Use a general linear model to explore the regional volume differences between the WD and HC groups, and analyze the intracranial volume (ICV), gender and age as covariates, and use the FDR (false discovery rate) method for multiple comparison correction (FDR<0.05 ). Use the shrinkage rate to get the most significant area of the area of interest volume reduction. The calculation method for the shrinkage rate is (the mean volume of the HC group-the mean volume of the WD group)/the mean volume of the HC group.

### **Statistical analysis**

Use SPSS 25.0 software for statistical analysis of demographic and clinical data. The Kolmogorov-Smirnov test was used to detect the normality of the data. Chi-square test, independent sample t test, Kruskal-Wallis test and Mann-Whitney U test were used to compare the clinical data. The correlation analysis between volume value and clinical score adopts Spearman correlation analysis method, using one-way analysis of variance between the volume values of type 4 patients, using ROC curve to get the best diagnostic value,  $P<0.05$  indicates that the difference is statistically significant.

## Result

### Demographic and clinical data

A total of 45 WD patients (including 31 males and 14 females) and 21 normal controls (9 males and 12 females) were enrolled in this study. The results of statistical analysis showed that there was no statistical difference in age between the two groups . The specific results are shown in Table 2. After grouping WD, 13 cases of dyskinesia type, 17 cases of Parkinson's symptom type, 11 cases of oral-mandibular dystonia type, and 4 cases of mental disorder type were obtained.

### Volume analysis

Bilateral caudate nucleus, putamen, globus pallidus, thalamus, corpus callosum, brainstem, hippocampus, amygdala, and nucleus accumbens were all detected in WD patients with reduced overall volume ( $P<0.05$ ), The difference between the anterior and middle part of the corpus callosum was not statistically significant ( $P>0.05$ ). According to the calculation formula of atrophy rate, the areas with the most obvious volume reduction were putamen, caudate nucleus, globus pallidus, and nucleus accumbens. Table 3 and Figure 1-3 .

### Clinical data analysis

The process of the disease is not significantly correlated with the volume. The serum ceruloplasmin value is negatively correlated with the volume of the left globus pallidus, bilateral amygdala, bilateral nucleus accumbens, and anterior corpus callosum ( $r=-0.321, -0.451, -0.348, -0.333, -0.429, -0.453, P=0.049, 0.005, 0.032, 0.041, 0.007, 0.004$ ), 24h urine copper value is negatively correlated with bilateral putamen volume ( $r=-0.434, -0.446, P=0.004, 0.003$ ), the Hamilton depression scale score was negatively correlated with the volume of the anterior corpus callosum and bilateral thalamus ( $r=-0.396, -0.437, -0.351, P=0.030, 0.001, 0.012$ ); MMSE score was correlated with bilateral putamen, right The volume of the lateral caudate nucleus and nucleus accumbens is negatively correlated ( $r=-0.473, -0.409, -0.381, -0.387, P=0.008, 0.025, 0.038, 0.035$ ); the correlation study with the modified YOUNG table found that the left The volume of the caudate nucleus was negatively correlated with the total score of the modified young ( $r=-0.424, P=0.049$ ), and the commode score was negatively correlated with the volume of the left globus pallidus ( $r=-0.430, P=0.046$ ), and the gait score was negatively correlated with The volume of the left caudate nucleus was negatively correlated ( $r=-0.489, P=0.021$ ). The results of intra-group difference analysis are shown in Picture 2. Type 1 and type 2 patients have significant differences

in the volume of the right globus pallidus, with the best discrimination at 1561.35, the area under the curve is 0.72, the sensitivity is 0.62, and the specificity is 0.82. Types 1 and 3 patients have significant differences in the brainstem, bilateral hippocampus, and left amygdala. The area under the ROC curve of the right hippocampus is the largest, with the best discrimination at 4143.50. The area under the curve is 0.82, and the sensitivity is 0.73, the specificity is 0.85. Type 2 and type 3 patients have significant differences in bilateral thalamus, bilateral hippocampus, brainstem, and left amygdala. The area under the ROC curve of the left thalamus is the largest, with the best distinction at 6513.10. The area under the curve is 0.79, the sensitivity is 0.73, and the specificity is 0.76. There is a significant difference between type 3 and type 4 patients in the right hippocampus area, and the area under the curve is 0.89, which has the best discrimination at 3,99.90, and the sensitivity is 0.91, the specificity is 0.75. Figure 4

## Discussion And Conclusion

This study is based on the T1 sequence of MR, using Freesurfer software to automatically outline the cortex and nucleus volume of the brain area of interest, by comparing the volume changes between WD and healthy controls, and analyzing the correlation between the volume changes and clinical data. To observe the volume changes of specific areas in the brain of WD patients, and analyze the correlation between the volume changes of different parts and clinical manifestations.

In WD patients, a large amount of copper accumulates in the central nervous system, which can cause nerve cell damage and necrosis in the brain. Through anatomy of 2 WD death cases, Mikol<sup>[6]</sup> was found that there are different degrees of necrosis and atrophy between gray and white matter and basal ganglia in the brain of WD, and the degree of necrosis on both sides is asymmetric; Many imaging methods have proved that the caudate nucleus and putamen are the most significant areas of copper-induced cell damage in WD patients. In our study, it was also observed that the volume of WD in the bilateral caudate nucleus, putamen, globus pallidus, thalamus, corpus callosum, brainstem, hippocampus, amygdala, and nucleus accumbens was significantly smaller than that of HC. According to the calculation formula of atrophy rate, the areas with the most obvious volume reduction are putamen, caudate nucleus, globus pallidus, and nucleus accumbens, which are consistent with the previous results. In previous studies on WD, everyone paid little attention to the nucleus accumbens area. In this study, we found that the nucleus accumbens is the most significant area of volume reduction after the globus pallidus, and the nucleus accumbens is located at the head of the caudate nucleus. 95% of its neurons are gamma-aminobutyric acid (GABA) projection neurons. Previous studies have found that the nucleus accumbens is associated with the effects of happiness, reward, motivation, reinforcement learning, fear, addiction, impulse, and placebo<sup>[7]</sup>. When liver function is impaired, the liver's ability to clear GABA decreases, and excessive GABA can cause damage to the nucleus accumbens<sup>[8]</sup>, which is consistent with the pathogenic mechanism of WD. After neurological WD suffers from liver function damage, the ability to process GABA is reduced, causing excessive GABA in the brain, resulting in damage to the nucleus accumbens. In the onset of many patients with WD, it is often accompanied by various mental symptoms

and emotional problems. The relationship between these and the nucleus accumbens is worthy of our in-depth study.

By analyzing the correlation between different parts of the brain and the Hamilton depression scale and MMSE, it was found that the anterior part of the corpus callosum and bilateral thalamus were negatively correlated with the Hamilton depression scale, and the putamen, caudate, and nucleus accumbens volumes were negatively correlated with the MMSE score. In the past, with regard to the changes in the thalamus and putamen of WD patients, more attention has been paid to the effects of muscle tone, movement, executive function, articulation and Parkinson's symptoms. In our study, We get that the depression score of WD patients is related to the volume of the thalamus. The smaller the volume of the thalamus, the more severe the depression of WD, This is consistent with the results of the coenen study [9]. He has confirmed that the thalamus plays a vital role in arousal function, and is important in emotion and reward processing and the development of depression, and that the thalamus is associated with the amygdala, prefrontal cortex, parietal cortex, and medial temporal lobe. Connected, so it's no surprise that the thalamus is involved in various emotional and cognitive functions as well as reward-based functions [10]. Dichter [11] et al. also reported thalamus abnormalities in patients with depression. And When shen studied the cognition of patients with Parkinson's disease, she found that the putamen is related to cognitive function<sup>[12]</sup>. Previous studies have suggested that WD patients rarely affect the corpus callosum, Zhou et al. have used DTI to prove that WD patients have deep fiber bundle damage<sup>[13]</sup>, The corpus callosum is a fiber bundle plate made up of fibers that connect the left and right cerebral hemispheres. We suspect that patients with WD may have a decrease in the corpus callosum fiber bundles, which will cause a decrease in volume. By subdividing the corpus callosum into five regions, and After a correlation analysis of clinical symptoms, we found that the Hamilton depression scale score was negatively correlated with the anterior volume of the corpus callosum, combined with<sup>[14]</sup>DTI imaging on the study of corpus callosum fiber bundles, The anterior part of the corpus callosum is mainly assigned to the prefrontal lobe, anterior motor and auxiliary motor cortex areas, and the frontal lobe is related to emotion and cognition. These all support our results. It also reminds us that WD patients should pay more attention to the changes of the corpus callosum.

By analyzing the correlation between the different parts of the brain area and the modified Young scale, it was found that the left caudate nucleus volume was negatively correlated with the modified young total score, the ataxia score was negatively correlated with the left globus pallidus volume, and the gait score was negatively correlated with the left side. The volume of the caudate nucleus is negatively correlated. The above results indicate that with the severe atrophy of the caudate nucleus, the higher the modified YOUNG scale score, the more severe the patient's neurological symptoms. The more severe the atrophy of the left globus pallidus, the more it may affect the patient's mutual aid movement. The smaller the left caudate nucleus, the more difficult it is for the patient to walk independently. The selective deposition of copper in different parts of the brain in WD patients can cause a variety of clinical symptoms.

Ceruloplasmin (Cp) is a protein in human serum. It contains 95% serum copper<sup>[15]</sup>, copper metabolism disorder will aggravate the production of ceruloplasmin-free<sup>[16]</sup>, thus losing the ability to oxidize Fe 2+ to Fe 3+, Fe 2+ will produce harmful oxygen free radicals, causing cell damage, ceruloplasmin genes can be expressed in the liver, brain, lung, spleen and testis<sup>[17]</sup>, mainly involved in iron metabolism. In this experiment, we found that the serum ceruloplasmin value is negatively correlated with the left globus pallidus, bilateral amygdala, bilateral nucleus accumbens, and the anterior volume of the corpus callosum. This result may be meaningless, because WD patients There is not much difference between the ceruloplasmin.

In this study, there is no correlation between the course of the disease and the volume of the region of interest, which is a common clinical phenomenon. The disease of WD is relatively rare, with various onset modes and hidden onset. It is easy to be misdiagnosed in clinical work. The severity of the patient's condition and the rate of progress vary, and the severity is related to genetic types and environmental factors, so it is not simply Affected by the course of the disease.

Combined with the modified YOUNG scale, the patients were subdivided into type 4 (dyskinesia, Parkinson's symptom, oromo-mandibular dystonia, mental disorder). We found that the main difference between type 1 and type 2 lies in the right In the lateral globus pallidus, the volume of the globus pallidus in patients with dyskinesia-type WD is significantly smaller than that of patients with Parkinson's symptomatic WD. At 1561.35 on the right globus pallidus, the two types of WD can be best distinguished. Types 1 and 3 patients have significant differences in the brainstem, bilateral hippocampus, and left amygdala. The dyskinesia type is significantly smaller than the oro-mandibular dystonia type in the above-mentioned difference area, and it has 4143.50 in the right hippocampus. Best discrimination. Type 2 and type 3 patients have significant differences in bilateral thalamus, bilateral hippocampus, brainstem, and left amygdala. The Parkinson's symptom type is significantly smaller than the oral-mandibular dystonia type in the above-mentioned difference area, and it is in the left thalamus. 6513.10 has the best discrimination. Compared with the oral-mandibular dystonia, the mental disorder has a smaller volume in the right hippocampus, and has the best discrimination at 3,99.90 in the right hippocampus. Based on the above results, we can see the globus pallidus, brainstem, and hippocampus. Volume changes can cause dyskinesias and Parkinson-like symptoms in patients, and changes in hippocampal volume are related to patients' mental symptoms.

In this study, starting from the nucleus in the basal ganglia of WD patients, the local study of the nucleus volume change and the clinical correlation, we have the following conclusions: (1) The thickness of the cerebral cortex and nucleus volume of WD patients are generally reduced compared with normal controls; (2) The degree of regional volume reduction may be related to clinical manifestations; however, the overall changes in cortex and white matter may be ignored in this experiment. Zhou<sup>[18]</sup> and others found that by subdividing the volume difference of each subregion of the basal nucleus, the decrease in volume between different subregions of the basal nucleus corresponds to different cortical projection areas. Combining these findings, we have reason to believe that there is atrophy in both the cortex and the basal

nucleus area of WD, and the common changes of the two may lead to different clinical manifestations of WD patients.

## Conclusions

In this article, we found that compared with normal controls, Wilson disease(WD) patients have a significant reduction in subcortical volume, and the volume reduction in different areas is correlated with clinical symptoms.

## Declarations

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### Author information

X.X. and H.Z.H. contributed equally to this work.

Conception and study design (XX, HZH and LJ), data collection or acquisition (XX, HZH and LJ), statistical analysis (XX, LJ), interpretation of results (XX, HZH, LJ, ZXX and CJP), drafting the manuscript work or revising it critically for important intellectual content (XX, HZH, LJ and ZXX) and approval of final version to be published and agreement to be accountable for the integrity and accuracy of all aspects of the work (All authors).

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### Ethics declarations

#### Declaration of interest

The authors declare there is no financial conflict of interest.

### Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### Informed consent

Informed consent was obtained from all individual participants included in the study.

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## Tables

Table 1:Classification criteria for neurological WD based on the modified YOUNG scale

Types	YOUNG tables item	Score
Type 1:Dyskinesia	Item 9:Tremor parts Item 10:Tremor degree Item 11:Dance movement frequency Item 12:Dance movement degree	Total score $\geq 8$
Type2:Parkinson's symptom	Others item	Total score $\leq 2$
Type3 :oroma-mandibular dystonia	Item5:muscular tension elevate parts Item 6:muscular tension elevate degree Item 13:Gait abnormality degree Item 14:gait abnormality gesture	Total score $\geq 8$
	Others item	Total score $\leq 2$
Type4 :Mental disorder	Item1:speech articulation Item2:Linguistic coherence Item 3:deglutition Item 4:salivation	Total score $\geq 8$
	Others item	Total score $\leq 2$
	Item15:mental symptom Item 16:intelligence	Total score $\geq 4$

Table 2:Demographic and clinical data of all participating researchers

	WD	HC	P Value
Gender(male/female)	31/14 <sup>a</sup>	9/12 <sup>a</sup>	0.045
age	25.31±7.37	27.71±6.98	0.208
strong hand\right/left\	\45/0\	\21/0\	--
WD type	Nervous type	--	--
24h urine copper	891.45±611.01	--	--
ceruloplasmin	0.52±0.43	--	--
process\year\	6.29±3.98	--	--
K-Frings	100%	--	--

Data are shown as number or mean ± standard deviation. Process: the process of the time from diagnosis to the present

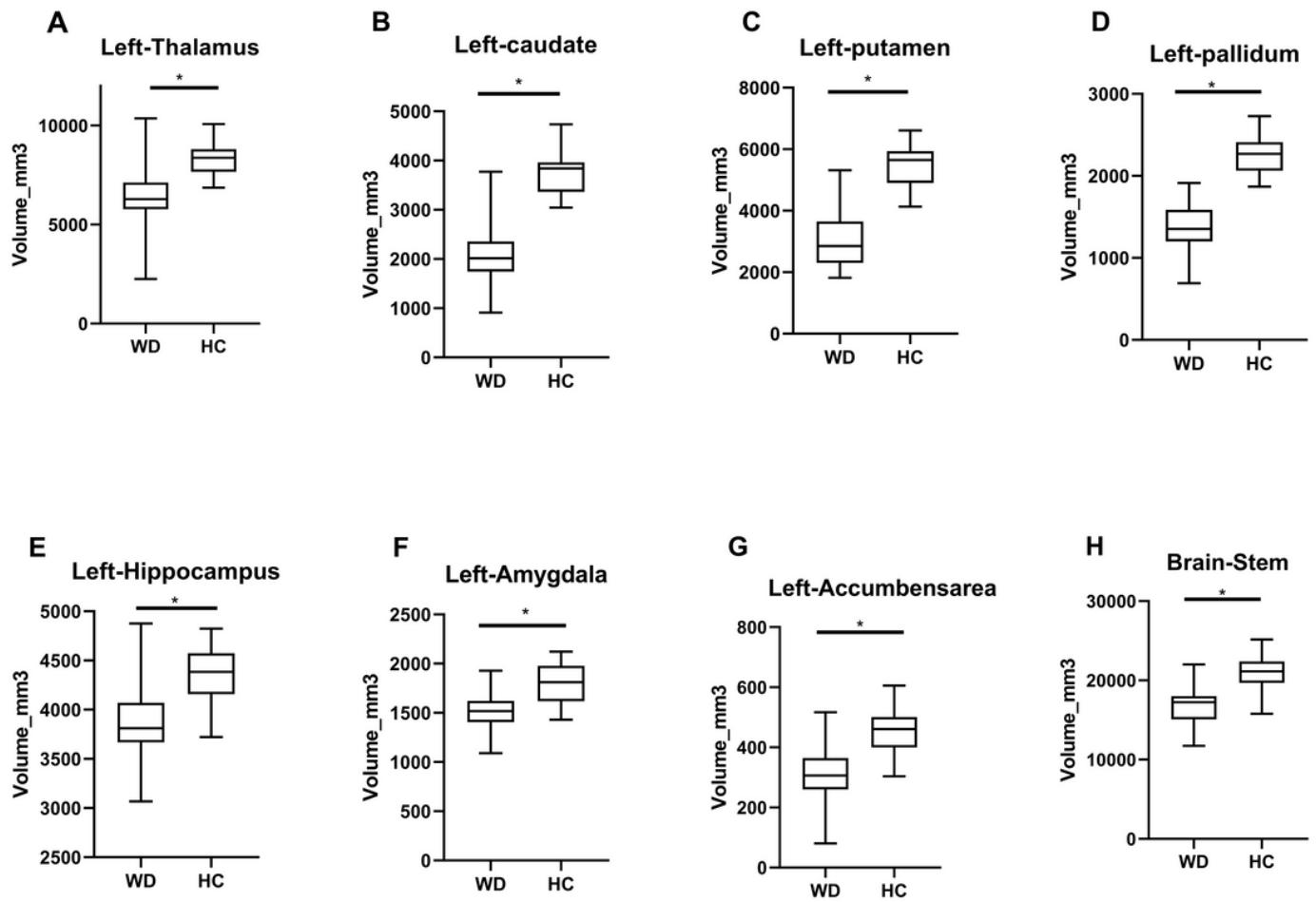
a Statistically significant compared to WD and the HC ( $P \leq 0.05$ )

**Table 3:Comparison of the volume of the two groups of regions of interest data**

	WD	HC	P Value	Rate
Left-Thalamus	6380.82±1329.47 <sup>a</sup>	8314.03±799.54	0.00	0.23
Left-Putamen	3065.38±923.04 <sup>a</sup>	5468.12±654.12	0.00	0.44
Left-Pallidum	1391.34±268.97 <sup>a</sup>	2250.82±215.74	0.00	0.38
Brain-Stem	16822.82±2252.33 <sup>a</sup>	21132.81±2261.36	0.00	0.20
Right-Thalamus	6309.71±1223.74 <sup>a</sup>	7816.33±727.70	0.00	0.19
Right-Putamen	3038.56±947.20 <sup>a</sup>	5587.27±561.08	0.00	0.46
Right-Pallidum	1413.89±323.56 <sup>a</sup>	2176.60±183.40	0.00	0.35
CC_Posterior	883.52±126.36 <sup>a</sup>	984.84±140.39	0.00	0.10
CC_Mid_Posterior	514.96±127.32 <sup>a</sup>	574.45±115.32	0.00	0.10
CC_Central	538.16±154.90 <sup>a</sup>	750.94±172.33	0.00	0.28
CC_Anterior	757.85±155.13 <sup>a</sup>	908.37±138.11	0.00	0.17
Left-Hippocampus	3853.20±366.71 <sup>a</sup>	4353.16±312.05	0.00	0.11
Left-Amygdala	1516.88±182.85 <sup>a</sup>	1807.07±213.44	0.00	0.16
Left-Accumbens-area	313.22±90.63 <sup>a</sup>	452.48±72.83	0.00	0.31
Right-Hippocampus	4053.42±368.79 <sup>a</sup>	4564.17±398.41	0.00	0.11
Right-Amygdala	1651.23±182.29 <sup>a</sup>	1884.17±203.24	0.00	0.12
Right-Accumbens-area	351.52±91.87 <sup>a</sup>	563.14±80.62	0.00	0.38
Left-Caudate	2138.36±593.48 <sup>a</sup>	3764.98±435.67	0.00	0.43
Right-Caudate	2262.46±586.85 <sup>a</sup>	3794.93±378.52	0.00	0.40

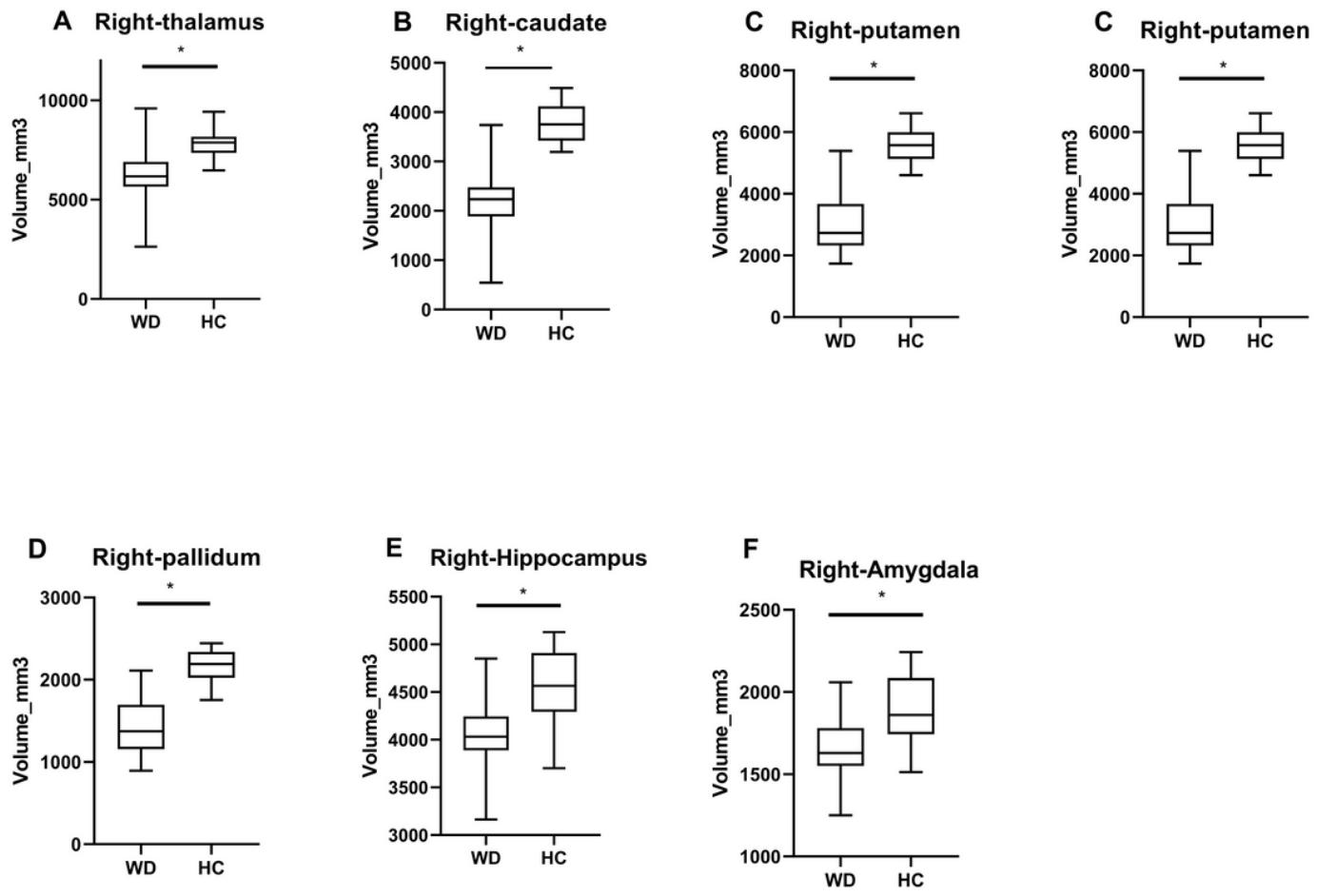
a Statistically significant compared to the normal control (P ≤ 0.05)

## Figures



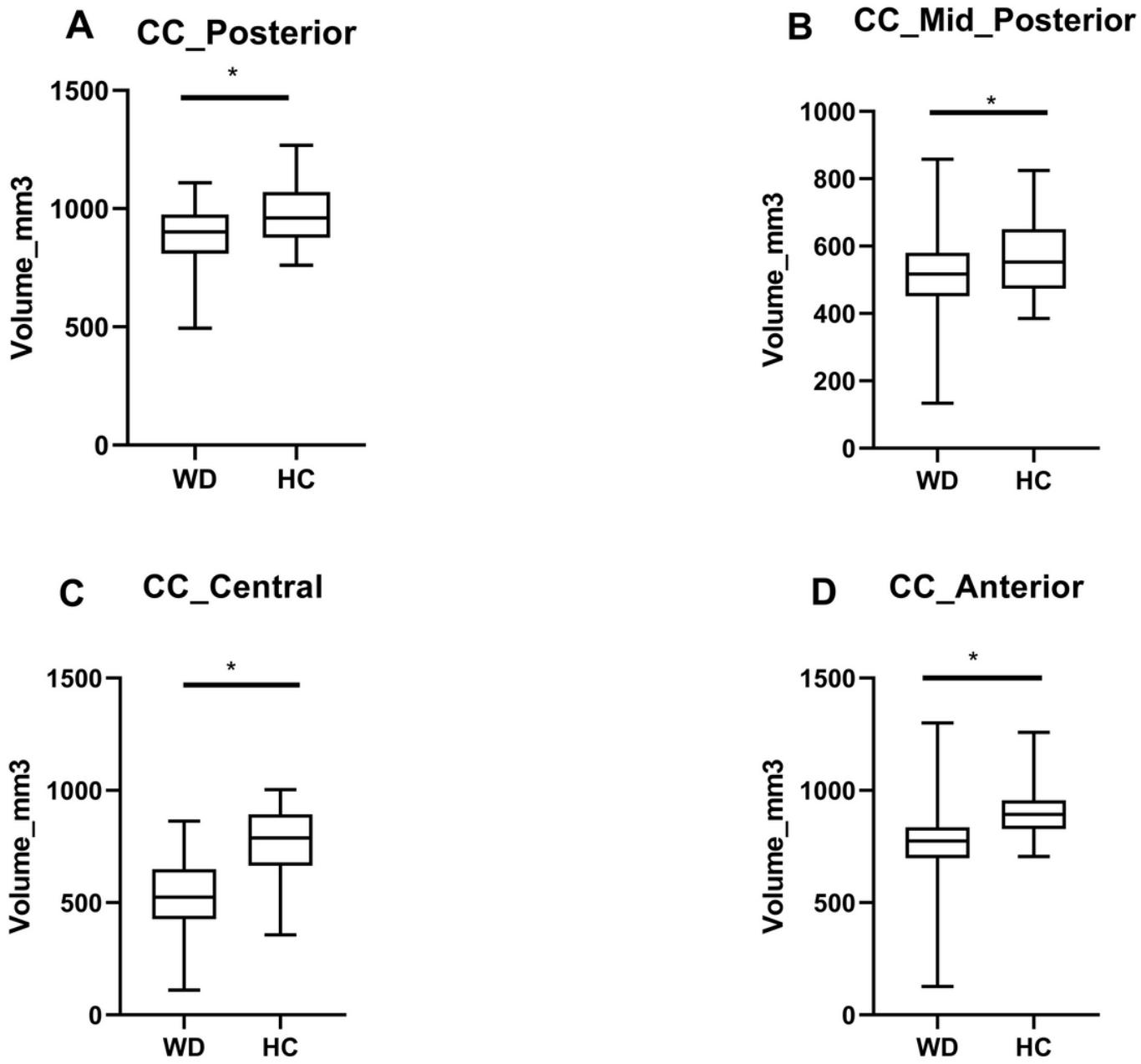
**Figure 1**

Differences in left subcortical volume and brainstem volume between WD and HC groups. The difference in the volume of the left subcortex and brainstem of the WD and HC groups. Figures A-H are thalamus, caudate nucleus, putamen, globus pallidus, hippocampus, amygdala, nucleus accumbens, and brainstem in order. \* Statistically significant compared to the normal control ( $P \leq 0.05$ )



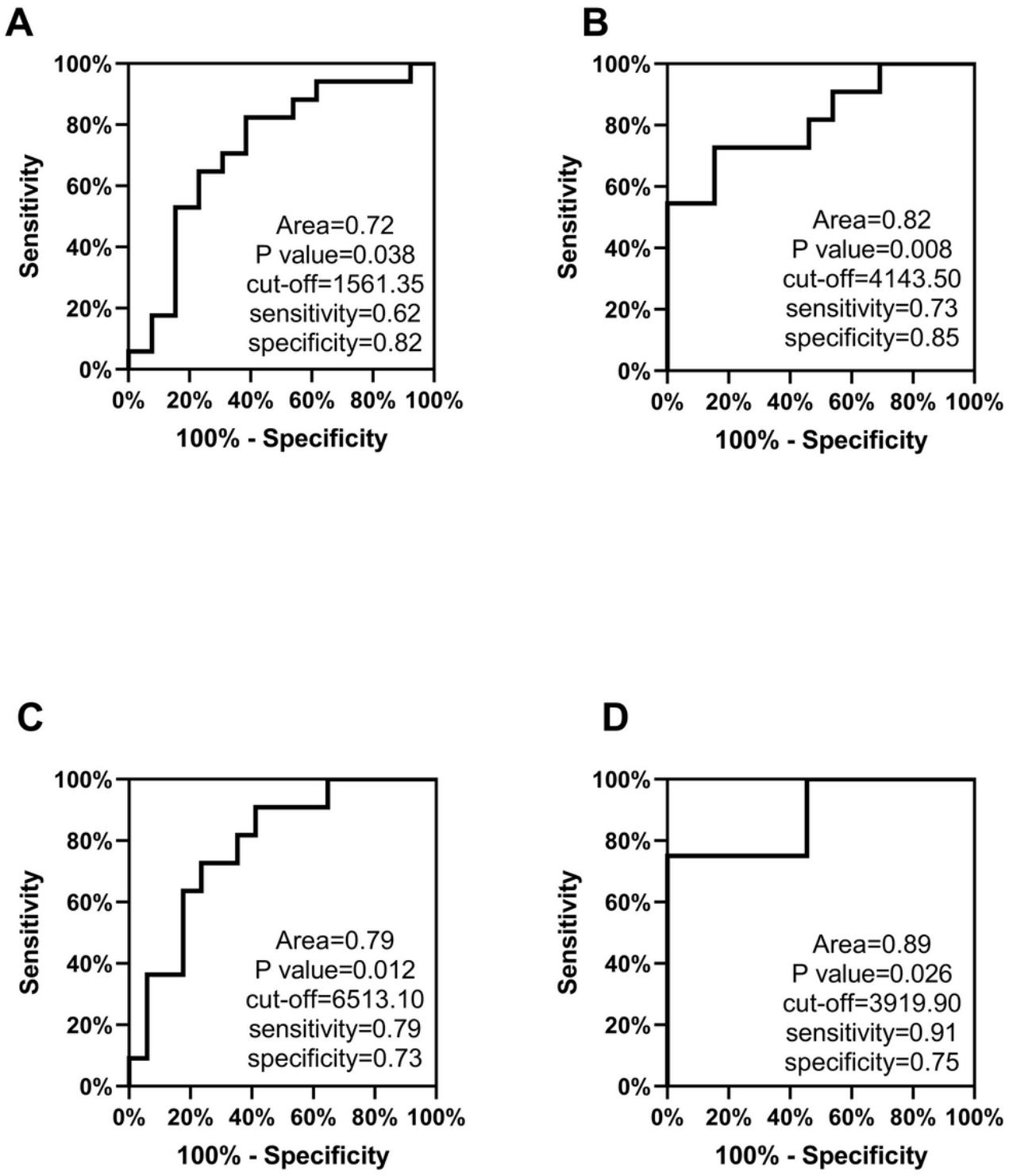
**Figure 2**

Differences in right subcortical volume between WD and HC groups. The difference in the volume of the right subcortex of the WD and HC groups. Figures A-G are thalamus, caudate nucleus, putamen, globus pallidus, hippocampus, amygdala, nucleus accumbens, and brainstem in order. \*Statistically significant compared to the normal control ( $P \leq 0.05$ )



**Figure 3**

Differences in Corpus callosum volume between WD and HC groups. The difference in the volume of the Corpus callosum of the WD and HC groups. Figures A-D are CC\_Posterior, CC\_Mid\_Posterior, CC\_Central, CC\_Anterior in order. \* Statistically significant compared to the normal control ( $P \leq 0.05$ )



**Figure 4**

Analysis of within-group differences Results of intra-group difference analysis, A shows that type 1 and type 2 patients have the best distinction in the right globus pallidus (Area=0.72,P=0.038).B shows that type 1 and type 3 patients have the best distinction in the right hippocampus (Area=0.82,P=0.008).C shows that type 2 and type 3 patients have the best distinction in the left thalamus

(Area=0.79,P=0.012).D shows that type 3 and type 4 patients have the best distinction in the right hippocampus (Area=0.89,P=0.026).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [BIBChecklist.pdf](#)