ACCSCIENCE PUBLISHING

Gene & Protein in Disease

ORIGINAL RESEARCH ARTICLE

Identifying regulatory variants in Indian Wilson's disease patients with missing heritability

Supplementary File

Table S1. The polymerase chain reaction primer sequences for screening *ATP7B cis*-regulatory elements in Wilson's disease patients

Gene	Exon/*cis*-regulatory element	Primers (5' → 3')	Amplicon length (bp)
ATP7B	Regulatory region 1	Forward: 5'GTGGGAAGAAGGCAAGTCTG3' Reverse: 5'ACCATGTTAGGTCTGTGGGC3'	478
	Regulatory region 2	Forward: 5'CAGCAACAAAAGCAAGGCACAG3' Reverse: 5'TCTCTGTATCCCAGCCACCTG3'	709
	Regulatory region 3	Forward: 5'CTCCTGTGTGCCCAATCAATG3' Reverse: 5'CCTGCCTGACTTTCTCAGCG3'	494

Abbreviation: bp: Base pair.

Table S2. Clinical details of Indian Wilson's disease patients with no coding mutations in *ATP7B*

Patient ID	[a]Sex	Age of onset (years)	Ceruloplasmin level (mg/dL)	Kayser–Fleischer ring (slit-lamp experiment)	Urinary copper level (μg/24 h)	Clinical phenotype
WD 151	M	9	6	Absent	90	Predominant neurological symptoms present with mild hepatic involvement. Dystonia, dysarthria, gait abnormality, and clumsiness in walking are present. Unable to recognize people, EEG shows sub-cortical dysrhythmia.
WD 306	M	NA	10.8	Present	NA	No hepatic symptoms are present. Neurological symptoms include dystonia, dysarthria, and gait disturbances. Generalized osteoporosis and decreased metaphyseal density were detected on the X-ray.
WD 375	F	17	8.3	Present	18.9	Both hepatic and neurological symptoms are present. USG shows a small shrunken liver with heterogeneous echotexture. Dystonia, dysarthria, dysphagia, drooling, and rigidity are present. T2 weighted MRI shows brain copper deposition.
WD 380	F	8.5	25	NA	192	Only hepatic symptoms are present. Enlargement and stiffness of the liver are observed, along with splenomegaly.
WD 405	M	8	12.72	Present	80	Only neurological symptoms are present. Neurological symptoms include dysarthria, tremor, and dystonia. Weakness in upper and lower limbs.
WD 429	M	16	16.5	Present	368	Both hepatic and neurological symptoms are present. Elevated serum AST and ALT levels. USG shows ascites in the liver and splenomegaly. MRI shows symmetrical hyper-density on T2W and FLAIR sequences in putamen and caudate.
WD 440	M	18.8	3.81	Present	230	Both hepatic and neurological symptoms present. Neurological symptoms involve dysarthria, tremors, drooling, dystonia, rigidity, and bradykinesia. Mild elevation in serum AST and ALT levels. USG shows hepatosplenomegaly with portal hypertension. MRI shows bilateral hyperintensity on T2WI and hypointensity on T1WI in bilateral putamen, thalamus, and caudate nucleus
WD 448	F	6	31	Present	282	Only neurological symptoms present. Neurological symptoms include dystonia, tremor, and cognitive impairment.
WD 511	F	16	27.05	Present	27.05	Predominant neurological manifestation with mild hepatic involvement. Neurological symptoms include dysarthria, tremor, dystonia, and rigidity in upper limbs.
WD 554	M	NA	19.83	NA	49.62	NA
WD 597	M	NA	16	Present	NA	Only hepatic symptoms are present. Mild elevation in liver enzymes. Gallstone present.
WD 695	F	4	1	Absent	472	Mild hepatic and neurological features are present.
WD 721	F	13.5	6	Present	750	Both hepatic and neurological manifestations present. USG shows hepatosplenomegaly. Neurological symptoms include dystonia and dysarthria.

Note: [a]Sex: M refers to male, and F refers to female.

Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate transferase; EEG: Electroencephalogram; ID: Identification; MRI: Magnetic resonance imaging; NA: Not available; USG: Ultrasonography; WD: Wilson's disease.

Table S3. Clinical details of Indian Wilson's disease patients with single coding mutation in *ATP7B*

Patient ID	[a]Sex	Age of onset (years)	Ceruloplasmin level (mg/dL)	Kayser–Fleischer ring (slit-lamp experiment)	Urinary copper level (µg/24 h)	Clinical phenotype
WD 17	F	11	3	Present	NA	Both hepatic and neurological symptoms are present. Neurological features include dystonia, dysarthria, rigidity, and dementia. USG shows hepatosplenomegaly. However, liver enzymes are within normal limits. A CT scan result shows diffuse symmetrical hypodensity at the basal ganglia region. Caudate nucleus atrophy resulting in mild dilation of the frontal horn was observed. Hypodensity in the frontoparietal and occipital areas was observed.
WD 183	M	9.5	NA	Present	NA	Only neurological symptoms are present. The neurological symptoms include dysarthria, dystonia, tremor, rigidity, and gait disturbances. A CT scan shows old infarcts in the basal ganglia.
WD 196	F	9	14	Present	NA	Both hepatic and neurological symptoms are present. USG shows cirrhotic changes in the liver. Neurological symptoms include dysarthria, drooling, dysphagia, and rigidity. Acute restlessness and aggressive behavior were observed.
WD 211	M	NA	NA	NA	NA	NA
WD 268	F	8	3	Present	210	Only neurological symptoms are present. The neurological symptoms include dysarthria, dystonia, dysphagia, rigidity, and gait disturbances. Masked facies and saccadic gaze disturbances were observed.
WD 290	M	14	15.5	Present	NA	Only hepatic symptoms are present. Hepatic encephalopathy with portal hypertension was observed. Elevated serum levels of AST, ALT, and alkaline phosphatase were observed.
WD 319	M	24	NA	Present	NA	Only neurological symptoms are present. The neurological symptoms include dysarthria, dystonia, tremor, rigidity, and gait disturbances.
WD 342	M	12	NA	Present	NA	Only neurological symptoms are present. The neurological symptoms include dysarthria, dystonia, tremor, and gait disturbances. Progressive scholastic deterioration was observed.
WD 365	F	3.6	18.7	NA	343	Predominant neurological symptoms with mild hepatic involvement. USG shows mild hepatomegaly. Neurological symptoms include psychotic changes and disoriented.
WD 395	M	28	10	Absent	8	Predominant neurological symptoms present with mild hepatic involvement. USG shows a mildly enlarged liver and mild splenomegaly. Neurological symptoms include rigidity and dystonia.
WD 509	M	7	25.4	Present	1,408	Both hepatic and neurological symptoms are present. Elevated serum AST, ALT, and alkaline phosphatase levels were observed. The neurological symptoms include dystonia, dysarthria, drooling, rigidity, and gait disturbances. The patient is present with psychotic symptoms.

(*Cont'd*...)

Table S3. (*Continued*)

Patient ID	[a]Sex	Age of onset (years)	Ceruloplasmin level (mg/dL)	Kayser–Fleischer ring (slit-lamp experiment)	Urinary copper level (μg/24 h)	Clinical phenotype
WD 515	M	13	5.54	Present	574.4	Both hepatic and neurological symptoms are present. Elevated serum AST and ALT levels were observed. Neurological symptoms include dystonia and dysarthria The patient shows aggressive behavior.
WD 542	M	9	6	Present	175	Only neurological symptoms are present. The neurological symptoms include tremors, dysarthria, and dystonia. The patient shows aggressive behavior and visual hallucinations.
WD 595	M	3	NA	NA	NA	Only neurological symptoms are present. The neurological symptoms include gait disturbances.
WD 642	F	9	2	Present	440	Predominant hepatic symptoms with mild neurological involvement. Hepatic symptoms include elevated AST and ALT levels. Cirrhotic changes observed in USG.
WD 634	M	9	2.5	Present	185	Only neurological symptoms are present. Neurological symptoms include tremors, dysarthria, and gait abnormality
WD 689	F	10	8	Present	155	Only neurological symptoms are present. Neurological symptoms include dysarthria and tremor. CT scan images revealed bilateral basal ganglia infarcts.

Note: [a]Sex: M refers to male, and F refers to female.
Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate transferase; CT: Computed tomography; ID: Identification; NA: Not available; USG: Ultrasonography; WD: Wilson's disease.