The changes of extrapyramidal network studied by Diffusion tensor imaging in WD patients after 5 years of copper excluding treatment

Xiang-xue ZHOU (✉ 1397880617@qq.com)

the east area of the first affiliated hospital of The Sun Yat-Sen University <https://orcid.org/0000-0002-6583-9734>

Hao-ling QIN

Sun Yat-Sen University

Jian LIAO

Sun Yat-Sen University

Xia XIAO

Sun Yat-Sen University

Research Article

Keywords: Wilson disease, diffusion tensor imaging, extracortical tract, network

Posted Date: February 2nd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1190627/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Objective: To evaluate the changes of extrapyramidal network in Wilson disease (WD) patients after 5 years of copper excluding treatment using diffusion tensor imaging (DTI).

Methods: 50 patients with WD, including 41 with cerebral type and 9 with hepatic type, and 10 age-matched healthy controls were enrolled. Neurological symptoms were scored using the modified Young Scale. Patients with cerebral type WD were divided into four subgroups: those with (1) hypokinesia, (2) parkinsonism, (3) mouth and throat dystonia, and (4) psychiatric symptoms. All study subjects underwent DTI of the brain. Five regions of interest (ROIs) were chosen. Fractional anisotropy (FA) and fiber volumes between ROIs were determined. After 5 years of copper excluding treatment, DTI review was performed.

Results: After 5 years of copper excluding treatment, the volumes of fibers between the globus pallidus (GP) and the putamen (PU), the GP and the caudate nucleus (CA), the substantia nigra (SN) and the PU, the SN and the thalamus (TH), the PU and the TH, the TH and the CA, the TH and the cerebellum were also significantly higher in WD patients ($P=0.013, 0.040, 0.037, 0.021, 0.042, 0.038, 0.019$). The volumes of fibers between the SN and the PU were higher in WD patients of group 3 after therapy ($P=0.027$). FA values of the fibers between the GP and the PU, the GP and the CA were significantly higher in WD patients of group 4 after therapy ($P=0.026, 0.047$). FA values of the fibers between the GP and the CA in WD patients with hepatic type were higher in WD patients after therapy ($P=0.019$).

Conclusions: After the treatment with metal chelating agent, the damage of extrapyramidal neural network connection fibers in WD patients were repaired to a certain extent. Different types of WD patients have different responses to copper excluding treatment.

1. Introduction

Wilson disease (WD) is an autosomal recessive inherited disorder of copper metabolism, characterized by excessive copper deposition, especially in the liver and brain. Patients with WD have many clinical manifestations and changes on magnetic resonance imaging (MRI), and show varied responses to treatment. Conventional MRI usually shows signal abnormalities in the subcortical nuclei, including the mesencephalon, thalamus and basal ganglia regions^[1]. Neurological symptoms in WD patients may be caused by damage to both the subcortical nuclei and the fiber projections between them^[1, 2, 3, 4]. We have previously demonstrated through DTI that WD patients have damage to the nerve connective fibers of the extrapyramidal network. Moreover, the injury of nerve fibers of the extrapyramidal system is correlated with the type and severity of neurological symptoms^[5]. Our previous study found that metal deposition in the brain of WD patients could be reduced after copper excluding treatment^[6]. However, there is no research on whether the damage of extrapyramidal network of WD patients can be improved after copper excluding treatment.

Diffusion tensor imaging (DTI) has been used to detect changes in white matter integrity [7]. DTI can identify and quantify microstructural damage in the brain, while providing directional information on the random movement of water molecules^[8, 9]. DTI metrics include fractional anisotropy (FA), radial diffusivity and axial diffusivity^[9]. DTI fiber tracking (FT) can show individual fiber tracts of the brain because of the anisotropy of water diffusion in central nervous system tissue^[3, 10]. Moreover, DTI can be used to trace subcortical gray matter connections. In this study, DTI was used to study the changes of brain extrapyramidal fibrous connection in WD patients after copper excluding treatment, and to analyze the influencing factors of fibrous connection improvement.

2. Materials And Methods

2.1. Participants

The study enrolled 50 WD patients (28 males and 22 females, mean age 21 ± 4 years) evaluated at the First Affiliated Hospital, Sun Yat-Sen University, between July 2007 and October 2021. Patients were categorized as cerebral type WD, presenting mainly with psychiatric and/or neurological symptoms, or hepatic type WD, presenting mainly with hepatic symptoms. The diagnosis of each patient was based on a combination of clinical symptoms and laboratory tests. Clinical indications included decreased serum ceruloplasmin concentration, low serum copper concentration, elevated 24 h urinary copper excretion and the presence of Kayser–Fleischer (K–F) rings on slit lamp examination. As a control group, 10 age- and sex-matched healthy individuals were recruited from the hospital. Informed consent was obtained from all participants prior to enrollment. This study was approved by the Ethics Committee of Sun Yat-sen University.

The severity of neurological symptoms was assessed using the modified Young Scale^[11]. This scale consists of items evaluating dysarthria, dysphagia, rigidity, ataxia, tremor, choreic movement, gait abnormality and psychogenia. Based on their neurological symptoms, patients with cerebral type WD were divided into four subgroups: those with (1) hypokinesia (tremor, choreic movement, ataxia), (2) parkinsonism (rigidity and gait abnormality), (3) mouth and throat dystonia (dysarthria and dysphagia), and (4) psychiatric symptoms. All patients received a combination treatment of sodium dimercaptopropansulfonate and dimercaptosuccinic acid. All the patients received DMPS treatment (Injects were given 1 to 2 times per year, each time 1g/d, for 10-14 days), DMSA treatment with a dose of 10mg/kg/day. The introduction of the medications was full dose immediately. All patients accepted treatment for 5 years. Patients who had obvious deterioration or adverse events would stop medication. DTI review was performed before and after treatment. Copper concentrations in serum and 24 h urinary copper excretion were measured before and after treatment.

2.2. MRI protocol

All participants underwent DTI tests of the brain before treatment. All MRI data were obtained using a 1.5 T scanner (Achieva Nova Dual; Philips Healthcare, Best, The Netherlands), equipped with eight-channel, phase-array head coils. Conventional MRI sequences included spin-echo (SE) T1-weighted images [488 ms repetition time (TR), 15 ms echo time (TE)] obtained in the axial and sagittal planes, with an acquisition time of 2 min, matrix of 256×256 , and a 250 mm field of view (FOV). Axial T2-weighted images were also acquired (3600 ms TR, 100 ms TE). The section thickness was 5 mm. DTI was performed using SE

echo-planar imaging (EPI) (9500 ms TR, 70 ms TE, 256×256 matrix size, 250 mm field of view, 2 mm section thickness with no gaps). Images were obtained with 32-direction diffusion-encoding (b =1000 s/mm2 for each direction) and without diffusion encoding (b =0 s/mm2) and were corrected for head and cardiac motion artifacts.

2.3. Image processing

All DTI data were transferred to a workstation (Philips extended MR workspace 2.6.3.2, Philips Medical Systems) and processed using DTI fiber-tracking software for DTI quantitative analysis. Regions of interest (ROIs) were drawn according to anatomical structures, including the globus pallidus (GP), head of the caudate nucleus (CA), putamen (PU), thalamus (TH), substantia nigra (SN), and cerebellum. Fiber tracts were reconstructed using the fiber assignment continuous tracking (FACT) method. DTI fibers were counted, and FA values along the tracts were determined. Two neuroradiologists separately and independently drew all ROIs on tensor color maps and measured FA and DTI fiber counts. To reduce random variability in the measurements, each value was an average of three different measurements. The reported values are the mean of the two observers.

2.4. Statistical analysis

Results are presented as mean±SEM. All statistical analyses were performed using SPSS 13.0. (SPSS Inc., Chicago, Illinois, USA). DTI parameters of WD patients and controls were compared using independent-sample t-tests. One-way analysis of variance (ANOVA) and least significance difference (LSD) tests were used to compare the DTI parameters of each group and before and after treatment. Pearson's correlation analysis was used to assess the correlations of DTI parameters with modified Young Scale scores. A P value < 0.05 was considered statistically significant.

3 Results

3.1. Demographic and clinical characteristics of each group

Gender distribution and age were similar in WD patients and healthy volunteers (Table 1).

Table 1
Clinical and demographic characteristics of patients and controls.

		Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4	Hepatic type WD	Controls	P
Number		12	10	11	8	9	10	
Gender (M/F)		6/6	4/6	7/4	5/3	6/3	5/5	0.094
Age (years)		23±3	20±5	21±3	20±2	18±5	21±3	0.079
Duration (months)		22±3	17±4	19±5	21±4	19±7	0	0.022
Modified Young Scale scores	before treatment	15.22±3.07	24.61±7.30	19.52±6.98	6.07±3.43	0	0	0.014
	after treatment	8.49±2.77 ^a	21.16±8.25	13.27±5.42 ^a	3.09±1.26 ^a	0		0.031
Child-Pugh liver grade	before treatment	A(12)	A(10)	A(11)	A(8)	A/B/C(4/3/2)		
	after treatment	A(12)	A(10)	A(11)	A(8)	A/B/C(6/3/0)		
Data are shown as number or mean±standard deviation.								
a: P≤0.05 compared with the baseline.								

3.2. DTI parameters of fibers between ROIs after treatment

FA values and the volume of fibers between ROIs of WD patients and healthy controls are shown in Table 2, Fig. 1.

Table 2
FA values and fiber volumes between ROIs of WD patients and healthy controls

		Subgroup 1		Subgroup 2		Subgroup 3		Subgroup 4		Hepatic type WD	
		FA	VOL	FA	VOL	FA	VOL	FA	VOL	FA	VOL
GP-SN	Before treatment	0.49±0.21	192±45	0.46±0.12	82±34 ^a	0.51±0.11	186±44	0.47±0.20	121±33 ^a	0.52±0.04	207±56
	After treatment	0.52±0.16	189±76	0.43±0.07	57±60 ^a _b	0.50±0.12	180±73	0.46±0.12	214±90 ^b	0.50±0.07	190±30 ^a
GP-PU	Before treatment	0.44±0.12	207±63 ^a	0.40±0.11 ^a	239±27 ^a	0.36±0.09 ^a	240±26 ^a	0.37±0.03 ^a	234±41	0.42±0.10 ^a	292±28
	After treatment	0.48±0.09	280±20 ^b	0.36±0.24 ^a	227±110 ^a	0.33±0.10 ^a	220±137 ^a	0.46±0.21 ^b	280±133	0.51±0.21	311±14
GP-TH	Before treatment	0.52±0.11	160±62	0.50±0.04	92±25 ^a	0.40±0.13 ^a	133±31	0.54±0.09	142±42	0.55±0.06	164±21 ^c
	After treatment	0.52±0.09	153±20	0.49±0.27	83±31 ^a	0.46±0.07	146±27	0.55±0.27	183±77	0.56±0.03	161±31
GP-CA	Before treatment	0.41±0.13	174±65 ^a	0.38±0.05 ^a	296±60 ^a	0.43±0.20	285±33 ^a	0.37±0.11 ^a	182±70 ^a	0.44±0.03	354±12
	After treatment	0.46±0.21	280±20 ^a _b	0.38±0.12	280±130 ^a	0.43±0.22	277±145 ^a	0.56±0.29 ^b	299±63 ^a _b	0.59±0.09 ^b	380±70
SN-PU	Before treatment	0.40±0.13	50±27 ^a	0.41±0.07	63±12 ^a	0.44±0.17	67±13 ^a	0.40±0.11	52±29 ^a	0.43±0.14	76±6
	After treatment	0.41±0.20	76±20 ^b	0.40±0.25	62±33 ^a	0.46±0.23	90±41 ^b	0.46±0.03	80±35 ^b	0.46±0.03	80±35
SN-TH	Before treatment	0.49±0.10	541±149 ^a	0.50±0.09	674±49	0.32±0.21 ^a	421±64 ^a	0.59±0.13	685±41	0.53±0.07	723±12
	After treatment	0.46±0.19	790±220 ^b	0.50±0.17	680±72	0.31±0.04 ^a	410±60 ^a	0.53±0.17	680±322	0.52±0.13	710±21
SN-CA	Before treatment	0.55±0.07	110±27	0.56±0.15	114±23	0.59±0.22	116±35	0.60±0.05	120±21	0.60±0.03	134±9
	After treatment	0.54±0.14	100±41	0.57±0.27	104±57	0.56±0.33	110±33	0.59±0.24	116±34	0.55±0.04	120±34
SN-cerebellum	Before treatment	0.50±0.03	544±49	0.48±0.17	312±107 ^a	0.51±0.05	469±103 ^a	0.53±0.09	507±180	0.55±0.08	566±28 ^c
	After treatment	0.49±0.04	480±120 ^a	0.50±0.29	320±122 ^a	0.53±0.16	510±132	0.53±0.16	512±207	0.56±0.24	580±38 ^c
PU-TH	Before treatment	0.31±0.15 ^a	113±57 ^a	0.55±0.07	298±65	0.49±0.06	282±112	0.47±0.10	107±79 ^a	0.52±0.05	304±77
	After treatment	0.46±0.09 ^b	280±120 ^b	0.46±0.29	280±33	0.50±0.11	280±37	0.48±0.21	276±38 ^b	0.51±0.04	301±40
PU-CA	Before treatment	0.41±0.22	294±90	0.40±0.12	230±57	0.37±0.09	221±24	0.40±0.07	240±56	0.42±0.10	241±79
	After treatment	0.40±0.11	248±102 ^a	0.41±0.19	211±30	0.36±0.15	190±43 ^a	0.34±0.31	209±103	0.50±0.05	250±49
TH-CA	Before treatment	0.22±0.10 ^a	76±41 ^a	0.47±0.08	186±37	0.36±0.05	106±37 ^a	0.37±0.06	166±39	0.43±0.04	196±47
	After treatment	0.36±0.07 ^b	182±40 ^b	0.46±0.22	169±133	0.37±0.04	130±21 ^a	0.36±0.14	151±61	0.41±0.05	180±63
TH-cerebellum	Before treatment	0.42±0.10	103±41 ^a	0.51±0.09	123±55 ^a	0.46±0.07	133±41 ^a	0.47±0.11	142±27	0.52±0.05	211±56
	After treatment	0.46±0.15	176±70 ^b	0.53±0.23	139±40 ^a	0.46±0.11	142±43 ^a	0.46±0.05	149±49	0.51±0.07	202±37 ^c

Abbreviations: PU, putamen; GP, globus pallidus; CA, head of the caudate nucleus; TH, thalamus; SN, substantia nigra,

Results reported as mean±SEM. a: P≤0.05 compared with healthy controls. b: P≤0.05 compared with the before treatment. c: P≤0.05 compared with the cer

After 5 years of copper excluding treatment, FA values of the fibers between the PU and the TH, the TH and the CA, were significantly higher in WD patients of group 1 than prior treatment ($P=0.031, 0.026$). The volumes of fibers between the GP and the PU, the GP and the CA, the SN and the PU, the SN and the TH, the PU and the TH, the TH and the CA, the TH and the cerebellum were also significantly higher in WD patients after therapy ($P=0.013, 0.040, 0.037, 0.021, 0.042, 0.038, 0.019$). The volumes of fibers between the SN and the PU were higher in WD patients of group 3 after therapy ($P=0.027$). FA values of the fibers between the GP and the PU, the GP and the CA were significantly higher in WD patients of group 4 after therapy ($P=0.026, 0.047$). The volumes of fibers between the GP and the SN, the GP and the CA, the SN and the PU, the PU and the TH were also higher in WD patients after therapy ($P=0.020, 0.014, 0.035, 0.045, 0.024, 0.024, 0.043$). FA values of the fibers between the GP and the CA in WD patients with hepatic type were higher in WD patients after therapy ($P=0.019$).

The volumes of fibers between the SN and the GP in WD patients of group 2 were lower after therapy ($P=0.043$).

3.4 Differences of DTI parameters among subgroups of WD patients

After copper removal treatment, the volumes of fibers between the SN and the GP, the GP and the TH, the SN and the cerebellum, the TH and cerebellum were lower in WD patients of group 2 than in the other subgroups of WD patients (Fig. 1). The FA values of fibers between the SN and the TH, the volumes of fibers between the SN and the TH, the PU and the CA, the TH and the CA were lower in WD patients of group 3 than in the other subgroups.

3.5 Differences of DTI parameters between hepatic and cerebral type of WD patients

After copper excluding treatment, the volumes of fibers between the cerebellum and SN ($P=0.033$), the cerebellum and TH ($P=0.026$) were lower in patients with cerebral type than hepatic type WD.

3.6 Correlations between DTI parameters and clinical parameters in patients with WD

After copper excluding treatment, the scores of modified Young scale of WD patients were lower than that before treatment. Among them, the scores of modified Young Scale in Group 1 ($P=0.019$), Group 3 ($P=0.042$) and Group 4 ($P=0.035$) were lower after treatment than before (Table 1). Neurological symptoms were aggravated in 5 of the 41 patients with cerebral WD after copper excluding treatment. 4 patients in group 2 showed aggravation of neurological symptoms, and 1 patient in group 3 showed aggravation of neurological symptoms. After treatment, the serum copper content of WD patients decreased ($P=0.042$), while the urine copper content increased ($P=0.042$) (Table 3). After treatment, liver function grade of WD patients with hepatic type was improved. DTI values were unrelated to Child-Pugh liver grade, the scores of modified Young scale and copper concentration.

Table 3
Scores of the modified Young Scale and copper parameters of WD patients and healthy controls

	Modified Young Scale scores	Serum copper (mg/L)	Urinary copper ($\mu\text{g}/24\text{h}$)
before treatment	21.33 $\pm$ 8.56 ^a	0.38 $\pm$ 0.09 ^a	141 $\pm$ 56 ^a
After treatment	13.07 $\pm$ 8.25 ^{a b}	0.21 $\pm$ 0.04 ^{a b}	286 $\pm$ 163 ^{a b}
Controls	0	0.81 $\pm$ 0.10	27 $\pm$ 6
Results reported as mean $\pm$ SEM. a : $P\leq 0.05$ compared with healthy controls. b: $P\leq 0.05$ compared with before treatment.			

4. Discussion

The neural connections in the brain include anatomical connections, functional connections and effective connections, forming a complex brain network. At present, the research is more clear in pyramidal tract system. Extrapyramidal neural network is also an important part of brain network and plays an important role in motor regulation. Extrapyramidal nuclei have extensive fiber connections, forming a complex extrapyramidal neural network. Functional imaging has indicated that cognitive dysfunction and movement disorders are predominantly caused by neural network dysfunction^[2]. Hippocampal-basal ganglia and thalamic-cortical networks are associated with cognitive impairment^[12]. The neural connections between basal ganglia and cortex are not only involved in motor regulation, but also in various cognitive tasks. Studies have found that WD patients have attentional network function due to impaired fibrous connection between basal ganglia region and frontal parietal cortex, especially vigilance network disorder^[13] and impaired attentional network function^[12].

DTI is a method to study the structural connections of neural networks. It is a method of visualizing white matter fibers in vivo, has applications in neurodegeneration. We previously confirmed that the FA value of DTI reflected axon and myelin sheath injury through the correlation analysis of DTI indexes and pathological indexes^[14, 15]. At the same time, DTI is an effective method to observe the path of fiber bundles and can be used to study the structural connections of fibers^[16, 17].

Previous studies have confirmed that the brain extrapyramidal nerve fibers are damaged in chronic degenerative diseases. Decreased FA values in the subcortical nuclei have been observed in PD and AD^[2, 18]. Copper deposits in the extrapyramidal system of WD patients lead to neurological symptoms, especially extrapyramidal symptoms. The structural connections of the extrapyramidal neural network were damaged in WD patients. DTI of Toxic Milk mice (TX mice) and WD patients confirmed the existence of extrapyramidal network injury in WD brain. Through the DTI study of WD patients, it was found that the FA value of the extrapyramidal system in WD patients decreased and the number of contact fibers decreased, suggesting the existence of structural damage in the extrapyramidal network in WD patients^[5]. We confirmed that the injury of the extrapyramidal network has a significant influence on the type, severity and symmetry of WD neurological symptoms^[5, 19]. WD patients can show different types of parkinson-like movement, dance-like movement and ataxia, and the severity is different. Through DTI studies, we confirmed that involuntary movement was related to afferent fiber damage in putamen and caudate nuclei.

Increased muscular tension is associated with injury of efferent fibers in the globus pallidus and substantia nigra [5,19]. In addition, the left and right differences in the damage of the connective fibers between the nuclei were the main cause of the asymmetry of neurological symptoms. Therefore, the improvement of extrapyramidal network injury in WD patients affects the improvement of neurological symptoms. Our previous study found that after copper removal treatment, metal deposition in the brain of WD patients could be significantly reduced [6,15]. However, there has been no study on whether WD patients with extrapyramidal neural network damage can be improved after copper removal treatment. In this study, DTI fiber tracer method was used to observe the repair of brain extrapyramidal neural network damage in WD patients after 5 years of copper removal treatment, so as to clarify the effect of copper excluding treatment.

We found that after 5 years of copper excluding treatment in WD patients, the FA value of the connection fibers between some extrapyramidal nuclei increased, and the number of the connection fibers between some nuclei increased. It is suggested that the structural damage of the brain extrapyramidal system in WD patients can be repaired to a certain extent after the treatment. However, we also noticed that the changes of the connective fibers between the extrapyramidal nuclei were not uniform after copper excluding treatment. The number of connective fibers between some nuclei decreased after treatment. It is suggested that the treatment effect of copper removal on WD brain structural injury is limited.

We found that DTI values were unrelated the scores of modified Young scale. However, we found that different types of WD patients had different degrees of damage to the extrapyramidal nerve connection fibers in the brain. According to the clinical symptoms of WD patients, WD patients with cerebral type were divided into involuntary motor type (type 1), Parkinson's symptom type (type 2), oromandibular dystonia type (type 3) and mental disorder type (type 4). Before and after treatment, WD patients of Parkinson's symptom type had the highest scores on the modified Young Scale and the most severe neurological symptoms. Before and after treatment, the number of some nuclear contact fibers in type 2 and type 3 WD patients was less than that in other types of WD patients, suggesting that the damage of extrapyramidal nerve contact fibers in type 2 and type 3 WD patients was more serious than that in other types of WD patients. These results also suggest that the severity of extrapyramidal nerve fiber injury is related to the severity of neurological symptoms.

Different types of WD patients have different responses to copper excluding treatment. Neurological symptom scores of type 2 WD patients did not significantly decrease After copper excluding treatment. Some type 2 and type 3 WD patients showed aggravation of neurological symptoms after copper excluding treatment. It is suggested that WD patients with increased muscle tension of limbs and mandibular muscles mainly have a poor response to the copper excluding treatment. Neurological symptom scores were significantly reduced in type 1 and type 4 WD patients. After copper excluding treatment, the number of fibers associated with multiple nuclei in type 1 WD patients was significantly increased compared with that before treatment, suggesting that type 1 WD patients had the best efficacy to copper excluding treatment. Previous studies have suggested that the cerebello-thalamo-cortical-cerebellar and cortico-thalamo-cortical loops are regarded as being involved in tremor activity and whole-body ataxia [20]. Part of the cerebello-thalamo-cortical network, the dentato-rubro-thalamic tract (DRT), has been associated with tremor control [21]. We observed correlations between DTI metrics and type of WD and that tremor was associated with the cerebello-thalamo-cortical network. In addition, we found that the inflow path of the GP was associated with hypokinesia including tremor, choreic movement and ataxia. Combined with the results of this study, the fiber may be damaged, there is a certain possibility of repair. Type 2 WD patients had poor results after treatment. Our results indicate that damage to the outflow path of the GP and SN may be an important cause of hypermyotonia. These fibers are less likely to repair damage. Our study suggests that the connection fibers in different parts of the extrapyramidal system may have different therapeutic effects on copper excluding treatment and have different repair capacities.

Currently, most people believe that hepatic type and cerebral type are different course states of WD, rather than different types of diseases. However, in clinical practice, we did find that many WD patients with hepatic type showed long-term liver symptoms without obvious neuropsychiatric symptoms. We have previously compared hepatic and cerebral WD patients. It was found that there were differences in the amount of metal deposition and neurostructural damage in the brain of WD patients with hepatic and cerebral types [5,6]. In this study, we also found that the damage of extrapyramidal nerve connection fibers in WD patients with hepatic type after copper excluding treatment was less than that in WD patients with cerebral type. The degree of brain structural damage in WD patients with hepatic type is relatively mild, which may be the reason for the absence of obvious neuropsychiatric symptoms in WD patients with hepatic type.

Limitations

This study had several limitations. First, DTI is not the best method for evaluating nerve fibers at present. Since this study required a comparison before and after the treatment of copper, DTI was still used to evaluate the nerve fibers in order to ensure the consistency of the evaluation methods. Second, as ROIs were drawn by radiologists relative to anatomical structures, there may have been random variability in the measurements. To reduce this variability, all measurements were made by two neuroradiologists independently, with the value for each being an average of three different measurements. Furthermore, Because of the need for 5 years of copper therapy observation, 6 WD patients gave up treatment and were lost to follow-up due to the aggravation of symptoms during treatment, and 5 patients were lost to follow-up due to economic reasons. Because no post-treatment data were available for comparison, this subset of patients was not included in the analysis. The lost follow-up cases may cause some bias to the final results. A larger sample size and longer follow-up may be needed in the future.

Conclusion

After the treatment with metal chelating agent, the extrapyramidal neural network connection fibers in WD patients were damaged and repaired to a certain extent. Different types of WD patients have different responses to copper excluding treatment. WD patients with involuntary movement can have damage and repair of extrapyramidal nerve fibers after copper excluding treatment. However, the neurological symptoms of WD patients with increased muscle tone were not significantly improved after treatment, and the repair effect of nerve fiber injury in the extrapyramidal system was poor.

Declarations

Ethical Approval: This study was approved by the Ethics Committee of Sun Yat-sen University. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent to Participate: Informed consent was obtained from all participants prior to enrollment.

Consent to Publish: The publication has been approved by all co-authors.

Authors Contributions

Xiangxue ZHOU : Project design, case collection, data collection, paper writing

Hao-ling QIN: Imaging data acquisition

Jian LIAO: Case collection, data collection

Xia XIAO: Case collection, data collection

Funding: This study was funded by the Natural Science Foundation of Guangdong Province (2021A515012407). This study was supported by grants from the Southern China International Cooperation Base for Early Intervention and Functional Rehabilitation of Neurological Diseases (2015B050501003), Guangdong Provincial Engineering Center For Major Neurological Disease Treatment, Guangdong Provincial Translational Medicine Innovation Platform for Diagnosis and Treatment of Major Neurological Disease, Guangdong Provincial Clinical Research Center for Neurological Diseases.

Competing Interests: All Authors declare that they have no conflict of interest.

Availability of data and materials: The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Favrole, P., Chabriat, J. P., Guichard, F., & Woimant, F. (2006). Clinical correlates of cerebral water diffusion in Wilson disease. *Neurology*, 66, 384–389
2. Yael, D., Reijmer, A., Leemans, K., Caeyenberghs, Sophie, M., & Heringa, H. L. (2013). Koek, Geert Jan Biessels. Disruption of cerebral networks and cognitive impairment in Alzheimer disease. *neurology* ;80:1370–1377
3. Sedrak, M., Gorgulho, A., Frew, A., Behnke, E., & DeSalles, A. (2011). Nader Pouratian. Diffusion Tensor Imaging and Colored Fractional Anisotropy Mapping of the Ventralis Intermedius Nucleus of the Thalamus. *Neurosurgery*, 69, 1124–1130
4. Kaan Yagmurlu, A. L., Vlasak Albert, L., & Rhoton Jr. (2015). Three-Dimensional Topographic Fiber Tract A Anatomy of the Cerebrum. *Operative Neurosurgery*, 11, 274–305
5. Xiangxue Zhou, X., Li, H., Qin, G., Li, H., Huang, Y., Liang, X., & Liang (2016). XiaoYong Pu. Diffusion tensor imaging of the extracortical network in the brains of patients with Wilson disease. *Journal of the Neurological Sciences*, 362(6), 292–298
6. Xiangxue Zhou, X., Xiao, X., Li, H., Qin, X., Pu, D., Chen, C. ... Feng (2020). Xiuling Liang. A study of susceptibility weighted imaging in patients with Wilson disease during the treatment of metal chelator. *Journal of Neurology*, 267(6), 1643–1650
7. Le Bihan, D. (2003). Looking into the functional architecture of the brain with diffusion MRI. *Nat Rev Neurosci*, 4, 469–480
8. Meijer, F. J., Bloem, B. R., Mahlknecht, P., Seppi, K., & Goraj, B. (2013). Update on diffusion MRI in Parkinson's disease and atypical parkinsonism. *Journal of the Neurological Sciences*, 332, 21–29
9. Metwalli, N. S., Benatar, M., Nair, G., Usher, S., Hu, X., & Carew, J. D. (2010). Utility of axial and radial diffusivity from diffusion tensor MRI as markers of neurodegeneration in amyotrophic lateral sclerosis. *Brain Research*, 1348, 156–164
10. Volker, A., Coenen, B. M., Töller, H., Schiffbauer, H., & Urbach, N. A. (2011). Individual Fiber Anatomy of the Subthalamic Region Revealed With Diffusion Tensor Imaging: A Concept to Identify the Deep Brain Stimulation Target for Tremor Suppression. *Neurosurgery*, 68, 1069–1076
11. Xiang-xue, Z. H. O. U., Xun-hua, L. I., & Hai-wei, H. U. A. N. G. (2011). Improved Young Scale – a scale for the neural syndrome of Wilson disease. *Chinese Journal of Nervous and Mental Disease*, 37, 171–172
12. Han, Y., Cheng, H., & Toledo, J. B., Xun Wang, Bo Li, Yongzhu Han Wang, K. (2016). Yong Fan Impaired functional default mode network in patients with mild neurological Wilson's disease. *Parkinsonism and Related Disorders*, 30, 46e51
13. Federica, C., b, & Van Gorder, R. A. (2019). The role of network structure and time delay in a metapopulation Wilson–Cowan model. *Journal of Theoretical Biology* ; 4771–13
14. Zhou, X. X., Qin, H. L., Li, X. H., Huang, H. W., Liang, Y. Y., & Liang, X. L. (2014). Xiao-Yong Pu. Characterizing brain mineral deposition in patients with Wilson disease using susceptibility-weighted imaging. *Neurology India*, 62(4), 362–366
15. Xiangxue Zhou, X., Xiao, H., Qin, D., Chen, C., & Wu (2021). Study on different pathogenic factors in different disease stages of patients with Wilson disease. *Neurol Sci*. doi: 10.1007/s10072-020-04973-7
16. Ghasempour, M., & Ashrafpour, R. R. (2015). Effect of milk and yogurt on streptococcus sobrinus counts and caries score in rats. *Dental Research J (Isfahan)*, 12(6), 569–573

17. Xiang-xue, Z. H. O. U., Li, X., Chen, D., Wu, C., & Feng, L. (2019). Hao-lin Qin, Xiao-Yong Pu, Xiu-ling Liang. Injury factors and pathological features of toxic milk mice during different disease stages. *Brain and Behavior*, 00, e01459
18. Zhang, K., Yu, C., Zhang, Y., Wu, X., Zhu, C., Chan, P., & Li, K. (2011). Voxel-based analysis of diffusion tensor indices in the brain in patients with Parkinson's disease. *European Journal of Radiology*, 77, 269–273
19. Xiang-xue, Z. H. O. U., Xun-hua LI, Ding-bang, C. H. E. N., Chao, W. U., Li, F. E. N. G., Jian-ping, C. H. U., Zhi-yun, Y. A. N. G., & Xin-bei, L. I. (2018). Gui-dian LI, Hai-wei HUANG, Ying-ying LIANG, Xiu-ling LIANG. The asymmetry of neural symptoms in Wilson disease patients detecting by diffusion tensor imaging, resting state functional MRI and susceptibility weighted imaging. *Brain and behavior*, 8(5), e00930
20. Volker, A., Coenen, N., Allert, S., & Paus (2014). Martin Kronenburger, Horst Urbach, Burkhard Maedler. Modulation of the Cerebello-Thalamo-Cortical Network in Thalamic Deep Brain Stimulation for Tremor: A Diffusion Tensor Imaging Study. *Neurosurgery*, 75, 657–670
21. Volker, A., Coenen, B. M., Maedler, H., Schiffbauer, H., & Urbach, N. A. (2011). Individual Fiber Anatomy of the Subthalamic Region Revealed With Diffusion Tensor Imaging: A Concept to Identify the Deep Brain Stimulation Target for Tremor Suppression. *Neurosurgery*, 68, 1069–1076

Figures

Figure 1

DTI results showing the volumes of fibers between regions of interest (ROIs) in the brains of individual WD patients. A: The first row is PU-GP contact fiber of WD patients with type 1, type 2, type 3, type 4 and liver type before treatment; the second row is PU-GP contact fiber of WD patients with type 1, type 2, type 3, and type 4 and liver type after treatment. B: The first row is PU-SN contact fiber of WD patients with type 1, type 2, type 3, type 4 and liver type before treatment; the second row is PU-SN contact fiber of WD patients with type 1, type 2, type 3, and type 4 and liver type after treatment. C: The first row is CA-GP contact fiber of WD patients with type 1, type 2, type 3, type 4 and liver type before treatment; the second row is CA-GP contact fiber of WD patients with type 1, type 2, type 3, and type 4 and liver type after treatment. D: The first row is the CA-TH contact fiber of WD patients with type 1, type 2, type 3, type 4 and liver type before treatment; the second row is the CA-TH contact fiber of WD patients with type 1, type 2, type 3, and type 4 and liver type after treatment. E: In the first row, TH-Cerebellum connectors were used for WD patients with type 1, type 2, type 3, type 4 and liver before treatment, and in the second row, TH-Cerebellum connectors were used for WD patients with type 1, type 2, type 3, and type 4 after treatment. F: The first row is the SN-TH contact fiber of WD patients with type 1, type 2, type 3, type 4 and liver type before treatment; the second row is the SN-TH contact fiber of WD patients with type 1, type 2, type 3, and type 4 after treatment, respectively. Abbreviations: PU, putamen; GP, globus pallidus; CA, head of the caudate nucleus; TH, thalamus; SN, substantia nigra.

Supplementary Files

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

- [AuthorChecklist.docx](#)