Genes and metals : a deadly combination

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Abstract

Wilson's disease is an autosomal recessive disease of copper metabolism, with an estimated prevalence of 1:30000. The most common presentations of WD are liver disease and neurological disturbance. For many years the diagnosis was based on the results of several clinical and biochemical tests, for which several limitations had been reported. In recent years the developments of new techniques in genetic and molecular biology have provided useful tools in the diagnosis of Wilson's disease. However, the presence of several mutations and the fact that most patients are compound heterozygote means that the problem is not completely resolved. Chelators and zinc salts have been largely used in the treatment of WD patients with a favorable outcome, but the debate continues as to the agents of first choice. Liver transplantation is a cure for patients with decompensated liver disease but its effect on the neurological outcome is still not clear. (Acta gastroenterol. belg., **2005**, 68, **26-32**).

Key words : Wilson's Disease, genetics, diagnosis, treatment, fulminant hepatic failure.

Wilson's disease is an autosomal recessive disease of copper metabolism, with an estimated prevalence of 1:30000 (1). In normal conditions, copper is absorbed in the small intestine and distributed to tissues and organs. In the liver, copper incorporated into metallothionein is stored in hepatocytes (a non-toxic way of storing copper in cells). The excess copper is released and immediately bound to the chaperone protein ATOX and transported to the Golgi apparatus. Copper-transport adenosine triphosphatase (ATP7B) located at trans golgi directs copper into the secretory pathway, where it is incorporated into apocaeruloplasmin and excreted into the bile. ATP7B is a membrane P-type ATPase (160 KDa) with six copper-binding motifs, a phosphorylation motif, ATP- binding site and eight transmembrane regions. In Wilson's disease patients a defect of ATP7B involved in biliary copper excretion and in the biosynthesis of caeruloplasmin, is responsible for the toxic accumulation of copper in the liver, brain, cornea and kidney. To date more than 200 mutations affecting this protein's ability to eliminate the excess copper have been identified (database maintained at the University of Alberta, www.medgen.med.ualberta.ca). ATOX is not the only protein involved in the chelation of copper. Other chaperon proteins called Sco1, Sco2 and CCS, which do not interact with ATP7B, are involved in the formation of other copper proteins resulting in chelation of copper. However in Wilson's disease, despite a high level of copper in the cells, low amounts of copper are available to these proteins (2,3,4,5,6).

Clinical presentation

The clinical manifestations of the disease are usually observed in teenage years, however symptomatic patients have been reported as early as 3 years of age and as late as 76 years of age. The clinical presentation of Wilson's disease is extraordinarily diverse (Table I). The most common clinical presentations are liver disease and neurological disturbance (1,7). The disease begins with a pre-symptomatic period, during which copper accumulation in the liver causes subclinical hepatitis. Early diagnosis, therefore, of pre-symptomatic patients, followed by adequate treatment, can prevent the onset of clinical symptoms. Screening of all first-degree relatives of a WD patient is highly recommended for prompt detection of asymptomatic WD individuals ; however mass screening has been shown to have a limited success rate, as reported recently by Yamaguchi *et al.* (1999). Serum caeruloplasmin, liver function tests, urinary copper, including penicillamine challenge, molecular testing (if available) and ophthalmic examination for KF rings should be performed in all siblings aged more than 3 years.

Wilson's disease Diagnosis

The diagnosis of WD is based on the results of several clinical and biochemical tests as displayed in table II (1,7,8). However, each of these tests has its limitations.

Plasma Caeruloplasmin

Although, low plasma caeruloplasmin is reported in 73% of WD patients (9), false negatives have been observed in cases of infection, pregnancy and estrogens intake. On the other hand, false positive data may be observed in heterozygotes (20%), protein-losing enteropathy, aceruloplasminemia and severe hepatic insufficiency. The method used by the laboratory (the oxidative assay or nephelometric assay) may also affect the results of caeruloplasmin measurement (10).

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Table I. — **Clinical manifestation of Wilson's disease**

	Clinical presentations
Hepatological	Hepatomegaly, jaundice, acute hepatitis, portal hypertension, cirrhosis, fulminant hepatic failure
Neurological	Coordination difficulties, gait disturbance, balance disorders, stiffness or rigidity, abnormal reflexes, abnormal speech, drooling, difficulty swallowing
Psychiatric	Reduced school or work performances, paranoia, hallucinations, delusions and sexual dysinhibition
Ocular	Kayser-Fleisher (KF) rings present in all patients with neurological or psychiatric symptoms. In hepatic patients approximately 40% had KF rings.
Other	Renal, cardiac, bone, haem presentations

Table II. — **Tests routinely performed for the diagnosis of Wilson's disease**

Urinary Copper No therapy Penicillamine Challenge (500mg × 2, 12h apart)	24h excretion > 100 µg in 65% of WD patients > 1600 µg or > 25 mmol, in 90% of patients
Serum Copper	Free Copper > 25µg/dl
Serum Caeruloplasmin	< 20 mg/dl (in 95% of WD patients)
KF rings	Identification in most patients requires an experienced observer
Liver Copper	> 250 µg/gm of dry weight liver
Coombs negative haemolytic anaemia	
Molecular diagnosis	Over 200 mutations are known
MRI Scan	

24h urinary copper

The 24h urinary copper value may be misleading because of incorrect 24h urine collection, especially in pediatric patients, for whom 24h urine collection is not very easy.

Serum copper

The serum copper value suffers from several limitations, and it is no longer considered to be a reliable diagnostic tool.

Liver copper

Liver copper values equal to or higher than 250 µg/gm of dry weight are considered to be the gold standard in the diagnosis of Wilson's disease. However, in chronic cholestatic conditions the liver copper content is also elevated. In fact, hepatic manifestations of Wilson's disease are very similar to those observed in autoimmune hepatitis, steatosis, chronic cholestatic conditions and fulminant hepatic failure. In a study to be published in the J. Clinical Gastroenterology and Hepatology (2005), Ferenci *et al.* assessed the hepatic copper content of 114 patients at the time of diagnosis of Wilson's disease. The distribution of hepatic copper concentration as a function of histological findings showed that 19 Wilson's disease patients had a liver copper concentration below 250 µg/g dry weight. The sensitivity analysis based on comparison of these 106 patients to 244 other patients without Wilson's disease showed that the upper limit of diagnosis (> 250 µg/g dry weight) has a poor sensitivity (82%) and very good specificity. The low range (75 µg/g dry weight) has a higher sensitivity, but lower specificity as well as a positive predictive value. The negative predictive value shows a major gain. Further studies are required to confirm these data.

MRI

MRI is an efficient method for documenting involvement of the central nervous system in WD, allowing better anatomical and clinical correlations (11).

Molecular biology

In recent years the developments of new techniques in genetic and molecular biology have provided useful tools in the diagnosis of Wilson's disease. By using these new techniques, Ferenci et al has extensively studied the mutations affecting the ATP7B gene, and the subsequent protein abnormalities and dysfunction in Wilson's disease patients. In an ongoing study Ferenci *et al.* collected the DNA from 951 patients, mainly of German, Austrian, Eastern European and Turkish origin, as well as a standardized questionnaire asking for age, symptoms, and biochemical and histological findings. In every single case, the validity of the diagnosis was reassessed based on the international consensus statement on diagnostic criteria for Wilson's disease (8). Mutation analysis was performed only in confirmed cases of Wilson's disease. Using polymerase chain reaction (PCR), mutation analysis was first performed to detect the H1069Q mutation, which is the most common mutation among the WD patients of central, eastern and northern European origin. Further mutation analysis was performed in the absence of the H1069Q mutation. The sequence of analysed exons has been displayed in Fig. 1. Amongst 820 index cases studied so far, 90% of patients had at least one known mutation (in 60% both mutations were identified, 30% had only one known mutation). In 10% of cases no mutation was identified. This is to some extent due to the fact that this is an ongoing study, where exons 2 to 5, and 21 were not yet analysed. In H1069Q homozygotes or compound (44% of all patients), copper transport in to the Golgi apparatus, although lower than normal, was still maintained. Folding of ATP7B was impaired at high copper concentrations, leading to impaired copper excretion.

The distributions of WD mutations according to clinical presentation of the disease as well as the age at onset of either neurological or hepatic symptoms were also assessed (Table III). The data indicated that the H1069Q

Table III. — **Distribution of WD mutations according to clinical presentation and age at onset of symptoms**

	H1069Q/ H1069Q	*H1069Q/ Exon 14*	*H1069Q/ Exon 8*	*H1069Q/ 3400delC*	*H1069Q/ Exon13*	*H1069Q/ ?*	*H1069Q/ Other*	*Exon 8§*	*Exon 15§*	*Other*	*?/?*
Clinical presentation											
Neurologic	97	1	13	12	2	52	13	25	5	27	33
*Hepatic**	75	5	29	6	13	58	12	39	14	43	51
Other	1								1		
Age at onset of neurological symptoms											
< 10	0		1			2	2	2	1	3	
11-20	28		5	8	1	22	7	13	3	12	12
21-35	60		6	4	1	32	4	8	1	12	13
> 35	6		1			2		1	0	5	8
Age at onset of hepatological symptoms											
< 10	11		4	2	1	11	2	7	3	11	9
11-20	38	4	22	2	4	16		19	7	27	21
21-35	24		4	1	5	10	5	4	3	13	16
> 35	2		1	1	3	6	2	1		2	4

§ Homozygote or second mutation unknown.

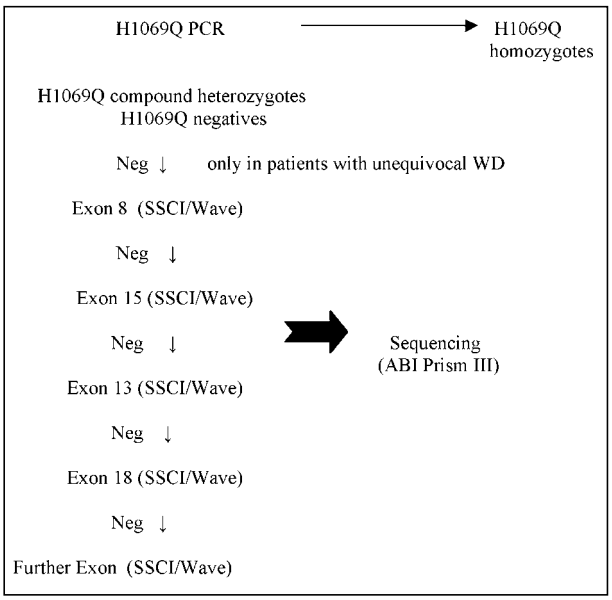

Fig. 1. — Mutation detection strategy

homozygote mutation, associated with late onset neurologic disease, was mainly detected in neurological presentations. This was also the case of the H1069Q/ 3400delC mutation. Hepatic presentation of WD mutations was mainly associated with mutations affecting exon 8.

The relevance of this test in the diagnosis of WD patients was evaluated in 82 patients already diagnosed with WD (Ferenci *et al.*). The results of a limited mutation analysis performed only on exon 8, 13, 14, 15 revealed a mutation on either one or both chromosomes in 35 and 27 patients respectively. In the remaining 12 patients no mutation was detected. These results indicated that, by using this technique, a rapid (within a week) diagnosis of WD would have been possible in more than 80% of patients. Furthermore, in pre-symptomatic siblings and first-degree relatives, the mutation analysis is the unique test allowing a quick diagnosis of WD, prior to the presentation of clinical and biochemical presentations.

Clinical tests

The accuracy of clinical tests is not the same for patients with neurological and hepatic disease. In a study conducted by Steindl *et al.* (9) only 50% of hepatic patients were found to have KF rings, while KF rings were detected in 90% of neurological patients. Only 7 to 10% of siblings had KF rings.

In conclusion, the combination of genetic, clinical and biochemical tests provides a powerful and reliable tool for the diagnosis of WD.

Scoring system

In 2001, during the 8th international conference on WD and Menkes disease, a scoring system for the diagnosis of WD was discussed (12). The aim was to provide objective criteria with high sensitivity and specificity for the diagnosis of Wilson disease. A combination of clinical and biochemical tests with a score ranging from 0 to 4 for each test was developed (Table IV). The patients with a total score of at least 4 were diagnosed with Wilson's disease. The patients with a total score of two to three were considered as "likely to have Wilson's disease, yet more investigations had to be performed". The

Table IV. — **Different criteria involved in the Diagnosis of Wilson's disease based on scoring system developed by Ferenci *et al.***

Liver copper (in absence of cholestasis)		*Serum Caeruloplasmin*	
Normal (< 50 µg/g)	-1	Normal (> 0.2 g/l)	0
< 5 × ULN (50-250 µg/g)	1	0.1-0.2 g/l	1
> 5 × ULN (> 250 µg/g)	2	< 0.1 g/l	2
Rhodanine Stain			
Absent	0		
Present	1		
Mutation Analysis		*Clinical symptoms and signs*	
2 chromosome mutations	4	KF rings	
1 chromosome mutation	1	Present	2
No mutations detected	0	Absent	0
Urinary copper (in absence of acute hapatitis)			
Normal	0	Severe	2
1-2 × ULN	1	Mild	1
> 2 × ULN	2	Absent	0
Normal but > 5 × ULN after penicillamine	2	Coombs' negative haem anemia	
		Present	1
		Absent	0

Table V. — **Evaluation of the Leipzig meeting score**

	Score			
	≥ 4	*2-3*	*≤ 1*	*Total*
Wilson's disease Patients	50	3	0	53
Other diagnosis	5	40	45	90
	True +	*False –*	*False +*	*True –*
Wilson's disease Patients	50	3		
Other diagnosis			5	85
	Sensitivity	*Specificity*	*+ predictive value*	*Predictive value*
	94.339%	94.444%	90.909%	96.590%

diagnosis of Wilson's disease was judged to be improbable for scores between zero and one. With respect to molecular analysis, it should be noted that more than 200 different mutations have been identified. It has been difficult to devise a simple genetic screening test for the disease. Thus only the H1069Q (exon 14), was researched.

In order to test this scoring system, 143 children with chronic liver disease, aged at least 5 years, were reviewed. All patients had urinary copper assessments and a liver biopsy as part of the diagnostic work up. The results of this retrospective review were displayed in table V. Fifty patients with Wilson's disease had a score ≥ 4 (true positives). A total of 85 true negatives with a score of either 2-3 (40 children) or < 1 (45 children) were observed. Only 3 patients with Wilson's disease had a score of 2 to 3 (false negatives), while 5 non Wilson's disease patients had a score of at least 4 (False positives). Both sensitivity and specificity of this scoring system was higher than 94%. In addition, positive predictive value and negative predictive values were higher than 90% (90.9% and 96.59% respectively).

Fulminant hepatic failure

As previously mentioned, hepatic failure is a common feature of WD, predominantly reported in females (75% versus 25% in males). The patients with fulminant presentation of WD, defined as acute liver disease with encephalopathy, have a high mortality (almost 100%) in the absence of transplantation. In order to assess survival in FHF patients a prognostic index based on the

Table VI. — **Diagnosis of Wilson's disease**

	Number (%)	*Media (range)*
Family History	17 (22.7)	
Kayser-Feischer Rings	38 (50.7)	
Coombs' negative haemolytic anaemia	1	
Serum Caeruloplasmin (g/l)	45/58 (77.6)	0.07 (0-0.82)
24h Urinary Copper (µmol/24 hr) *Post-penicillamine*	54/57 (94.7) 21/30 (70)	10.3 (0.7-192) 34.9 (12.6-381.6)
Liver Copper (µg/g of dry weight)	20/25 (80)	458(5-2358)

Table VII. — **Clinical presentations of Wilson's disease 74 children admitted to king's college Hospital**

Major Symptoms			
Jaundice	54.7%	Ascites	25.3%
Acute liver failure	36%	Hepatomegaly	24%
Abdominal pain	32%	Splenomegaly	22.7%
Gastrointestinal Symptoms n(%)			
Abdominal distension	16 (21.3)	Pale Stool	8 (10.7)
Anorexia	15 (20)	Diarrhoea	7 (9.3)
Vomiting	13 (17.3)	Melaena	1 (1.3)
Neurological Symptoms			
Lethargy	22 (29.3)	Vertigo	2 (2.7)
Encephalopathy	20 (27)	Tremors	1 (1.3)
Behavioural changes	5 (6.7)	Developmental delay	1 (1.3)
Headaches	4 (5.3)		
Other			
Peripheral Oedema	13 (17.3)	Pruritus	6 (8)
Dark Urine	10 (13.3)	Gynaecomastia	4 (5.3)
Fever	10 (13.3)	Joint Pain	4 (5.3)
Epistaxis	6 (8)		

SBR, AST and INR values has been developed (13). The results showed that among the 27 patients included in this study all patients with a score of at least 7 died. The sensitivity and specificity of the test were respectively 87% and 90%, with a likelihood ratio of 8.7. This scoring system has been re-evaluated by Dhawan *et al.* The medical records of children with Wilson's disease, in particular those with fulminant Wilson's disease, admitted to King's College Hospital (London, UK) were reviewed retrospectively. Between 1967 and 2000, 74 children (46 boys and 28 girls) with a median age of 11.7 years (2.6-17.9 years) were admitted to the hospital. All children with at least two positive tests out of the following list were diagnosed with Wilson's disease.

– Family history
– KF rings
– Low Caeruloplasmin
– Coombs' negative haemolytic anaemia
– Elevated 24-Urinary copper
– Elevated liver copper
– Positive penicillamine challenge

As displayed in tables VI and VII, elevated urinary copper, low caeruloplasmin, KF rings and anemia were reported in half of patients. Elevated liver copper and family history were noted in respectively 76% and 17% of patients (Table VI). More than half of the children (54.7%) had jaundice. Acute liver failure and abdominal pain were reported in respectively 36% and 32% of patients. Lethargy and encephalopathy were observed in almost one third of patients (Table VII).

Among these patients, 17 fulminant presentations of WD were observed. Nine were male and 8 female, with a median age of 11.9 (8.6-16) years. In WD patients with fulminant presentation, diagnosis is even more challenging, due to the lethal condition of the disease, which requires a rapid diagnosis associated with difficulties performing biochemical tests, especially the 24h urinary copper (caused by renal insufficiency).

The data obtained in all patients were analysed retrospectively, using bilirubin, white cell counts, INR, albumin and AST values at presentation as predictors of mortality. Authors proposed a new predictive index value of 11 with a higher likelihood ratio than the previous scoring system (22.8 vs 8.7), as well as higher sensitivity and specificity (93% and 96% compared to 87% and 90% respectively).

Treatment

Chelators and zinc

The safety and efficacy of zinc and chelators (D-penicillamine, Trientine and Tetrathiomolybdate) in the treatment of Wilson's disease was presented. A systematic review of scientific literature revealed that chelators or zinc have been administered at different stages of the disease. Published data showed that, during the initial phase of treatment, penicillamine therapy improves the clinical condition of WD patients, despite some minor or major residual disabilities. In addition, in some patients neurological symptoms may initially worsen on treatment with penicillamine. Therefore, in patients with neurological Wilson's disease zinc might be an option. However no randomized controlled zinc vs penicillamine trial in this patient group has been performed so far. For well controlled and decoppered patients as well as presymptomatic patients articles show that zinc is an effective treatment with a very good tolerance. Various zinc salts are available. The use of zinc sulfate has been associated with gastrointestinal intolerance (epigastric or abdominal pain, 14,15,16), while zinc acetate (Wilzin™) is known to have a better gastric tolerability (17).

Liver transplant

Fulminant hepatitis and severe hepatic insufficiency are two major indications for liver transplant in WD patients. As previously reported patients with fulminant presentation of WD and prognostic index of 11 had no chance of survival in the absence of liver transplant. Therefore for these patients liver transplant is not only a treatment but also a life saving procedure.

Since the late 80's, 58.000 liver transplantations performed in Europe were recorded in the European liver transplant registry. This registry is audit based and has at least 95% correlation with the clinical reality. A total of 2155 adults and children (5%) with metabolic disease underwent liver transplant during the last 14 years. In paediatric patients, one out of 5 liver transplanted patients aged between 2 and 15 years, had a metabolic liver disease. Among these patients, 382 (15%) were WD patients. The ten year follow up showed a survival rate of 79%.

In these studies, the low number of patients remains the major limiting factor. However a Wilson's disease European six framework program (EuroWilson) has been established. The aims are to create a Wilson's disease clinical database providing information on the incidence, prevalence of sub-types, current treatments and short term outcomes and to design randomized controlled clinical trials. The EuroWilson progress can be checked on www.eurowilson.org.

Conclusion

In a proportion of patients, the diagnosis of WD is difficult to establish. The development in the field of molecular genetics has addressed this issue, but, presence of several mutations and the fact that most patients are compound heterozygote means that the problem is not completely resolved. The outcome on medical therapy is very good but debate continues as to the agents of first choice between chelators and zinc. Liver transplantation is a cure for patients with decompensated liver disease but its effect on the neurological outcome is still not clear.

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