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REGULAR RESEARCH ARTICLE

Correlations Among mRNA Expression Levels of ATP7A, Serum Ceruloplasmin Levels, and Neuronal Metabolism in Unmedicated Major Depressive Disorder

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Abstract

Background: Previous studies have found that elevated copper levels induce oxidation, which correlates with the occurrence of major depressive disorder (MDD). However, the mechanism of abnormal cerebral metabolism of MDD patients remains ambiguous. The main function of the enzyme ATPase copper-transporting alpha (*ATP7A*) is to transport copper across the membrane to retain copper homeostasis, which is closely associated with the onset of mental disorders and cognitive impairment. However, less is known regarding the association of *ATP7A* expression in MDD patients.
Methods: A total of 31 MDD patients and 21 healthy controls were recruited in the present study. Proton magnetic resonance spectroscopy was used to assess the concentration levels of N-acetylaspartate, choline (Cho), and creatine (Cr) in brain regions of interest, including prefrontal white matter (PWM), anterior cingulate cortex (ACC), thalamus, lentiform nucleus, and cerebellum. The mRNA expression levels of *ATP7A* were measured using polymerase chain reaction (SYBR Green method). The correlations between mRNA expression levels of *ATP7A* and/or ceruloplasmin levels and neuronal biochemical metabolite ratio in the brain regions of interest were evaluated.
Results: The decline in the mRNA expression levels of *ATP7A* and the increase in ceruloplasmin levels exhibited a significant correlation in MDD patients. In addition, negative correlations were noted between the decline in mRNA expression levels of *ATP7A* and the increased Cho/Cr ratios of the left PWM, right PWM, and right ACC in MDD patients. A positive correlation between elevated ceruloplasmin levels and increased Cho/Cr ratio of the left PWM was noted in MDD patients.
Conclusions: The findings suggested that the decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels induced oxidation that led to the disturbance of neuronal metabolism in the brain, which played important roles in the pathophysiology of MDD. The decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels affected neuronal membrane metabolic impairment in the left PWM, right PWM, and right ACC of MDD patients.

Key Words: Major depressive disorder, copper metabolism, prefrontal white matter, anterior cingulate cortex, neuronal metabolism

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Significance Statement

Previous studies have shown that elevated copper levels induce oxidation, which could correlate to the occurrence of MDD. We performed a correlation analysis among mRNA expression levels of ATP7A, serum ceruloplasmin levels, and neuronal metabolism in 31 MDD patients and 21 HC participants. The data presented may have important implications in identifying MDD patients based on the decline in the mRNA expression levels of *ATP7A*. In addition, these patients may have a risk of neuronal impairment in the left PWM, right PWM, and right ACC. Elevated ceruloplasmin levels exhibit an inverse effect on the neuronal metabolism of the left PWM in MDD. The decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels exhibit a close association with neuronal damage and are considered to play important roles in the pathophysiology of MDD. These results indicate new directions for further research.

Introduction

A report from the depression fact sheet of the World Health Organization in 2017 stated that major depressive disorder (MDD) has affected more than 300 million individuals. MDD is a severe disorder with high mortality (van Zoonen et al., 2014). Features of MDD contain emotional, somatic, and functional impairments, which lead to reduced activity with regard to family and work responsibilities (Sheehan et al., 2017). Extensive research studies were recently performed, and accumulating evidence has suggested that the brain metabolic alteration is associated with increasing depressive symptoms (Su et al., 2018; Khan et al., 2018; Tang et al., 2018). However, the detailed mechanism of abnormal cerebral metabolism in MDD is unclear. Therefore, valid information of the evaluation of brain metabolic alteration is necessary for the comprehensive understanding of the pathophysiology of MDD.

It has been suggested that copper imbalance is involved in the reduction of neuronal metabolism (Zhao et al., 2013). Researchers have verified that decreased brain metabolism exhibits a major impact on the development of depression (Chu et al., 2017; Liu et al., 2018). Copper is important in regulating catecholamine-type neurotransmitters associated with neurological management (Schmidt et al., 2018). In addition, it has been shown that abnormal copper levels exhibit an essential role in the development of depressive disorder (Ni et al., 2018; Ullas Kamath et al., 2019). However, the exact mechanism by which copper imbalance manifests in MDD remains uncertain.

ATPase copper-transporting alpha (*ATP7A*), known as an ATP-driven copper transporter, is a highly sensitive enzyme. It plays an important role in balancing copper levels in the brain. In addition, it has been reported that the inactivation of *ATP7A* can cause downregulation of copper transition (Inesi et al., 2014). In addition, the inactivation of *ATP7A* originates from a gene mutation and decreases *ATP7A* mRNA expression to a large extent (Bennett-Baker et al., 2003). Previous evidence has shown that the gene mutation of *ATP7A* was taken into account for the presence of Menkes disease (Cao et al., 2017). Nevertheless, the results of the correlation between mRNA expression levels of *ATP7A* and MDD remain unclear. In addition to *ATP7A*, ceruloplasmin is also an important copper-transporting serum protein (Linder, 2016). Following the elevation of copper levels, increased ceruloplasmin levels bind to large amounts of copper participating in mental disease (Morera et al., 2007). However, the association between ceruloplasmin levels and the incidence of MDD has been explained ambiguously. Therefore, it is necessary to investigate the underlying associations among the mRNA expression levels of *ATP7A*, serum ceruloplasmin levels, and neuronal metabolism in MDD patients.

Copper imbalance can cause severe damage to neuronal metabolic alteration. Our previous study has shown that MDD patients exhibit biochemical abnormalities in prefrontal white matter (PWM) (Jia et al., 2015). PWM is susceptible to copper imbalance, and dysfunction of whiter matter is associated with several symptoms of depression (Barredo et al., 2019). The anterior cingulate cortex (ACC) is the key component of multiple neural networks involved in cognitive and emotional processes (Kowal et al., 2013). However, the impaired cerebral regions of changeable neuronal biochemical metabolites are inclusive. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a noninvasive technique, which can accurately quantify the biochemical metabolite ratios in brain (Lai et al., 2018). With regard to major neuronal biochemical metabolites, we focused on N-acetylaspartate (NAA), choline (Cho), and creatine (Cr) levels (Vythilingam et al., 2003). Cr levels are considered relatively constant and are mainly used as an internal reference to assess the metabolites of NAA and Cho (Kaymak et al., 2009). Accumulating evidence has shown that the alterations in the levels of NAA and Cho are associated with the development of depression (McNamara et al., 2016; Njau et al., 2017). Therefore, more precise indicators (NAA/Cr and Cho/Cr) were selected to establish metabolic markers for the changes of neuronal activity in MDD patients.

A previous study showed that discrepant degrees of neuronal metabolism are present in different brain regions (Dauth et al., 2017). We hypothesized that the decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels were potentially associated with alterations of neuronal biochemical metabolism in PWM and ACC of MDD patients. Therefore, the goal of the present study was to explore the correlations among the mRNA expression levels of *ATP7A*, serum ceruloplasmin levels, and neuronal biochemical metabolism of copper-rich cerebral regions in MDD patients. This was performed by assessing the levels of NAA/Cr and Cho/Cr. In this way, we aimed to evaluate the alteration of neuronal metabolism derived from copper imbalance in MDD patients and further provide effective methods for the treatment of this disease.

Materials And Methods

Human Participants

A total of 31 MDD patients (26.84±8.74 years old) and 21 age- and gender-matched healthy control (HC) participants (25.10±6.59 years old) were recruited in the present study. All the participants were right-handed and were of Han nationality. The demographics and clinical characteristics of all the participants were assessed by psychiatrists.

MDD Group

A total of 31 participants were diagnosed with MDD and were recruited from the Psychiatric Department of The First Affiliated Hospital of Jinan University (Guangzhou, China).

The inclusion criteria of the MDD group were the following: (1) MDD was diagnosed by a psychiatrist based on DSM-5, (2) a score of ≥20 on the Hamilton Depression Scale-24 version (24-item HAMD), (3) a score <6 on the Young Manic Rating Scale (YMRS), (4) psychiatric drug naive and treatment naïve status, and (5) voluntary participation in the study and signed informed consent.

The exclusion criteria of the MDD group were the following: (1) the presence of any other mental illness or neurological disease, (2) the presence of brain organic mental disorders, (3) pregnancy or lactation for female individuals, (4) abnormal brain structure or magnetic resonance contraindications, and (5) antipsychotic drug treatment.

HC Group

A sample of 21 healthy participants was recruited by advertising. The inclusion criteria of the HC group were the following: (1) the absence of any underlying disease or condition, (2) a score of <8 on the 24-item HAMD, (3) a score of <6 on the YMRS, and (4) voluntary participation in the study and signed informed consent.

The exclusion criteria of the HC group were as follows: (1) psychiatric disease or family history of mental disorder, (2) pregnancy or lactation for the female individuals, and (3) abnormal brain structure or magnetic resonance contraindications.

The present study was approved by the Ethics Committee of The First Affiliated Hospital of Jinan University.

MRI and ^{1}H-MRS Acquisition

Both MRI and ^{1}H-MRS were performed with the Discovery MR 750 3.0T MRI scanner (General Electric, Milwaukee, WI) with a conventional gradient system. The participants were equipped with ear plugs in their external auditory canal, and their heads were fixed by foam pad prior to the scanning to reduce noise and minimize head motion. They were asked to close their eyes and stay still during the scanning.

The voxel of interest was identified by the same experienced researcher in order to avoid bias from human errors. The voxel of interest of the bilateral PWM, ACC, thalamus, lentiform nucleus (LN), and cerebellum was localized at the midline and adjacent to the anterior margin of the anterior horn of the lateral ventricle (Fig. 1).

The ^{1}H-MRS dataset was analyzed by the Function Tool of the GE 3.0T workstation (Sun, Advantage Windows ADW4.5). The distribution maps of the metabolites were collected automatically and merged with the MRI images to obtain the 2D ^{1}H-MRS curves by the software. The software automatically processed the baseline calibration, signal average, and metabolite identification of the spectrum and subsequently measured the peak area of NAA, Cho, and Cr. Each spectrum was evaluated for the peak area of Cho at 3.22 ppm, Cr at 3.03 ppm, and NAA at 2.02 ppm. We calculated the ratios of NAA/Cr and Cho/Cr for analyses. The voxel placement, peak area determination, and data analyses were conducted by an experienced researcher who was blind to the participant diagnosis to ensure consistency of sampling and analysis.

Blood Sample Collection and Analysis

The blood samples of the participants were obtained within 1 week of their ^{1}H-MRS scan. A total 2 mL of blood sample was obtained from each participant in the morning. The blood samples analyses were performed in the biochemical laboratory of The First Affiliated Hospital of Jinan University (Guangzhou, China). The sample analyses were conducted by a Hitachi 7600 automatic biochemical analyzer.

Detection of mRNA Expression Levels of *ATP7A*

Primer Design and Synthesis

The following primer sequences were designed, synthesized, and used for the experiments.

Sequence name: *ATP7A* (the length of amplified fragment is 85bp): target gene

Forward primer: 5'- TGCTTATCTGCGCAACACATG-3'

Reverse primer: 5'- GCCTTTGATGTTTGTGCCTCTT-3'

Sequence name: *GAPDH* (the length of amplified fragment is 95 bp): internal reference gene Forward Primer: 5'-GATTCCACCCATGGCAAATT-3' Reverse Primer: 5'-TCTCGCTCCTGGAAGATGGT-3';

1. Total RNA extraction from whole blood;
2. Total RNA integrity detection;
3. Reverse transcription reaction (cDNA synthesis);
4. Fluorescence quantitative PCR reaction; and
5. Estimation of mRNA expression levels of *ATP7A*

The relative quantification method was used as follows: -ΔΔCT value quantitative method. Three major steps were used for analysis:

Step 1: ΔCT = CT target gene -CT internal reference gene.

Step 2: ΔCT = ΔCT-ΔCT control group/maximum value.

Step 3: Relative expression of sample = $2^{-\Delta\Delta CT}$.

Statistical Analysis

Statistical analysis was conducted by using the Statistical Package for Social Sciences (IBM SPSS) version 22.0 (SPSS, Chicago, IL). The age of the 2 groups (MDD and HC) was compared using the independent-samples *t* test. The differences regarding the gender of the 2 groups (MDD and HC) were assessed using the chi-square test. The 24-item HAMD and YMRS scores of the 2 groups (MDD and HC) were compared using the Mann-Whitney U test. The mRNA expression levels of *ATP7A* of the 2 groups (MDD and HC) were compared using the Mann-Whitney U test. The copper levels, ceruloplasmin levels, and biochemical metabolite ratios (NAA/Cr and Cho/Cr) of the 2 groups (MDD and HC) were compared using the independent-samples *t* test. The Spearman correlation was used to analyze the associations between abnormal biochemical metabolite ratios (Cho/Cr) in the brain regions of interest (PWM, ACC, thalamus, LN, and cerebellum) and abnormal *ATP7A* mRNA levels of MDD patients. The Pearson correlation was used to analyze the associations between abnormal biochemical metabolite ratios (Cho/Cr) in the brain regions of interest (PWM, ACC, thalamus, LN, and cerebellum) and abnormal ceruloplasmin levels in MDD patients. The Spearman correlation was used to analyze the association between the mRNA expression levels of *ATP7A* and ceruloplasmin levels in MDD patients. The Spearman correlation was used to analyze the associations between the clinical characteristics (age of onset, course of disease, number of episodes, 24-item HAMD scores) and significant indicators of MDD patients. The *P* values were adjusted using the false discovery rate correction. $P < .05$ was considered significantly different.

Results

Demographics and Clinical Characteristics

Table 1 indicated the demographics and clinical characteristics of all participants. A total of 31 MDD patients (14 males, 17 females; 26.84 ± 8.74 years of age) and 21 HC participants (7 males,

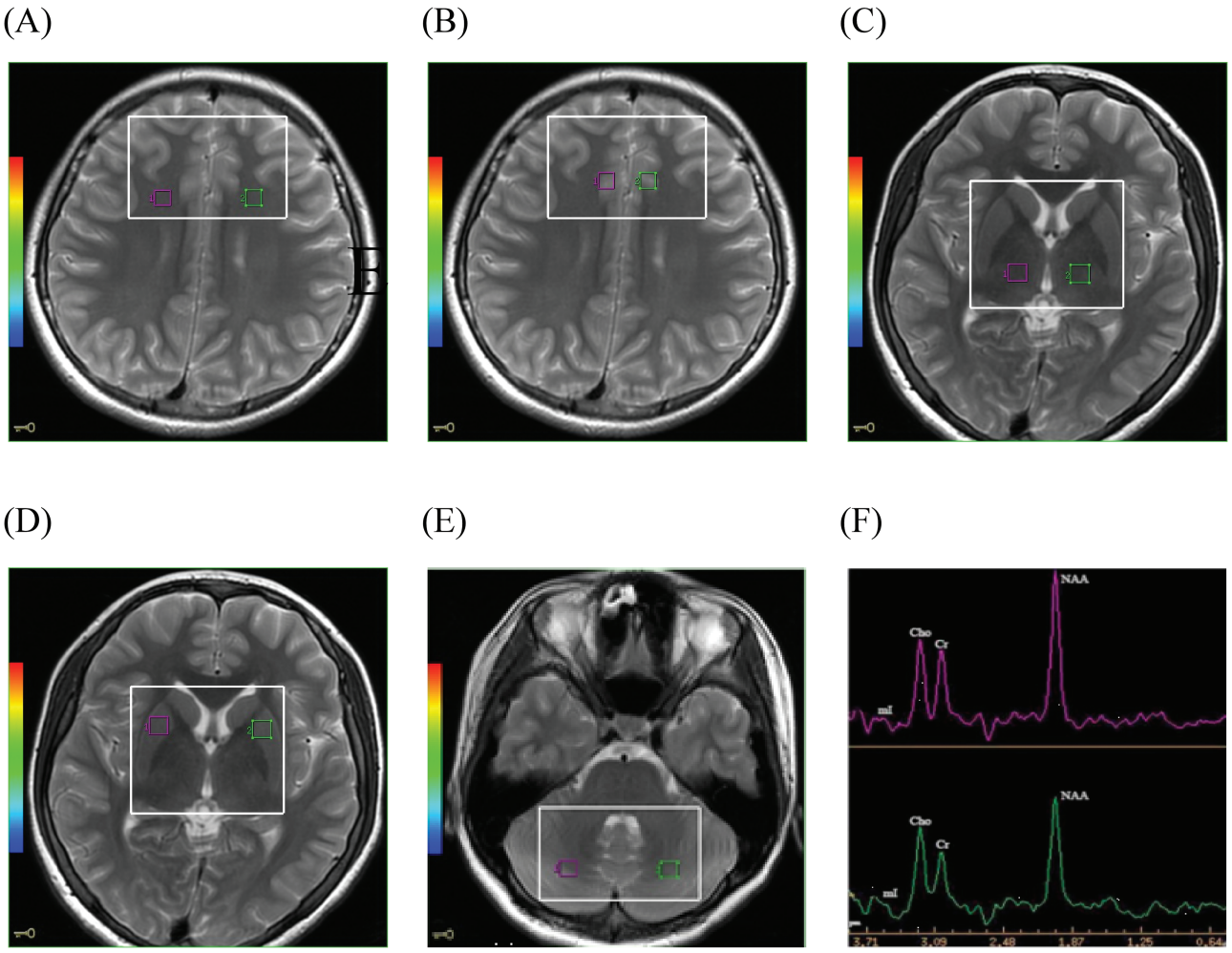

Figure 1. Magnetic Resonance Imaging (MRI) and Proton magnetic resonance spectroscopy (^{1}H-MRS) acquisition. The participants were equipped with ear plugs in their external auditory canal, and their heads were fixed by foam pad prior to the scanning to reduce noise and minimize head motion. They were asked to close their eyes and stay still during the scanning of MRI. The same experienced researcher scanned 5 brain regions of interest of every participant, including prefrontal white matter, anterior cingulate cortex, thalamus, lentiform nucleus, and cerebellum. (A) The voxel of interest of bilateral prefrontal white matter was localized at the midline via using ^{1}H-MRS technology. 1 represents the right side of prefrontal white matter, and 2 represents the left side of prefrontal white matter. (B) The voxel of interest of bilateral anterior cingulate cortex was localized at the midline via using ^{1}H-MRS technology. 1 represents the right side of anterior cingulate cortex, and 2 represents the left side of anterior cingulate cortex. (C) The voxel of interest of bilateral thalamus was localized at the midline via using ^{1}H-MRS technology. 1 represents the right side of thalamus, and 2 represents the left side of thalamus. (D) The voxel of interest of bilateral lentiform nucleus was localized at the midline via using ^{1}H-MRS technology. 1 represents the right side of lentiform nucleus, and 2 represents the left side of lentiform nucleus. (E) The voxel of interest of bilateral cerebellum was localized at the midline via using ^{1}H-MRS technology. 1 represents the right side of cerebellum, and 2 represents the left side of cerebellum. (F) The ^{1}H-MRS dataset was analyzed by the Function Tool of the GE 3.0T workstation. The distribution maps of the metabolites were collected automatically and merged with the MRI images to obtain the 2D ^{1}H-MRS curves by the software. The software automatically processed the baseline calibration, signal average, and metabolite identification of the spectrum and subsequently measured the peak area of NAA, Cho, and Cr. Each spectrum was evaluated for the peak area of Cho at 3.22 ppm, Cr at 3.03 ppm, and NAA at 2.02 ppm.

14 females; 25.10 ± 6.59 years of age) were enrolled in the present study. No significant differences were noted in the parameters of age ($t = 0.776$, $P = .442$) or gender ($\chi^2 = 0.727$, $P = .525$) between MDD patients and HC participants. Following comparison of the clinical scales, the HAMD scores of the MDD patients were significantly higher than those of the HC participants ($z = -6.091$, $P < .001$). No significant difference was noted in the YMRS scores between the MDD patients and the HC participants ($z = -1.515$, $P = .260$).

Comparison of Copper-Associated Indices Between MDD Patients and HC Participants

Table 2 and Figure 2 depict the results of the comparison of copper-associated indices of the MDD patients and HC participants. In the present study, we demonstrated that the mRNA expression levels of *ATP7A* in MDD patients (13.64 [24.46]) were lower than those noted in HC participants (27.79 [24.80]) ($z = -2.695$, $P = .011$). The data indicated that ceruloplasmin levels in MDD patients [0.43 (0.12)] were higher than those noted in HC participants (0.35 [0.06]) ($t = 3.156$, $P = .009$). No significant differences in the copper levels were noted between the 2 groups.

Comparison of NAA/Cr Ratio in Different Brain Regions Between MDD Patients and HC Participants

Table 3 indicated the results of the comparison of the NAA/Cr ratios in different brain regions between MDD patients and HC participants. No significant differences were noted in the NAA/Cr ratio of the different brain regions between MDD patients and

Table 1. Demographics and Clinical Characteristics of MDD Patients and HC Participants

Variables	MDD	HC	χ^2/t/Z	Adjusted *P*
Gender (male/female)	14 (17)	7 (14)	0.727	.525
Age	26.84 (8.74)	25.10 (6.59)	0.776	.442
Age of onset	25.16 (9.31)	NA		
Duration of disease (mo)	25.39 (37.09)	NA		
No. of episodes	2.06 (1.09)	NA		
24-item HAMD scores	23.10 (4.94)	2.14 (1.76)	−6.091	<.001[a]
YMRS scores	0.94 (1.20)	0.52 (0.98)	−1.515	.260

Abbreviations: HAMD, Hamilton Depression Scale; HC, healthy control; MDD, major depressive disorder; YMRS, Young Manic Rating Scale.
Adjusted *P*: *P* values were adjusted using false discovery rate correction. Adjusted *P* value for gender was obtained by the chi-square test. Adjusted *P* value for age was obtained by the independent-samples *t* test. Adjusted *P* values for 24-item Hamilton Depression Scale (HAMD) scores and Young Manic Rating Scale (YMRS) scores were obtained by the Mann-Whitney U test. Values for MDD and HC are presented as mean (SD) unless otherwise specified.
[a]Adjusted $P < .05$.

Table 2. Comparison of Copper-Associated Indices Between MDD Patients and HC Participants

Variables	MDD	HC	Z/t	Adjusted *P*
mRNA expression levels of ATP7A	13.64 (24.46)	27.79 (24.80)	−2.695	.011[a]
Copper levels	13.41 (3.26)	13.48 (3.27)	−0.074	.942
Ceruloplasmin levels	0.43 (0.12)	0.35 (0.06)	3.156	.009[a]

Abbreviations: HC, healthy control; MDD, major depressive disorder.
Adjusted *P*: *P* values were adjusted using false discovery rate correction. Adjusted *P* values for copper and ceruloplasmin levels were obtained by the independent-samples *t* test. Adjusted *P* value for mRNA expression levels of ATP7A was obtained by the Mann-Whitney U test. Values for MDD and HC are presented as mean (SD) unless otherwise specified.
[a]Adjusted $P < .05$.

HC participants, including the left PWM, right PWM, left ACC, right ACC, left thalamus, right thalamus, left LN, right LN, left cerebellum, and right cerebellum. ($t = -0.443$, $P = .825$; $t = 0.036$, $P = .971$; $t = 1.809$, $P = .256$; $t = -0.629$, $P = .760$; $t = -2.928$, $P = .051$; $t = -2.644$, $P = .055$; $t = -0.958$, $P = .686$; $t = -0.171$, $P = .961$; $t = 0.707$, $P = .760$; $t = -1.369$, $P = .442$, respectively).

Comparison of Cho/Cr Ratio in Different Brain Regions Between MDD Patients and HC Participants

The Cho/Cr ratio of different brain regions between MDD patients and HC participants was analyzed (Table 4; Fig. 3). The Cho/Cr ratio was assessed in significant brain regions, including the left PWM, right PWM, left ACC, right ACC, left thalamus, right thalamus, left LN, and right cerebellum. This parameter was significantly increased in MDD patients compared with that noted in HC participants ($t = 5.019$, $P < .001$; $t = 5.129$, $P < .001$; $t = 5.882$, $P < .001$; $t = 0.899$, $P < .001$; $t = 5.420$, $P < .001$; $t = 4.577$, $P < .001$; $t = 2.982$, $P = .005$; $t = 3.657$, $P = .001$, respectively). No significant differences were noted in the Cho/Cr ratio with regard to the right LN and left cerebellum between MDD patients and HC participants ($t = 1.285$, $P = .205$; $t = 1.999$, $P = .056$, respectively).

Correlation Between mRNA Expression Levels of *ATP7A* and Ceruloplasmin Levels in MDD Patients

The results of the correlation analysis between mRNA expression levels of *ATP7A* and ceruloplasmin levels in MDD patients are depicted in Table 5 and Figure 4. A significant negative correlation was noted between the mRNA expression levels of *ATP7A* and ceruloplasmin levels ($r = -0.389$, $P = .031$). This result indicated that the decline in mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels interacted, resulting in the retention of copper homeostasis, which may have an important influence on MDD.

Correlations Among mRNA Expression Levels of *ATP7A*, Ceruloplasmin Levels, and Cho/Cr Ratio in MDD Patients

The mRNA expression levels of *ATP7A*, ceruloplasmin levels, and the Cho/Cr ratio were selected as indexes for further analysis due to the significant differences noted between MDD patients and HC participants. The results of the correlation among the mRNA expression levels of *ATP7A*, ceruloplasmin levels, and the Cho/Cr ratio in MDD patients are shown in Table 6 and Figure 5. A negative correlation between mRNA expression levels of *ATP7A* and the Cho/Cr ratio was noted in the left PWM, right PWM, and right ACC ($r = -0.598$, $P < .001$; $r = -0.421$, $P = .036$; $r = -0.497$, $P = .016$, respectively). In addition, a significant positive correlation was noted between the ceruloplasmin levels and the Cho/Cr ratio of the left PWM ($r = 0.523$, $P = .024$). These results suggested that the decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels exhibited an impaired influence on neuronal metabolism, which was attributed to the development of MDD.

Correlation Between Participant Clinical Characteristics and Values of Significant Indicators in MDD Patients

Table 7 shows that no significant correlations were noted between clinical characteristics (age of onset, course of disease, number of episodes, and 24-item HAMD scores) and the significant indicators of MDD patients.

Discussion

To the best of our knowledge, this is the first early study that has explored the association between copper imbalance derived from *ATP7A* inactivity and the alteration of brain metabolites

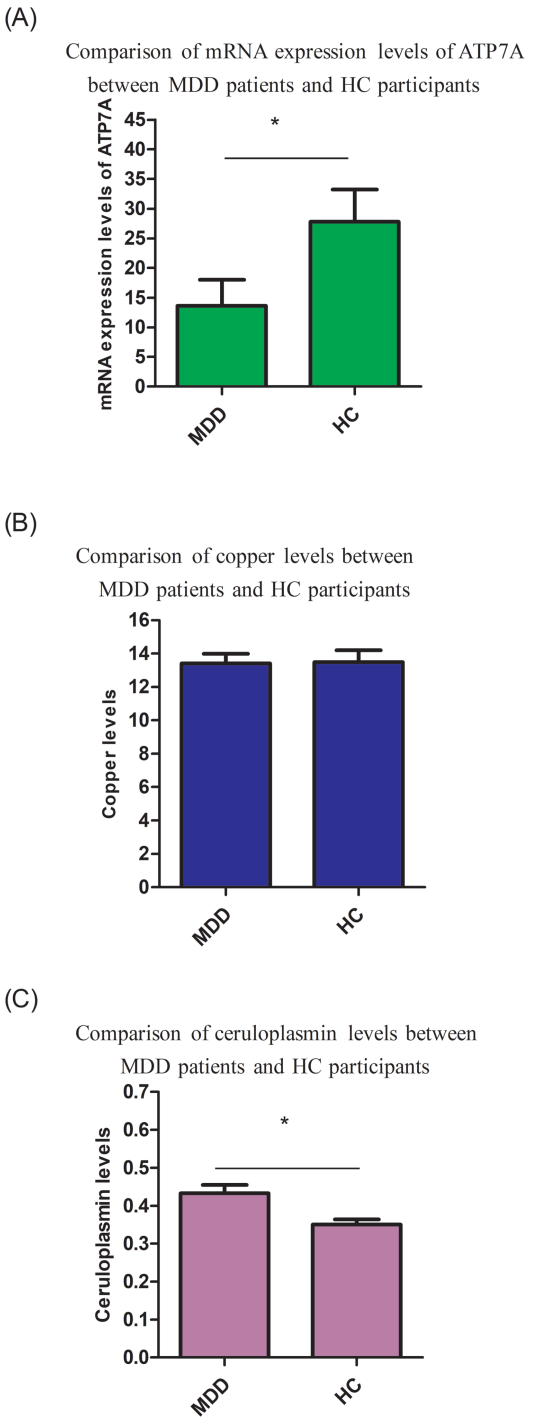

Figure 2. Comparison of copper-associated indices between major depressive disorder (MDD) patients and healthy control (HC) participants. (A) The mRNA expression levels of *ATP7A* of the MDD patients (n = 31) and the HC participants (n = 21) were compared using the Mann-Whitney U test. The mRNA expression levels of *ATP7A* in MDD patients were significantly lower than those noted in HC participants. The symbol (–) shows the comparison of mRNA expression levels of *ATP7A* between MDD patients and HC participants. *$P < .05$. (B) The copper levels of the MDD patients (n = 31) and the HC participants (n = 21) were compared using the independent-samples *t* test. No significant difference in the copper levels was noted between MDD patients and HC participants. (C) The ceruloplasmin levels of the MDD patients (n = 31) and the HC participants (n = 21) were compared using the independent-samples *t* test. The symbol (–) was used to compare ceruloplasmin levels between MDD patients and HC participants. *$P < .05$.

in MDD patients. To investigate biochemical abnormalities, the 3.0T multi-voxed ^{1}H-MRS was used to detect reactive brain regions in the presence of depression. A decline in mRNA expression levels of *ATP7A* and an increase in ceruloplasmin levels were noted. These trends exhibited a significant correlation in MDD patients. The decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels exhibited an effect of neuronal membrane metabolic impairment in the left PWM, right PWM, and right ACC of the MDD patients.

ATP7A is a primary activity membrane transporter. It can use the free energy of ATP hydrolysis to transport copper across the membrane to retain copper homeostasis (Inesi, 2017). It has been reported that the activities of *ATP7A* largely depend on the mRNA expression levels of *ATP7A*, which may influence copper transmission (Zheng et al., 2014). According to the Schoonover study, impaired copper-associated charge transfer through *ATP7A* was observed in schizophrenia (Schoonover et al., 2018). However, limited research has been conducted to investigate the association between *ATP7A* and MDD. The main finding of the present study was that the mRNA expression levels of *ATP7A* in the MDD group were lower than those of the HC participants. The results supported the hypothesis that the inactivity of the copper transporter *ATP7A* caused by lower mRNA expression exhibited an association with the development of MDD. In conclusion, we speculate that the decline in the mRNA expression levels of *ATP7A* can lead to copper imbalance, which may have a beneficial effect on depression.

Copper concentration is not maintained at a steady state in the brain following the decline in the mRNA expression levels of *ATP7A*. Reduced copper is transported across the membrane, and an increase in the copper concentration can produce oxidative damage to the neurons (White et al., 2001). Ceruloplasmin is an acute-phase reactant activated following oxidative damage in the central nervous system (Barbariga et al., 2015). Ceruloplasmin is an important copper-transporting serum protein that can couple with copper and carry it across the membrane to the extracellular medium (Das et al., 2007). However, a limited number of studies have explored the role of ceruloplasmin in the development of MDD. In the present study, increased ceruloplasmin levels were observed in the MDD group. In addition, we also found a significant correlation between the decline in mRNA expression levels of *ATP7A* and elevated ceruloplasmin levels in MDD patients, confirming that both of these parameters exhibited an interaction that could regulate copper homeostasis. In conclusion, these results suggested that both the decline in the mRNA expression levels of *ATP7A* and the elevated serum ceruloplasmin levels exhibited a significant contribution to the development of MDD.

According to a previous study, certain alterations were noted in specific biochemical metabolites that were associated with the development of MDD (Ende et al., 2006). The impairment of neurons leads to the reduction of cell metabolism. The membrane phospholipid metabolism is increased to supply energy in a compensation-type mechanism in mental disease (Smesny et al., 2012). NAA is synthesized in the mitochondria and reflects the structure and functional integrity of the neuron (Strakowski et al., 2005). Cho represents the metabolism of the cell membrane. Changes in Cho levels significantly affect neuronal metabolism (Husarova et al., 2012). We selected the indices NAA/Cr and Cho/Cr based on the exclusion of unavoidable factors, such as difference in height and weight in certain individuals. However, the impaired cerebral regions of altered neuronal biochemical metabolites are inclusive. Based on previous reports,

Table 3. Comparison of NAA/Cr Ratio in Different Brain Regions Between MDD Patients and HC Participants

Variables	MDD	HC	T	Adjusted P
Left prefrontal white matter	2.09 (0.29)	2.13 (0.37)	−0.443	.825
Right prefrontal white matter	2.18 (0.37)	2.18 (0.22)	0.036	.971
Left anterior cingulate cortex	1.80 (0.23)	1.68 (0.22)	1.809	.256
Right anterior cingulate cortex	1.77 (0.29)	1.82 (0.18)	−0.629	.760
Left thalamus	1.93 (0.39)	2.25 (0.38)	−2.928	.051
Right thalamus	1.91(0.31)	2.14 (0.28)	−2.644	.055
Left lentiform nucleus	1.40 (0.34)	1.50 (0.36)	−0.958	.686
Right lentiform nucleus	1.57 (0.34)	1.59 (0.37)	−0.171	.961
Left cerebellum	1.46 (0.56)	1.36 (0.28)	0.707	.760
Right cerebellum	1.37 (0.32)	1.51 (0.42)	−1.369	.442

Abbreviations: Cr, creatine; HC, healthy control; MDD, major depressive disorder; NAA, N-acetylaspartate.
Adjusted P: P values were adjusted using false discovery rate correction. The adjusted P value was obtained by the independent-samples t test. Values for MDD and HC are presented as mean (SD) unless otherwise specified.

Table 4. Comparison of the Cho/Cr Ratio Between MDD Patients and HC Participants

Variables	MDD	HC	T	Adjusted P
Left prefrontal white matter	1.52 (0.42)	1.06 (0.23)	5.019	<.001[a]
Right prefrontal white matter	1.53 (0.46)	1.07 (0.14)	5.129	<.001[a]
Left anterior cingulate cortex	1.44 (0.38)	0.96 (0.19)	5.882	<.001[a]
Right anterior cingulate cortex	1.35 (0.33)	1.08 (0.16)	0.899	<.001[a]
Left thalamus	1.67 (0.74)	0.92 (0.17)	5.420	<.001[a]
Right thalamus	1.65 (0.96)	0.85 (0.15)	4.577	<.001[a]
Left lentiform nucleus	1.48 (0.70)	0.99 (0.47)	2.982	.005[a]
Right lentiform nucleus	1.57 (0.78)	1.27 (0.85)	1.285	.205
Left cerebellum	1.43 (1.00)	0.99 (0.22)	1.999	.056
Right cerebellum	1.38 (0.57)	0.96 (0.23)	3.657	.001[a]

Abbreviations: Cho, choline; Cr, creatine; HC, healthy control; MDD, major depressive disorder.
Adjusted P: P values were adjusted using false discovery rate correction; the adjusted P value was obtained by the independent-samples t test. Values for MDD and HC are presented as mean (SD) unless otherwise specified.
[a]Adjusted P < .05.

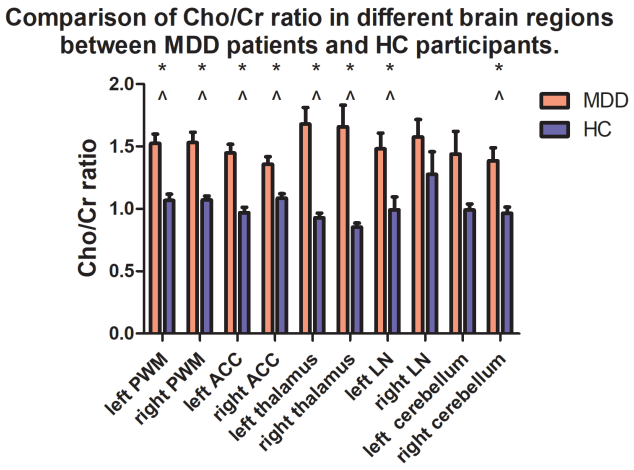

Figure 3. Comparison of the Cho/Cr ratio in different brain regions between major depressive disorder (MDD) patients and healthy control (HC) participants. The biochemical metabolite ratio (Cho/Cr) ratio of bilateral brain regions of interest between MDD patients (n = 31) and the HC participants (n = 21) was analyzed via using the independent-samples t test. The symbol (^) was used to compare the Cho/Cr ratio in the left prefrontal white matter (PWM), right PWM, left anterior cingulate cortex (ACC), right ACC, left thalamus, right thalamus, left lentiform nucleus (LN), and right cerebellum between the 2 groups. This parameter was significantly increased in MDD patients compared with that noted in HC participants. *P < .05. No significant differences were noted in the Cho/Cr ratio with regard to the right LN and left cerebellum between MDD patients and HC participants.

Table 5. Correlation Between mRNA Expression Levels of ATP7A and Ceruloplasmin Levels in MDD Patients

	Ceruloplasmin levels	
Variables	r	P
mRNA expression levels of ATP7A	−0.389	.031[a]

Abbreviation: MDD, major depressive disorder.
The P value was obtained by Spearman correlation coefficient.
[a]P < .05.

we confirmed that PWM exhibited a crucial effect on the development of depression (Bhatia et al., 2018). It was reported that MDD patients exhibited lower activity of PWM following processing of emotional stimuli (Jia et al., 2015). The ACC is the key component of multiple neural networks involved in cognitive and emotional processes (Kowal et al., 2013). The thalamus is a core part of the limbic-thalamic circuit involved in regulating emotional conditions (Takeuchi et al., 2017). The role of the thalamus in depression has been investigated in animals (Huang et al., 2017). The LN is an indispensable part of the basal ganglia that is affected in depression (Su et al., 2014). The cerebellum is well known as an important somatic homeostatic organ, which can also process depressive behaviors (Yamamoto et al., 2015). Therefore, the present study was based on the left and right cerebral regions to investigate the correlation among

mRNA expression levels of *ATP7A*, serum ceruloplasmin levels, and neuronal metabolism in unmedicated MDD patients.

In the current study, no significant differences were noted in the NAA/Cr ratios of the 5 brain regions of interest between the MDD and the HC groups. These findings may result from certain clinical features, including dietary habits and disease states. Therefore, a larger sample size is required to assess the effects of NAA in MDD. In addition, significantly increased Cho/Cr ratios were noted in the left PWM, right PWM, left ACC, right ACC, left thalamus, right thalamus, left LN, and right cerebellum in MDD patients, suggesting that the decreased neuronal metabolism of the above brain regions of interest was involved in the development of MDD.

ATP7A is an essential copper-transporting enzyme, and abnormal copper levels may generate psychogenic behaviors (Schoonover et al., 2018). According to these results, further analysis indicated that inactivation of *ATP7A* caused by the decline in its mRNA expression levels could lead to copper-associated damage on neuronal membrane metabolism, notably in the PWM and right ACC. *ATP7A* has an inverse effect on neuronal impairment. Our results indicated that elevated ceruloplasmin levels were accompanied by elevated copper levels and could induce oxidation, leading to disturbed neuronal metabolism in the left PWM. Depression is a common psychological disorder that can be influenced by the dysfunction of the PWM. Previous study has supported the evidence that MDD patients exhibit decreased metabolism in the PWM (Carceller-Sindreu et al., 2019). Wang et al. found that abnormalities in the metabolic levels of PWM might be involved in the development of MDD (Wang et al., 2012), which is in agreement with our findings. Depressive mood is also the result of dysfunction in complex brain networks. It has been shown that the PWM is connected with the ACC through complex brain networks to induce negative feelings (Gujral et al., 2017). The ACC mainly regulates emotional activity and has a great impact during the formation of depression (Godlewska et al., 2018). In the study reported by Gabbay et al., low metabolism in ACC corresponded to depressive symptoms (Gabbay et al., 2017). The study reported by Conklin et al. is in agreement with the present results, demonstrating that MDD patients exhibited disturbance in neuronal metabolism in the ACC (Conklin et al., 2010). Therefore, we speculated that MDD patients presenting with a decline in mRNA expression levels of *ATP7A* and elevated ceruloplasmin levels had increased risk of neuronal impairment in the left PWM, right PWM, and right ACC. In conclusion, our study provides a promising insight in the association between copper imbalance and decreased neuronal biochemical metabolism of MDD.

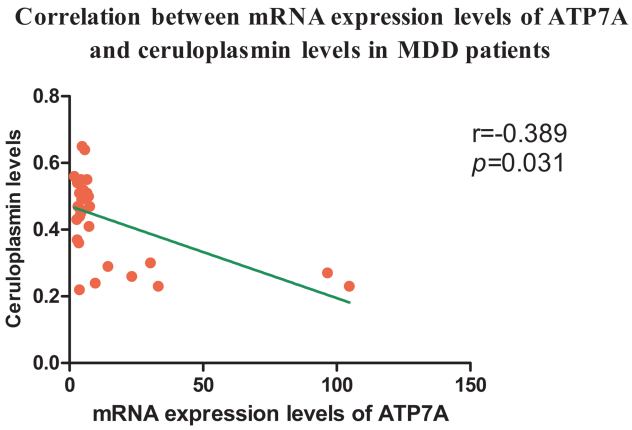

Figure 4. Correlation between mRNA expression levels of ATP7A and ceruloplasmin levels in major depressive disorder (MDD) patients. The Spearman correlation was used to analyze the association between the mRNA expression levels of *ATP7A* and ceruloplasmin levels in MDD patients (n = 31). A significant negative correlation was noted between the mRNA expression levels of *ATP7A* and ceruloplasmin levels (r = –0.389, *P* = .031).

However, in the present study, the correlation between elevated ceruloplasmin levels and increased Cho/Cr ratio was only noted in the left PWM of MDD patients. The correlation between the decline in the mRNA expression levels of *ATP7A* and increased Cho/Cr ratio was only found in the right ACC of the MDD patients. The different brain regions exhibited dissimilar associations between the aforementioned parameters, possibly due to different mechanisms involved. This resulted in the decline in *ATP7A* mRNA expression levels and the increase in ceruloplasmin levels, which affected the function of impaired neurons. In addition, it should be noted that MDD patients and HC participants did not reveal a significant correlation in serum copper levels. This result may be explained possibly due to the facts that individuals differences, sample-size and the serum copper levels do not entirely reflect copper metabolism. Copper levels in the cerebrospinal fluid, even in the intracellular fluid, could accurately present the state of copper metabolism. Therefore, further studies including more MDD patients are required to confirm these results.

This is the first study to our knowledge to evaluate the correlation between the mRNA expression levels of *ATP7A* and the alteration in the concentration of neuronal metabolites in brain

Table 6. Correlations Among mRNA Expression Levels of ATP7A, Ceruloplasmin Levels, and Cho/Cr Ratio in MDD Patients

	mRNA expression levels of ATP7A		Ceruloplasmin levels	
Variables	r	Adjusted *P*	r	Adjusted *P*
Left prefrontal white matter	–0.598	0.001[a]	0.523	0.024[a]
Right prefrontal white matter	–0.421	0.036[a]	0.360	0.189
Left anterior cingulate cortex	0.052	0.934	–0.112	0.548
Right anterior cingulate cortex	–0.497	0.016[a]	0.320	0.210
Left thalamus	0.017	0.944	–0.121	0.548
Right thalamus	–0.438	0.056	0.306	0.188
Left lentiform nucleus	–0.013	0.944	–0.280	0.169
Right cerebellum	–0.373	0.062	0.280	0.169

Abbreviations: Cho, choline; Cr, creatine; MDD, major depressive disorder.
Adjusted *P*: *P* values were adjusted using false discovery rate correction. Adjusted *P* value for mRNA expression levels of ATP7A was obtained by the Spearman correlation coefficient. Adjusted *P* value for ceruloplasmin levels was obtained by Pearson's correlation coefficient.
[a]Adjusted *P* < .05.

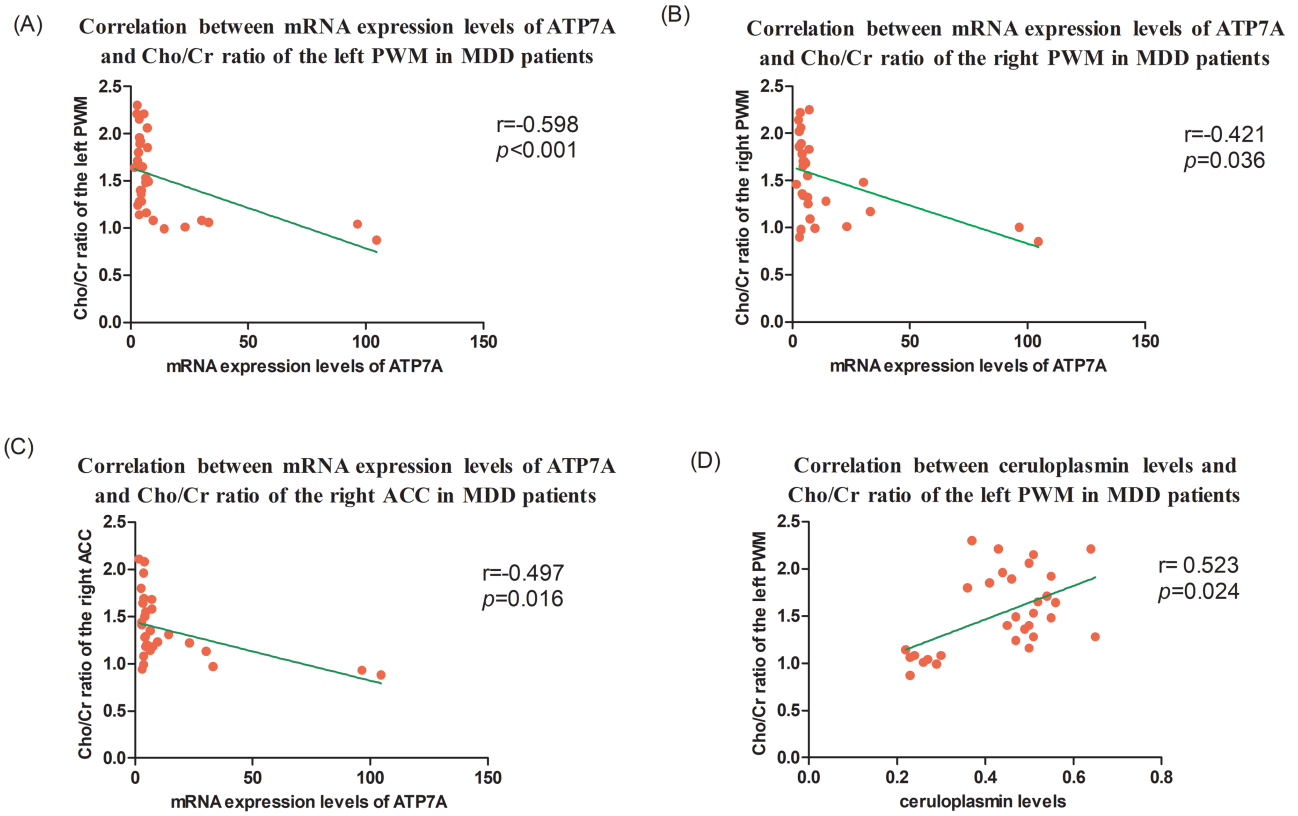

Figure 5. Correlations among mRNA expression levels of ATP7A, ceruloplasmin levels, and the Cho/Cr ratio in major depressive disorder (MDD) patients. The mRNA expression levels of ATP7A, ceruloplasmin levels, and the Cho/Cr ratio were selected as indexes for further analysis due to the significant differences noted between MDD patients and HC participants. (A) The Spearman correlation was used to analyze the association between the increased Cho/Cr ratio in the left PWM and the reduced mRNA expression levels of *ATP7A* of MDD patients (n = 31). A negative significantly correlation between the reduced mRNA expression levels of *ATP7A* and the Cho/Cr ratio was noted in the left PWM ($r = -0.598$, $P < .001$). (B)The Spearman correlation was used to analyze the association between the increased Cho/Cr ratio in the right PWM and the reduced mRNA expression levels of MDD patients (n = 31). A significantly negative correlation between the reduced mRNA expression levels of *ATP7A* and the increased Cho/Cr ratio was noted in the right PWM ($r = -0.421$, $P = .036$). (C) The Spearman correlation was used to analyze the association between the increased Cho/Cr ratio in the right ACC and the reduced *ATP7A* mRNA levels of MDD patients (n = 31). A significantly negative correlation between the reduced mRNA expression levels of *ATP7A* and the increased Cho/Cr ratio was noted in the right ACC ($r = -0.497$, $P = 0.016$). (D) The Pearson correlation was used to analyze the association between the increased Cho/Cr ratio in the left PWM and the elevated ceruloplasmin levels in MDD patients (n = 31). A significant positive correlation was noted between the elevated ceruloplasmin levels and the increased Cho/Cr ratio of the left PWM ($r = 0.523$, $P = .024$).

Table 7. Correlation Between Clinical Characteristics of Participants and Values of Significant Indicators in MDD Patients

	Age of onset		Course of disease		Number of episodes		24-item HAMD scores	
Variables	r	Adjusted *P*	r	Adjusted *P*	r	Adjusted *P*	r	Adjusted *P*
mRNA expression levels of ATP7A	0.022	.906	0.360	.235	0.262	.155	0.160	.390
Ceruloplasmin levels	0.035	.853	−0.183	.406	− 0.090	.631	−0.137	.461
Cho/Cr in left prefrontal white matter	−0.082	.288	−0.203	.680	−0.215	.244	−0.199	.283
Cho/Cr in right prefrontal white matter	−0.133	.475	−0.184	.536	−0.286	.119	−0.197	.288
Cho/Cr in right anterior cingulate cortex	0.057	.759	−0.169	.364	−0.298	.103	−0.330	.070

Abbreviations: Cho, choline; Cr, creatine; HAMD, Hamilton Depression Scale; MDD, major depressive disorder.
Adjusted P: P values were adjusted using false discovery rate correction. Adjusted P value was obtained by the Spearman correlation coefficient.

regions of interest in MDD patients. Nevertheless, some potential limitations of the present study should be taken into consideration. Firstly, the sample size was relatively small and this may limit the generalizability of our results. A higher sample size is required in future investigations. Secondly, we used serum copper-related indicators to reflect copper metabolism. The estimation of the copper levels in the cerebrospinal fluid may provide more accurate results in future studies. Thirdly, only the metabolic conditions of the left PWM, right PWM, and right ACC exhibited a significant association in the present study. Further exploration on other brain regions should be taken into account in future research studies. Finally, multiple confounding factors (e.g., nutritional habits, specificity of the scan) were present that may have influenced the variability on the measured results. Further studies are required to exclude the association of the aforementioned confounding factors.

Conclusions

In conclusion, these preliminary data suggested that the decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels exhibited a strong correlation in MDD. Both of these factors contributed to neuronal damage by affecting the neuronal membrane metabolic condition of specific brain regions. In addition, MDD patients with decreased mRNA expression levels of *ATP7A* exhibited a high risk of neuronal impairment in the left PWM, right PWM, and right ACC. Moreover, elevated ceruloplasmin levels had an inverse effect on the neuronal metabolism of the left PWM in MDD. The decline in the mRNA expression levels of *ATP7A* and the elevated ceruloplasmin levels play important roles in the pathophysiology of MDD. However, our investigation was preliminary, and further studies based on a larger population of MDD patients should be performed to widen the knowledge on the roles of *ATP7A* and ceruloplasmin in the pathophysiology of MDD.

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Statement of Interest

None.

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