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Neuropsychiatric Manifestations of Wilson Disease: Correlation with MRI and Glutamate Excitotoxicity

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Abstract

This study aims to identify neuropsychiatric manifestations in neurological Wilson disease (NWD), and their correlation with MRI changes and glutamate excitotoxicity. Forty-three consecutive patients with NWD from a tertiary care teaching hospital were evaluated prospectively who fulfilled the inclusion criteria. The neuropsychiatric evaluation was done using Neuropsychiatric Inventory (NPI) battery that assesses 12 domains including delusion, hallucination, agitation/aggression, dysphoria/depression, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor activity, appetite change, and abnormal nighttime behavior. Cranial MRI was done using a 3 T machine, and locations of signal changes were noted including the total number of MRI lesions. Serum glutamate level was measured by a fluorescence microplate reader. Abnormal NPI in various domains and total NPI scores were correlated with MRI lesions, serum and urinary copper, and glutamate level. The median age of the patients was 16 years. Forty-one (48.8%) patients had cognitive impairment and 37 (86%) had movement disorder. Neurobehavioral abnormality was detected in all—commonest being agitation (90.7%) followed by appetite change (81.4%), elation (74.4%), irritability (69.8%), anxiety (67.4%), depression (65.1%), apathy (44.2%), night time abnormal behavior (32.6%), aberrant motor behavior (20.9%), delusions (16.3%), and hallucination (18.6%). The thalamic lesion was associated with depression, globus pallidus with depression and anxiety, caudate with anxiety and agitation, brainstem with irritability, and frontal cortex with apathy. Serum glutamate level was higher in NWD. NPI sum score correlated with MRI load and glutamate level. Varying severity of neurobehavioral abnormalities are common in the patients with NWD and correlate with the location of MRI lesion and glutamate level.

Keywords Wilson disease; Copper; Neuropsychiatric inventory · MRI · Depression · Anxiety · Irritability · Delusion · Hallucination · Glutamate

Abbreviations

ADL	Activity of daily living
BFM	Burke-Fahn-Marsden
CNS	Central nervous system
Cu	Copper
MRI	Magnetic resonance imaging
MMSE	Mini-Mental State Examination
NPI	Neuropsychiatric Inventory
NMDA	N-methyl-D-aspartate
WD	Wilson disease

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Introduction

Wilson disease (WD) is an autosomal recessive copper (Cu) metabolism disorder due to ATP7B gene mutation in chromosome 13q14.3 that codes Cu transporting ATPase protein. This Cu-transporting ATPase is essential for the excretion of Cu into the bile and delivering Cu to the synthesis of ceruloplasmin [1, 2]. The absence of this transporter leads to excessive accumulation of Cu in the liver; later Cu is spilled over to the circulation and is deposited in multiple organs including cornea, ocular lens, brain, liver, heart, and bone. WD is a rare disease, and its worldwide prevalence is 1:30,000–1:50,000 population [3, 4]. About 40–60% of WD patients present with

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hepatic, 40–50% neurological, and 10–25% psychiatric symptoms [4, 5]. Nearly 95% of symptomatic neurological WD (NWD) patients have Kayser–Fleischer (KF) rings [5, 6]. During disease, almost all have hepatic, neurological, and psychiatric involvement [7–9]. Neuropsychiatric symptoms are although an important manifestation of WD, but till 2018, there were only 91 articles including 57 case reports, 12 cohort studies, 3 case-cohort studies, and 4 case series [7]. None of these studies comprehensively evaluated the correlation of neuropsychiatric manifestation with MRI changes. In NWD, the involvement of caudate (30–65%), putamen (45–85%), and thalamus (30–60%) is quite common, and 90–100% of patients had abnormal MRI [10–13]. These anatomical structures are linked to the limbic system and frontal network and have been evaluated as an anatomical substrate for various neuropsychiatric manifestations in Huntington's disease, Parkinson's disease, caudate hemorrhage, and subacute combined degeneration [14]. Excess of free Cu in WD has been reported to induce oxidative stress, cytokine release, and glutamate excitotoxicity [15, 16]. In an experimental study on the rat model, memory impairment has been linked to glutamate and its receptors. The rats were fed 100 mg of $CuSO_4$ for a period of 30 to 90 days and Y-maze was performed for memory and learning. Immunohistochemistry of hippocampus and frontal cortex revealed higher expression of the glial fibrillary acidic protein (GFAP) and caspase-3 in $CuSO_4$-fed rats compared to the controls. The glutamate level was higher in the hippocampus and frontal cortex, and expression of N-methyl-D-aspartate receptors (NMDARs) was lower in the hippocampus in the exposed group compared to the controls. These changes correlated with tissue copper, oxidative stress, and Y-maze attention score [17]. Higher expression of GFAP and caspase-3 has also been reported in the corpus striatum of $CuSO_4$ exposed rats [18]. In this communication, we report neuropsychiatric abnormality in a cohort of NWD and correlate these with MRI findings and serum glutamate level.

Subjects and Methods

Inclusion Criteria

The consecutive patients with NWD whose NPI scores and laboratory and MRI findings were available for review were included from a prospectively maintained NWD registry. These patients were managed by us during 2010–2019. Some of these patients have been included in our earlier publications [10, 16, 19]. The diagnosis was based on clinical features, KF ring on slit-lamp examination, low ceruloplasmin (< 20 mg/dl), and high urinary Cu (> 40 µg/24 h) [4, 5].

Exclusion Criteria

Patients with only hepatic WD, neurologic WD with hepatic encephalopathy, kidney failure, family history of major psychiatric disorder, malignancy, pregnancy, and those on psychotropic drugs were excluded.

Clinical Evaluation

A detailed medical history including age, gender, duration of illness, and presenting symptoms was noted. A pedigree chart was drawn of the indexed patient. History of jaundice and hemolysis in the past was enquired, and past medical records were evaluated. Presenting symptoms and signs were also noted. Movement disorders including dystonia, chorea, athetosis, myoclonus, or tremor were noted. The severity of dystonia was rated using Burke-Fahn-Marsden (BFM) score [20]. The neurological severity was categorized as grade 0-III based on 5 signs [dysarthria, ataxia, tremor, rigidity/bradykinesia, chorea/dystonia, and activity of daily living ADL]. Each sign is given a score of 0–3 (0 = none, 1 = mild, 2 = moderate, 3 = severe). The neurological disability is considered grade 0 (sum score 0, no disability); grade I (sum score 1, mild disability), grade II (sum score 2–7, moderate disability but independent for the activity of daily living), and grade III (sum score > 7, dependent for the activity of daily living) [21–24]. The other movement disorders were also graded on a 0–3 scale.

Neuropsychiatric Evaluation

Neuropsychiatric evaluation was done using Neuropsychiatric Inventory which provides a comprehensive assessment of psychopathology in dementia. Twelve behavioral domains including delusion, hallucination, agitation/aggression, dysphoria/depression, anxiety, euphorbia, apathy, disinhibition, irritability, aberrant motor activity, change in appetite, and night time behavior changes were evaluated. A screening questionnaire is used for each sub-area and if the response was no, a further question was not asked. If the response was yes, then the sub-questions were asked to record the frequency and severity. Based on the response, the frequency was categorized as 1 = occasionally, 2 = often, 3 = frequent, and 4 = very frequent. The severity was graded as mild = 1, moderate = 2, and severe = 3. The composite score was calculated by multiplying severity and frequency rating. The depression score > 6, disinhibition score > 4, irritability score > 4, and any score in the remaining domains are considered abnormal [25, 26]. Neuropsychiatric Inventory was used before the initiation of treatment in 34 and after a median duration of 14 months (6–30 months) of

penicillamine and zinc therapy in 8 and only zinc therapy in 1 patient. None of the patients was on dopamine, and those receiving anticholinergic and or dopamine antagonists were advised to stop for a week before the NPI evaluation.

Investigation

Blood counts, hemoglobin, blood glucose, serum creatinine, lactate dehydrogenase, transaminases, bilirubin, alkaline phosphatase, albumin, sodium, potassium, Cu, and ceruloplasmin were measured. A coagulation profile was done. Twenty-four-hour urinary Cu was measured by atomic absorption spectroscopy. Slit-lamp examination was done for KF ring and ultrasound abdomen for liver echotexture, portal vein diameter, splenomegaly, and ascites. Cranial MRI was done using a 3 T MRI machine (Signa GE Medical System, Wisconsin, USA). Axial images were obtained in T1, T2, FLAIR, and DW sequences. The anatomical locations of abnormal signal changes were noted. For calculating MRI load, 1 point was given to each lesion and a summation of these points was considered as MRI load.

Glutamate Assay

Serum glutamate level was measured by a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm (Thermo Fisher, USA). Briefly, 50 µL of the serum sample was collected in each well and mixed with 50 µL of a reaction buffer containing amplex red, glutamate oxidase, glutamate pyruvate transaminase, alanine, and horseradish peroxidase. After incubation at 37 °C, the fluorescence of the reaction mixture was detected by a fluorescence microplate reader. Serum glutamate was measured in 33 WD and 35 age (18.33 vs. 20.08 years; $p = 0.169$) and gender (male: female 28/5 vs. 27/8; $p = 0.54$) matched healthy controls. The control samples were collected from the attending personal of admitted patients in our ward or the hospital employee.

Statistical Analysis

The normality of all parameters was evaluated by the Shapiro–Wilk test. Categorical variables were expressed as the percentages and number of cases. Continuous variables were expressed as mean and standard deviation for normally distributed data, and median and inter-quartiles for non-normally distributed data. Nominal or dichotomous variables were compared using the Chi-square test or Fisher's exact test. Normally distributed continuous variables were compared using the independent-sample *t*-test, and non-normally distributed variables were compared with the Mann–Whitney *U* test. The abnormal features in the different domains of NPI were compared with MRI findings using the Chi-square test. To derive the best predicting MRI lesion for various domains of NPI, a multivariate logistic regression analysis was done including the different locations of MRI lesions. The sum score of NPI was correlated with age, duration of illness, neurological severity, BFM score, biochemical parameters, and MRI load using the Karl-Pearson or Spearman rank correlation test. Serum glutamate level was compared with abnormal NPI in different domains using an independent *t*-test. Glutamate levels were also correlated with the total NPI score and MRI load. These analyses were run using SPSS version 25.0 (SPSS Inc., Chicago IL, USA), and graphs were prepared by GraphPad Prism-5. A two-tailed statistical significance was accepted when exact $p < 0.05$.

Results

There were 43 patients with neurological WD, and their ages ranged between 11 and 34 years (median 16 years). Thirty-five patients were males, and 15 (34.9%) had a family history of WD. The median duration of neurological manifestation was 18 (6–60) months, and their median age of onset was 13 (9–31) years. History of jaundice was present in 11 (25.6%). The mean serum bilirubin was 0.98 ± 0.43 mg/dl (> 1.2 mg/dl in 13), glutamic pyruvate transaminase 41.35 ± 19.46 U/L (> 60 U/L in 12), albumin 4.00 ± 0.68 gm/dl (< 3.5 gm/dl in 9), and none had INR > 1.4. None had clinical or biochemical evidence of hepatic encephalopathy. Abdominal ultrasound revealed abnormal hepatic echotexture in 27 (62.8%), splenomegaly in 17 (39.5%), increased hepatic vein diameter in 9 (20.9%), and none had ascites. Thirty-seven (86%) patients had movement disorders and included dystonia in 31 (72.1%), chorea in 14 (32.6%), athetosis in 9 (20.9%), myoclonus in 5 (11.6%), and tremor in 16 (37.2%). Only 7 (16.3%) patients had a seizure. Cranial MRI was done in 36 and was abnormal in 35 patients. The abnormalities included involvement of caudate in 26 (60.5%), putamen in 28 (65.1%), globus pallidus in 28 (65.1%), thalamus in 27 (62.8%), brainstem in 15 (34.9%), cerebellum in 5 (11.6%), cerebral cortex in 9 (20.9%), subcortical white matter in 8 (frontal in 6 and parieto-occipital 2), and cortical atrophy in 7 (16.3%) patients. The details are present in Table 1.

Neurobehavioral Abnormality

Based on NPI, the neurobehavioral abnormality was detected in all—the commonest being agitation in 39 (90.7%) followed by appetite change in 35 (81.4%), elation in 32 (74.4%), irritability in 30 (69.8%), anxiety in 29 (67.4%), depression in 28 (65.1%), apathy in 19 (44.2%), disinhibition in 17 (39.5%), night time abnormal behavior in 14 (32.6%), aberrant motor behavior in 9 (20.9%), hallucinations in 8 (18.6%), and delusion in 7 (16.3%) patients. In 8 patients, the

Table 1 Clinical characteristics and MRI findings

Parameter		Number (%)	MRI	Number (%)
No. of patients		43	Caudate	26 (60.5%)
Age (years)#		16 (11–34)	Putamen	28 (65.1%)
Gender (Male/Female)		35/8	Globus pallidus	28 (65.1%)
History of jaundice		11 (25.6%)	Thalamus	27 (62.8%)
Age at onset (years)#		13 (6–34)	Brainstem	15 (34.9%)
NPI total score#		24 (4–115)	Cerebellum	5 (11.6%)
Severity	Severity I	9 (20.9%)	Cerebral Cortex	9 (20.9%)
	Severity II	23 (53.5%)	MMSE#	25.11 (16–30)
	Severity III	11 (25.6%)	MMSE abnormal	21 (48.8%)

#Median (range); *NPI*, neuropsychiatric inventory

NPI was significant to prescribe quetiapine. In each patient, multiple domains of NPI were abnormal with varying severity (Fig. 1).

Correlation of NPI with MRI Changes

Thalamic lesion on MRI is associated with depression ($p=0.019$), globus pallidus with depression ($p=0.046$) and anxiety ($p=0.036$), and caudate lesion with anxiety ($p=0.026$) and agitation ($p=0.028$). Brainstem lesion is associated with irritability ($p=0.011$) and frontal cortical lesion with apathy ($p=0.019$) and hallucination ($p=0.050$) (Fig. 2). Cerebellar and subcortical lesions were not associated with any abnormal domain of NPI. The sum score of NPI correlated to MRI lesion score ($r=0.397$; $p=0.017$; Fig. 3). On univariate regression analysis, agitation was predicted by the putaminal lesion (OR = 0.062, 95% CI = 0.005–0.720, $p=0.026$), hallucination by the cortical lesion (OR = 6.40, 95% CI = 1.08–37.96, $p=0.041$), irritability by the brainstem lesion (OR = 12.73, 95% CI = 1.41–115.11, $p=0.024$), and apathy by the cortical lesion (OR = 0.019, 95% CI = 1.41–49.06, $p=0.19$). On univariate regression analysis, depression was predicted by the thalamus, globus pallidus and putaminal lesion and anxiety was predicted by the globus pallidus, caudate, and putaminal lesion. On multivariate analysis, thalamus involvement (OR = 0.15595% CI = 0.024–0.983, $p=0.048$) was independent predictors of depression after adjusting for putamen lesions on MRI. The details of univariate analysis are presented in Table 2.

Correlation of NPI with Clinical Findings

The sum score of NPI correlated with age ($r=0.424$; $p=0.005$), neurological severity grade ($p=0.003$), BFM score ($r=0.497$; $p=0.001$), urinary Cu ($r=0.371$; $p=0.031$), and reticulocyte count ($r=0.509$; $p=0.004$). NPI score however did not correlate with duration of illness, MMSE score, liver enzymes, serum bilirubin, prothrombin time, evidence of chronic liver disease, and ceruloplasmin. The variables having significant correlation with NPI are presented in Fig. 3.

Glutamate and its Correlation with NPI

Serum glutamate was measured in 33 WD patients and 35 controls. The glutamate level was higher in NWD patients compared to the controls (24.25 ± 3.37 vs. 21.11 ± 2.28 µmol/L, $p=0.001$). The sum score of NPI correlated with glutamate level ($r=0.57$; $p=0.001$). Analysis of glutamate with the different domains of abnormal NPI revealed a significant association with all the domains of NPI (Fig. 4). The serum glutamate level in 6 patients who needed quetiapine was 26.66 ± 3.08 µmol/L and in those not needed was 23.71 ± 3.20 µmol/L ($p=0.049$).

Discussion

Neurobehavioral abnormalities were detected in almost all the patients with NWD with varying frequency and severity. The common NPI abnormalities are agitation, irritability, elation, anxiety, appetite change, and depression. Multiple neurobehavioral abnormalities were seen in the same patient. In univariate analysis, depression was associated with the thalamus, globus pallidus, or putamen involvement; anxiety with caudate, putamen, or globus pallidus; irritability with the brain stem; and apathy with frontal cortex involvement. On multivariate analysis, depression was predicted by thalamic lesion after adjusting for putameninal lesion. NPI sum score correlated with MRI lesion load, glutamate level, neurological severity, and urinary Cu. Although there are few studies on neuropsychiatric abnormalities in WD [7–9, 27–30], there is a paucity of information about the association of neuropsychiatric symptoms with glutamate and MRI changes. In a study on 50 WD patients with the hepatic or neuropsychiatric presentation, 18% had one neuropsychiatric abnormality, 14%

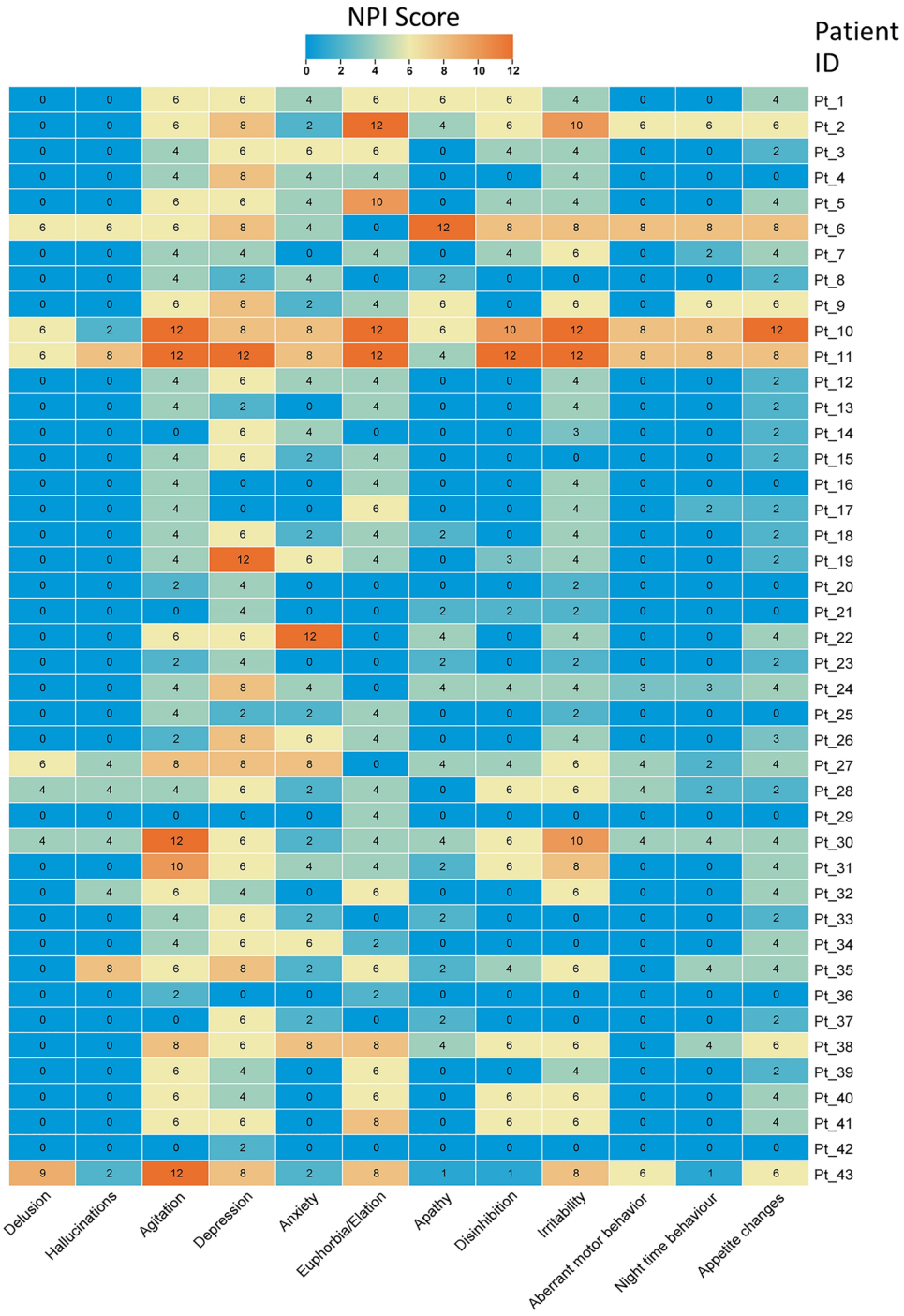

Fig. 1 Heatmap shows various neuropsychiatric manifestations and their severity in patients with neurological Wilson disease

had 2, and 40% had 3 or more symptoms. The commonest symptom was anxiety (62%) followed by depression (36%), irritability (26%), and disinhibition (24%) [29]. The neuropsychiatric manifestation may be a presenting feature or may develop during treatment [7, 28, 31]. Denning and Berrios have reported psychiatric manifestation in a longitudinal follow-up of WD at two different time points. The commonest psychiatric abnormalities in both the follow up were incongruous behavior, irritability, depression, and cognitive impairments. The incongruous behavior and cognitive impairment improved in the second follow-up. Patients with dysarthria, incongruous behavior, and hepatic symptoms had lesser improvement [27]. In various studies, neurobehavioral abnormalities have been reported in 46–71% of patients with WD, common manifestations being irritability, aggression, and anti-social behavior [27, 30, 32, 33]. We have also found irritability, aggression, and anxiety more frequently.

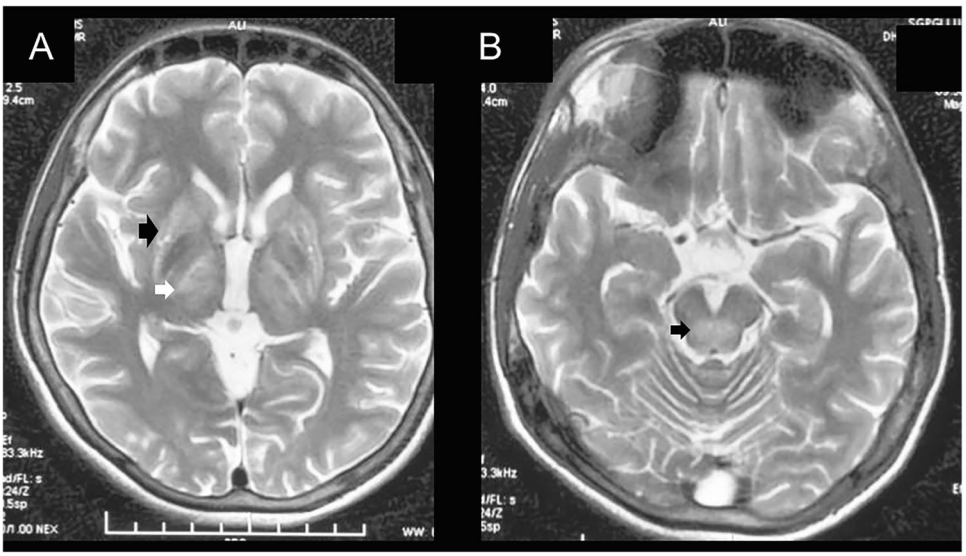

Fig. 2 Cranial MRI of patient # 2 shows **A** T2 hyper-intensity of caudate, putamen, and thalamus bilaterally; and **B** midbrain tegmentum. He had a neurobehavioral abnormality in multiple domains

Neurobehavioral abnormalities in WD have been categorized into (a) behavior and personality disorder (antisocial — aggression, substance abuse, poor school, and work performance; personality change — impulsiveness, lack of judgment, irresponsibility, and irritability), (b) disorder of affect (emotional liabilities, suicidal ideation, mania, major depression), (c) cognitive disorder (bradyphrenia, retrieval memory impairment), and (d) delusion (putative association with WD) [27]. In our patients, some of the abovementioned features were present in different frequencies, but agitation and irritability were more frequent suggesting abnormal behavior and personality disorder. Lesser frequency of delusion and hallucination in our study may be due to less frequent cortical lesions. In a review of 650 WD patients, neuropsychiatric symptoms were reported in 200 patients (30.8%), and the abnormalities were in the domains of affective, behavioral/personalities, schizophrenia-like, and cognitive [34]. Disorders in affect and behavior were the commonest [7, 30, 31]. The common occurrence of affect and behavior in NWD may be due to the commonest involvement of the corpus striatum, which receives massive input from the cortex especially from the frontal cortex [35]. Similar neuropsychiatric abnormalities have also been reported in Huntington's disease [36–38], Parkinson's disease [39, 40], progressive supranuclear palsy [41, 42], Fahr's disease [43], Tourette syndrome [44], vitamin B12 deficiency neurological syndrome [14], and Creutzfeldt-Jakob disease [45, 46].

Serotonin and dopamine dysregulation has been associated with various neuropsychiatric abnormality. Dorsal and medial Raphe nuclei and neurons in the brainstem reticular formation are mainly serotonergic, and these neurons have widespread projections to different regions of the brain including the thalamus, hypothalamus, corpus striatum, habenula, substantia nigra, hippocampus, amygdala, and prefrontal cortex. These areas are involved in determining mood, behavior, sleep, learning, memory, and many other neuropsychiatric functions [47]. Dopamine is mainly synthesized in substantia nigra and is the principal neurotransmitter of several CNS pathways, which regulates motor activity, motivation, and learning. The classical example of dopamine deficiency in Parkinson's disease and dopamine overactivity is schizophrenia [48]. In NWD, both the production and distribution sites are involved as evidenced by MRI involvement of corpus striatum, thalamus, brainstem, and frontal cortex. This may explain the high frequency of neuropsychiatric symptoms and movement disorders in NWD. Neuropsychiatric manifestation may also occur in WD due to hepatic encephalopathy. We, however, excluded WD patients with primary hepatic manifestation and NWD with hepatic encephalopathy.

We have used conventional MRI sequences for association studies with NPI and glutamate levels. Conventional MRI sequences reveal deep-seated gray matter abnormality more frequently in NWD, but there may be a functional abnormality in the other parts of the brain, which may be detected by MR spectroscopy, diffusion tensor MRI, single-photon emission computed tomography, or positron emission tomography (Li et al. 2019). A study on 36 patients with WD revealed decreased N-acetyl aspartate to creatine ratio in basal ganglia, parieto-occipital cortex, and frontal white matter compared with controls. Myo-inositol to creatine ratio was increased in basal ganglia [49]. Another study has also reported reduced N-acetyl aspartate/creatine (Cr) and choline (Cho)/Cr ratio in WD patients as compared with controls [50]. Subtle gray matter atrophy may not be revealing in conventional MRI, in which a T1W volumetric MRI study may be helpful. In a study on 10 patients with WD, widespread gray matter

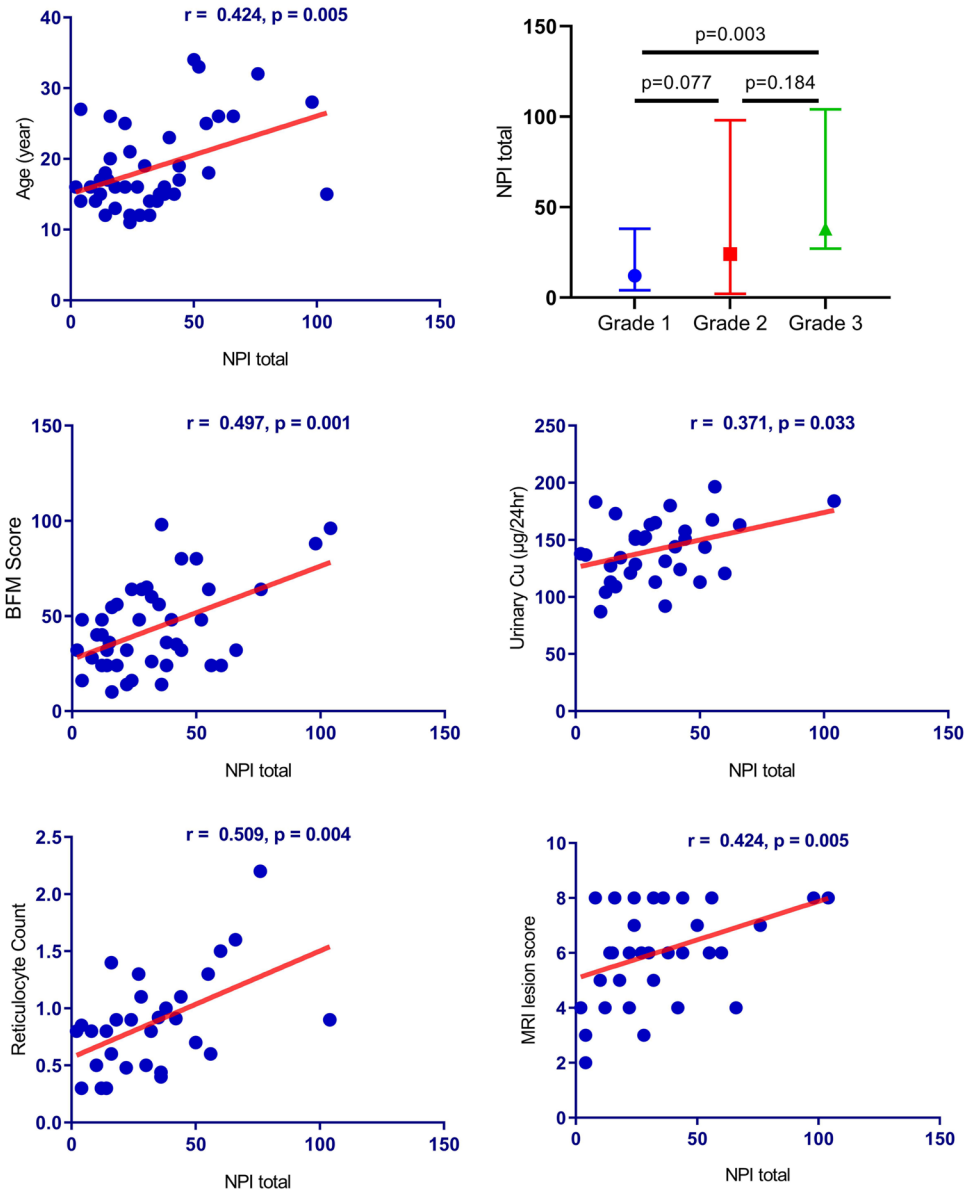

Fig. 3 Relationship of significant clinical and laboratory parameters with Neuropsychiatric Inventory (NPI) score

involvement was noted, which correlated with duration of illness and serum Cu level [51].

In our study, serum glutamate correlated with NPI sum score, various domains of NPI, and MRI load. Glutamate is the major excitatory neurotransmitter in more than 80% of central nervous system synapses. It plays a key role in regulating neuroplasticity, learning, and memory. It is synthesized in the presynaptic terminal by glutamine deamination or via the tricarboxylic acid cycle. Depolarization of the presynaptic membrane mediated by N-type and P-type calcium channels releases glutamate to the synaptic cleft. In a normal situation, synaptic glutamate causes stimulation. Synaptic glutamate may rise to 1 mM, but within a millisecond, it is cleared either by binding to a postsynaptic receptor or by reuptake by astrocyte in which it is broken down into glutamine for re-utilization [52–56]. In the Cu toxicity rat model, there was an increased expression of GFAP and caspase, suggesting inflammation and apoptosis resulting in impaired clearance of glutamate from the site [17, 18]. Basal ganglia and substantia nigra have higher metabolic demands renderings them vulnerable to oxidative stress and excitotoxic injury [18, 57]. Free Cu is highly reactive, and an increased expression of oxidative stress, cytokines, ER stress, and apoptosis markers in WD patients has been reported [16, 58–60]. MRI study in NWD has also shown the greater vulnerability of these brain structures (basal ganglia, thalamus, substantia nigra) [10, 12, 13]. The correlation of glutamate with MRI load and NPI sum score suggests their link in the pathogenesis and phenotypic expression. Therefore, both movement disorder and neuropsychiatric

Table 2 Association of MRI lesions with various domains of neurobehavioral abnormality detected on neuropsychiatric inventory

Brain region		Depression		*p*	Anxiety		*p*	Delusion		*p*	Hallucination		*p*	Agitation		*p*	Elation		*p*
		N	AN		N	AN		N	AN		N	AN		N	AN		N	AN	
Thalamus	−	7	2	0.019	7	2	0.14	9	0	0.302	9	0	0.156	1	8	1.00	1	8	0.65
	+	8	19		7	20		22	5		20	7		3	24		8	19	
GP	−	6	2	0.046	6	2	0.036	8	0	0.57	7	1	1.00	2	6	0.207	1	7	0.648
	+	9	19		8	20		23	5		22	6		2	26		8	20	
Putamen	−	6	2	0.046	6	2	0.036	8	0	0.57	8	0	0.309	3	5	0.028	3	5	0.384
	+	9	19		8	20		23	5		21	7		1	27		6	22	
Caudate	−	7	3	0.058	7	3	0.026	9	1	1.00	9	1	0.645	3	7	0.057	3	7	0.686
	+	8	18		7	19		22	4		20	6		1	25		6	20	
Brain stem	−	10	11	0.501	8	13	1.00	19	2	0.63	18	3	0.418	3	18	0.63	6	15	0.705
	+	5	10		6	9		12	3		11	4		1	14		3	12	
Cerebellar	−	13	18	1.00	12	19	1.00	26	5	1.00	24	7	0.564	4	27	1.00	7	24	0.581
	+	2	3		2	3		5	0		5	0		0	5		2	3	
SCWM	−	12	18	0.677	11	19	0.658	25	5	0.56	23	7	0.317	4	26	1.00	7	23	0.627
	+	3	3		3	3		6	0		6	0		0	6		2	4	
Cortex	−	13	14	0.252	12	15	0.432	25	2	0.088	24	3	0.050	4	23	0.55	6	21	0.660
	+	2	7		2	7		6	3		5	4		0	9		3	6	
Brain region		Apathy		*p*	Disinhibition		*p*	Irritability		*p*	Aberrant		*p*	Night-time behavior		*p*	Appetite change		*p*
		N	AN		N	AN		N	AN		N	AN		N	AN		N	AN	
Thalamus	−	6	3	0.705	6	3	1.00	3	6	1.00	8	1	1.00	6	3	1.00	3	6	0.384
	+	15	12		16	11		8	19		22	5		19	8		5	22	
GP	−	6	2	0.424	5	3	1.00	2	6	1.00	7	1	1.00	5	3	0.678	3	5	0.338
	+	15	13		17	11		9	19		23	5		20	8		5	23	
Putamen	−	4	4	0.694	5	3	1.00	4	4	0.214	8	0	0.302	5	3	0.678	3	5	0.338
	+	17	11		17	11		7	21		22	6		20	8		5	23	
Caudate	−	5	5	0.709	5	5	0.462	4	6	0.454	9	1	0.655	6	4	0.454	3	7	0.658
	+	16	10		17	9		7	19		21	5		19	7		5	21	
Brain stem	−	13	8	0.736	15	6	0.175	10	11	0.011	18	3	0.677	16	5	0.465	6	15	0.424
	+	8	7		7	8		1	14		12	3		9	6		2	13	
Cerebellar	−	18	13	1.00	18	13	0.628	9	22	0.631	25	6	0.564	22	9	0.631	6	25	0.305
	+	3	2		4	1		2	3		5	0		3	2		2	3	
SCWM	−	18	12	0.677	18	12	1.00	10	20	0.643	24	6	0.561	21	9	1.00	7	23	1.00
	+	3	3		4	2		1	5		6	0		4	2		1	5	
Cortex	−	19	8	0.019	18	9	0.267	9	18	0.690	24	3	0.151	21	6	0.096	7	20	0.648
	+	2	7		4	5		2	7		6	3		4	5		1	8	

+, present; −, absent; *N*, normal; *AN*, abnormal; *GP*, globus pallidus; *SCWM*, subcortical white matter

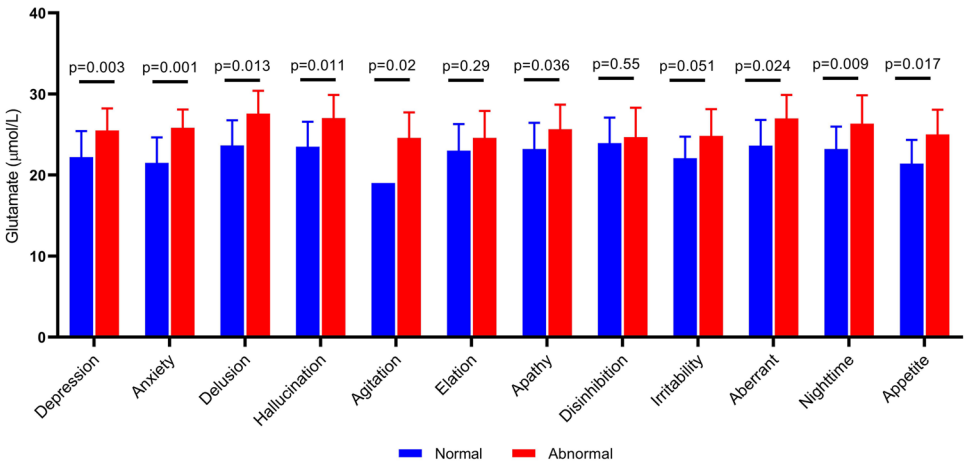

Fig. 4 Error bar diagram shows the relationship of serum glutamate with various domains of neuropsychiatric inventory (NPI)

abnormality are dominant neurological phenotypic expressions. The role of glutamate has also been reported in various psychiatric conditions including schizophrenia [61, 62], depression [63], and attention deficit [64]. In a study on mood disorder, glutamate level was elevated in the dorsomedial and dorso-anterolateral prefrontal cortex in H^1 MRI [65, 66]. Glutamate antagonist, NMDR agonist, and extracellular glutamate modulator (N-acetyl cysteine) are explored as treatment options in various psychiatric disorders [67, 68]. A meta-analysis of MR spectroscopy findings in schizophrenia has reported increased glutamate levels in the basal ganglia compared to the controls [69]. MR spectroscopy also revealed increased glutamate in the anterior cingulate cortex in the patients with the first episode of psychosis who were treatment naïve [69]. In the patients with depression, PET scan revealed improved glucose metabolism of the right ventral striatum and basal ganglia following injection of ketamine, an NMDA antagonist [70]. These findings suggest a role of glutamate in neuropsychiatric manifestations.

Limitation

We have not studied glutamate receptors and functional MRI. Glutamate level was not measured in CSF which might have been more specific for central nervous system changes. WD affects multiple organs; therefore, serum glutamate may reflect a global change. Lumbar puncture is an invasive procedure and was not found ethically appropriate.

Conclusion

Neuropsychiatric abnormality is a consistent feature of NWD especially in more severely affected NWD and if evaluated using an objective method. It correlates with the location of the MRI lesion, MRI lesion load, and glutamate level.

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Data Availability The data supporting the results of this investigation are available from the corresponding author upon request.

Declarations

Ethics Approval The research has been approved by the Institutional Ethics Committee (IEC), SGPGIMS, Lucknow, India (IEC Code: 2017–216-DM-100).

Consent to Participate Yes.

Consent for Publication Yes.

Conflict of Interest The authors declare no competing interests.

References

1. Thomas GR, Forbes JR, Roberts EA, Walshe JM, Cox DW (1995) The Wilson disease gene: spectrum of mutations and their consequences. Nat Genet 9(2):210–217. https://doi.org/10.1038/ng0295-210
2. Ferenci P (2006) Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. Hum Genet 120(2):151–159. https://doi.org/10.1007/s00439-006-0202-5

3. Sandahl TD, Laursen TL, Munk DE, Vilstrup H, Weiss KH, Ott P (2020) The prevalence of Wilson's disease: an update. Hepatology 71(2):722–732. https://doi.org/10.1002/hep.30911
4. Czlonkowska A, Litwin T, Dusek P, Ferenci P, Lutsenko S, Medici V, Rybakowski JK, Weiss KH, et al (2018) Wilson disease. Nat Rev Dis Prim 4(1):21. https://doi.org/10.1038/s41572-018-0018-3
5. European Association for Study of L (2012) EASL Clinical Practice Guidelines: Wilson's disease. J Hepatol 56(3):671–685. https://doi.org/10.1016/j.jhep.2011.11.007
6. Gow PJ, Smallwood RA, Angus PW, Smith AL, Wall AJ, Sewell RB (2000) Diagnosis of Wilson's disease: an experience over three decades. Gut 46(3):415–419. https://doi.org/10.1136/gut.46.3.415
7. Litwin T, Dusek P, Szafranski T, Dziezyc K, Czlonkowska A, Rybakowski JK (2018) Psychiatric manifestations in Wilson's disease: possibilities and difficulties for treatment. Ther Adv Psychopharmacol 8(7):199–211. https://doi.org/10.1177/2045125318759461
8. Akil M, Brewer GJ (1995) Psychiatric and behavioral abnormalities in Wilson's disease. Adv Neurol 65:171–178
9. Srinivas K, Sinha S, Taly AB, Prashanth LK, Arunodaya GR, Janardhana Reddy YC, Khanna S (2008) Dominant psychiatric manifestations in Wilson's disease: a diagnostic and therapeutic challenge! J Neurol Sci 266(1–2):104–108. https://doi.org/10.1016/j.jns.2007.09.009
10. Ranjan A, Kalita J, Kumar S, Bhoi SK, Misra UK (2015) A study of MRI changes in Wilson disease and its correlation with clinical features and outcome. Clin Neurol Neurosurg 138:31–36. https://doi.org/10.1016/j.clineuro.2015.07.013
11. Yu XE, Gao S, Yang RM, Han YZ (2019) MR imaging of the brain in neurologic Wilson disease. AJNR Am J Neuroradiol 40(1):178–183. https://doi.org/10.3174/ajnr.A5936
12. Zhong W, Huang Z, Tang X (2019) A study of brain MRI characteristics and clinical features in 76 cases of Wilson's disease. J Clin Neurosci 59:167–174. https://doi.org/10.1016/j.jocn.2018.10.096
13. Prashanth LK, Sinha S, Taly AB, Vasudev MK (2010) Do MRI features distinguish Wilson's disease from other early onset extrapyramidal disorders? An analysis of 100 cases. Mov Disord 25(6):672–678. https://doi.org/10.1002/mds.22689
14. Kalita J, Agarwal R, Chandra S, Misra UK (2013) A study of neurobehavioral, clinical psychometric, and P3 changes in vitamin B12 deficiency neurological syndrome. Nutr Neurosci 16(1):39–46. https://doi.org/10.1179/1476830512Y.0000000028
15. Nagasaka H, Takayanagi M, Tsukahara H (2009) Children's toxicology from bench to bed—liver Injury (3): oxidative stress and anti-oxidant systems in liver of patients with Wilson disease. J Toxicol Sci 34 Suppl 2:SP229–236. https://doi.org/10.2131/jts.34.sp229
16. Kalita J, Kumar V, Misra UK (2016) A study on apoptosis and anti-apoptotic status in Wilson disease. Mol Neurobiol 53(10):6659–6667. https://doi.org/10.1007/s12035-015-9570-y
17. Kalita J, Kumar V, Misra UK, Bora HK (2018) Memory and learning dysfunction following copper toxicity: biochemical and immunohistochemical basis. Mol Neurobiol 55(5):3800–3811. https://doi.org/10.1007/s12035-017-0619-y
18. Kalita J, Kumar V, Misra UK, Bora HK (2020) Movement disorder in copper toxicity rat model: role of inflammation and apoptosis in the corpus striatum. Neurotox Res 37(4):904–912. https://doi.org/10.1007/s12640-019-00140-9
19. Kalita J, Kumar V, Misra UK, Kumar S (2021) Movement disorder in Wilson disease: correlation with MRI and biomarkers of cell injury. J Mol Neuros: MN 71(2):338–346. https://doi.org/10.1007/s12031-020-01654-0
20. Krystkowiak P, du Montcel ST, Vercueil L, Houeto JL, Lagrange C, Cornu P, Blond S, Benabid AL, et al (2007) Reliability of the Burke-Fahn-Marsden scale in a multicenter trial for dystonia. Mov Disord 22(5):685–9.https://doi.org/10.1002/mds.21392
21. Kalita J, Misra UK, Pradhan PK (2011) Oromandibular dystonia in encephalitis. J Neurol Sci 304(1–2):107–110. https://doi.org/10.1016/j.jns.2011.02.001
22. Kalita J, Misra UK, Kumar V, Parashar V (2019) Predictors of seizure in Wilson disease: a clinico-radiological and biomarkers study. Neurotoxicology 71:87–92. https://doi.org/10.1016/j.neuro.2018.12.005
23. Grimm G, Prayer L, Oder W, Ferenci P, Madl C, Knoflach P, Schneider B, Imhof H, et al (1991) Comparison of functional and structural brain disturbances in Wilson's disease. Neurology 41 (2 (Pt 1)):272–276. https://doi.org/10.1212/wnl.41.2_part_1.272
24. Wiles CM (1990) Quantification of neurologic deficit. J Neurol Neurosurg Psychiatry 53(7):628–628. https://doi.org/10.1136/jnnp.53.7.628
25. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J (1994) The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia. Neurology 44(12):2308–2314. https://doi.org/10.1212/wnl.44.12.2308
26. Cummings JL (1997) The neuropsychiatric inventory: assessing psychopathology in dementia patients. Neurology 48(5 Suppl 6):S10-16. https://doi.org/10.1212/wnl.48.5_suppl_6.10s
27. Dening TR, Berrios GE (1989) Wilson's disease. Psychiatric symptoms in 195 cases. Arch Gen Psychiatry 46 (12):1126–1134. https://doi.org/10.1001/archpsyc.1989.01810120068011
28. Oder W, Grimm G, Kollegger H, Ferenci P, Schneider B, Deecke L (1991) Neurological and neuropsychiatric spectrum of Wilson's disease: a prospective study of 45 cases. J Neurol 238(5):281–287. https://doi.org/10.1007/BF00319740
29. Svetel M, Potrebic A, Pekmezovic T, Tomic A, Kresojevic N, Jesic R, Dragasevic N, Kostic VS (2009) Neuropsychiatric aspects of treated Wilson's disease. Parkinsonism Relat Disord 15(10):772–775. https://doi.org/10.1016/j.parkreldis.2009.01.010
30. Mura G, Zimbrean PC, Demelia L, Carta MG (2017) Psychiatric comorbidity in Wilson's disease. International review of psychiatry (Abingdon, England) 29(5):445–462. https://doi.org/10.1080/09540261.2017.1311845
31. Zimbrean PC, Schilsky ML (2014) Psychiatric aspects of Wilson disease: a review. Gen Hosp Psychiatry 36(1):53–62. https://doi.org/10.1016/j.genhosppsych.2013.08.007
32. Portala K, Westermark K, von Knorring L, Ekselius L (2000) Psychopathology in treated Wilson's disease determined by means of CPRS expert and self-ratings. Acta Psychiatr Scand 101(2):104–109. https://doi.org/10.1034/j.1600-0447.2000.90085.x
33. Carta M, Mura G, Sorbello O, Farina G, Demelia L (2012) Quality of life and psychiatric symptoms in Wilson's disease: the relevance of bipolar disorders. Clin Pract Epidemiol Ment Health 8:102–109. https://doi.org/10.2174/1745017901208010102
34. Dening TR (1985) Psychiatric aspects of Wilson's disease. Br J Psychiatry 147(6):677–682. https://doi.org/10.1192/bjp.147.6.677
35. Ring HA, Serra-Mestres J (2002) Neuropsychiatry of the basal ganglia. J Neurol Neurosurg Psychiatry 72(1):12–21. https://doi.org/10.1136/jnnp.72.1.12
36. Paulsen JS, Ready RE, Hamilton JM, Mega MS, Cummings JL (2001) Neuropsychiatric aspects of Huntington's disease. J Neurol Neurosurg Psychiatry 71(3):310–314. https://doi.org/10.1136/jnnp.71.3.310
37. Paoli RA, Botturi A, Ciammola A, Silani V, Prunas C, Lucchiari C, Zugno E, Caletti E (2017) Neuropsychiatric burden in Huntington's disease. Brain Sci 7(6):67. https://doi.org/10.3390/brainsci7060067

38. McColgan P, Tabrizi SJ (2018) Huntington's disease: a clinical review. Eur J Neurol 25(1):24–34. https://doi.org/10.1111/ene.13413
39. Schneider RB, Iourinets J, Richard IH (2017) Parkinson's disease psychosis: presentation, diagnosis and management. Neurodegener Dis Manag 7(6):365–376. https://doi.org/10.2217/nmt-2017-0028
40. Imperiale F, Agosta F, Canu E, Markovic V, Inuggi A, Jecmenica-Lukic M, Tomic A, Copetti M, et al (2018) Brain structural and functional signatures of impulsive-compulsive behaviours in Parkinson's disease. Mol Psychiatry 23(2):459–466. https://doi.org/10.1038/mp.2017.18
41. Flavell J, Nestor PJ (2021) A systematic review of apathy and depression in progressive supranuclear palsy. J Geriatr Psychiatry Neurol:891988721993545. https://doi.org/10.1177/0891988721993545
42. Jecmenica-Lukic M, Pekmezovic T, Petrovic IN, Tomic A, Svetel M, Kostic VS (2018) Use of the neuropsychiatric inventory to characterize the course of neuropsychiatric symptoms in progressive supranuclear palsy. J Neuropsychiatry Clin Neurosci 30(1):38–44. https://doi.org/10.1176/appi.neuropsych.17010012
43. Benke T, Karner E, Seppi K, Delazer M, Marksteiner J, Donnemiller E (2004) Subacute dementia and imaging correlates in a case of Fahr's disease. J Neurol Neurosurg Psychiatry 75(8):1163–1165. https://doi.org/10.1136/jnnp.2003.019547
44. Groth C, Mol Debes N, Rask CU, Lange T, Skov L (2017) Course of tourette syndrome and comorbidities in a large prospective clinical study. J Am Acad Child Adolesc Psychiatry 56(4):304–312. https://doi.org/10.1016/j.jaac.2017.01.010
45. Zeidler M, Johnstone EC, Bamber RW, Dickens CM, Fisher CJ, Francis AF, Goldbeck R, Higgo R, et al (1997) New variant Creutzfeldt-Jakob disease: psychiatric features. Lancet 350(9082):908–910. https://doi.org/10.1016/s0140-6736(97)03148-6
46. Spencer MD, Knight RS, Will RG (2002) First hundred cases of variant Creutzfeldt-Jakob disease: retrospective case note review of early psychiatric and neurological features. BMJ 324(7352):1479–1482. https://doi.org/10.1136/bmj.324.7352.1479
47. Pourhamzeh M, Moravej FG, Arabi M, Shahriari E, Mehrabi S, Ward R, Ahadi R, Joghataei MT (2021) The roles of serotonin in neuropsychiatric disorders. Cell Mol Neurobiol. https://doi.org/10.1007/s10571-021-01064-9
48. Foley PB (2019) Dopamine in psychiatry: a historical perspective. J Neural Transm (Vienna) 126(4):473–479. https://doi.org/10.1007/s00702-019-01987-0
49. Lucato LT, Otaduy MC, Barbosa ER, Machado AA, McKinney A, Bacheschi LA, Scaff M, Cerri GG, et al (2005) Proton MR spectroscopy in Wilson disease: analysis of 36 cases. AJNR Am J Neuroradiol 26(5):1066–1071
50. Pulai S, Biswas A, Roy A, Guin DS, Pandit A, Gangopadhyay G, Ghorai PK, Sarkhel S, et al (2014) Clinical features, MRI brain, and MRS abnormalities of drug-naive neurologic Wilson's disease. Neurol India 62(2):153–158. https://doi.org/10.4103/0028-3886.132349
51. Stezin A, George L, Jhunjhunwala K, Lenka A, Saini J, Netravathi M, Yadav R, Pal PK (2016) Exploring cortical atrophy and its clinical and biochemical correlates in Wilson's disease using voxel based morphometry. Parkinsonism Relat Disord 30:52–57. https://doi.org/10.1016/j.parkreldis.2016.06.017
52. Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 130(4S Suppl):1007S-1015S. https://doi.org/10.1093/jn/130.4.1007S
53. Zhou Y, Danbolt NC (2014) Glutamate as a neurotransmitter in the healthy brain. J Neural Transm (Vienna) 121(8):799–817. https://doi.org/10.1007/s00702-014-1180-8
54. Magi S, Piccirillo S, Amoroso S, Lariccia V (2019) Excitatory amino acid transporters (EAATs): glutamate transport and beyond. Int J Mol Sci 20 (22). https://doi.org/10.3390/ijms20225674
55. Hertz L (2006) Glutamate, a neurotransmitter—and so much more. A synopsis of Wierzba III. Neurochem Int 48 (6–7):416–425. https://doi.org/10.1016/j.neuint.2005.12.021
56. Nedergaard M, Takano T, Hansen AJ (2002) Beyond the role of glutamate as a neurotransmitter. Nat Rev Neurosci 3(9):748–755. https://doi.org/10.1038/nrn916
57. Jensen N, Oliveira JR (2014) Basal ganglia vulnerability to oxidative stress. Front Neurosci 8:80. https://doi.org/10.3389/fnins.2014.00080
58. Goyal MK, Sinha S, Patil SA, Jayalekshmy V, Taly AB (2008) Do cytokines have any role in Wilson's disease? Clin Exp Immunol 154(1):74–79. https://doi.org/10.1111/j.1365-2249.2008.03755.x
59. Wu P, Dong J, Cheng N, Yang R, Han Y, Han Y (2019) Inflammatory cytokines expression in Wilson's disease. Neurol Sci 40(5):1059–1066. https://doi.org/10.1007/s10072-018-3680-z
60. Nagasaka H, Inoue I, Inui A, Komatsu H, Sogo T, Murayama K, Murakami T, Yorifuji T, et al (2006) Relationship between oxidative stress and antioxidant systems in the liver of patients with Wilson disease: hepatic manifestation in Wilson disease as a consequence of augmented oxidative stress. Pediatr Res 60(4):472–477. https://doi.org/10.1203/01.pdr.0000238341.12229.d3
61. Marsman A, van den Heuvel MP, Klomp DWJ, Kahn RS, Luijten PR, Hulshoff Pol HE (2011) Glutamate in schizophrenia: a focused review and meta-analysis of 1H-MRS studies. Schizophr Bull 39(1):120–129. https://doi.org/10.1093/schbul/sbr069%JSchizophreniaBulletin
62. McCutcheon RA, Krystal JH, Howes OD (2020) Dopamine and glutamate in schizophrenia: biology, symptoms and treatment. World Psychiatry 19(1):15–33. https://doi.org/10.1002/wps.20693
63. Murrough JW, Abdallah CG, Mathew SJ (2017) Targeting glutamate signalling in depression: progress and prospects. Nat Rev Drug Discov 16(7):472–486. https://doi.org/10.1038/nrd.2017.16
64. Huang X, Wang M, Zhang Q, Chen X, Wu J (2019) The role of glutamate receptors in attention-deficit/hyperactivity disorder: from physiology to disease. Am J Med Genet B Neuropsychiatr Genet 180(4):272–286. https://doi.org/10.1002/ajmg.b.32726
65. Hasler G, Buchmann A, Haynes M, Muller ST, Ghisleni C, Brechbuhl S, Tuura R (2019) Association between prefrontal glutamine levels and neuroticism determined using proton magnetic resonance spectroscopy. Transl Psychiatry 9(1):170. https://doi.org/10.1038/s41398-019-0500-z
66. Arnone D, Mumuni AN, Jauhar S, Condon B, Cavanagh J (2015) Indirect evidence of selective glial involvement in glutamate-based mechanisms of mood regulation in depression: meta-analysis of absolute prefrontal neuro-metabolic concentrations. Eur Neuropsychopharmacol 25(8):1109–1117. https://doi.org/10.1016/j.euroneuro.2015.04.016
67. Goh KK, Wu TH, Chen CH, Lu ML (2021) Efficacy of N-methyl-D-aspartate receptor modulator augmentation in schizophrenia: a meta-analysis of randomised, placebo-controlled trials. J Psychopharmacol 35(3):236–252. https://doi.org/10.1177/0269881120965937
68. Singh SP, Singh V (2011) Meta-analysis of the efficacy of adjunctive NMDA receptor modulators in chronic schizophrenia. CNS Drugs 25(10):859–885. https://doi.org/10.2165/11586650-000000000-00000
69. Salavati B, Rajji TK, Price R, Sun Y, Graff-Guerrero A, Daskalakis ZJ (2015) Imaging-based neurochemistry in

schizophrenia: a systematic review and implications for dysfunctional long-term potentiation. Schizophr Bull 41(1):44–56. https://doi.org/10.1093/schbul/sbu132

70. Nugent AC, Diazgranados N, Carlson PJ, Ibrahim L, Luckenbaugh DA, Brutsche N, Herscovitch P, Drevets WC, Zarate CA Jr (2014) Neural correlates of rapid antidepressant response to ketamine in bipolar disorder. Bipolar Disord 16(2):119–128. https://doi.org/10.1111/bdi.12118