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WILSON'S DISEASE

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Abstract
Wilson's disease (WD) is a disorder of copper transport resulting from the defective function of a copper transporting P-type ATPase, ATP7B. The WD incidence is approximately 1/50–10,000 live births worldwide. Clinical manifestations of WD may be of any kind, but usually the symptoms of presentation are hepatic or neuropsychiatric, with a vast range of disturbances for both groups of symptoms. In children, however, clinical symptoms may be absent, making the diagnosis of the disease more difficult than in adults. Hepatic manifestations may range from asymptomatic minor biochemical disturbances, to acute, but mostly chronic, hepatitis, cirrhosis or severe fulminant hepatic failure. The spectrum of neurological manifestations is wide, including tremor, hypersalivation, Dysarthria, coordination defects, dystonia, ataxia. The spectrum of psychiatric manifestations is considerable and may include different disturbances such as altered working performance, anxiety, depression and antisocial behaviour. Kayser-Fleischer rings (KF) are present in 95% of patients with neurological symptoms and somewhat over half of those without neurological symptoms. In children presenting with liver disease, KF rings are usually absent. To obtain a more reliable diagnosis of WD, the Leipzig scoring system was proposed by an international consensus of experts. Wilson's disease copper overload is treated with chelating agents such as penicillamine, trientine and tetrathiomolybdate. Zinc is used mostly for mantainance therapy or the treatment of asymptomatic WD patients.

Key words: Wilson diseases, copper, cirrhosis, children.

Copper balance and pathogenesis of Wilson's disease

Copper is a trace element required for the survival of all organisms since it is an essential cofactor in many enzymatic pathways, in numerous cellular processes [1]. Thus copper deficiency decreases the activity of these enzymes and so adversely affects the corresponding physiological processes. Copper overload is also deleterious for cell metabolism since it can lead to cellular oxidative stress causing functional failure and cell death. Therefore, the level of copper in cells and tissue must be tightly regulated [1]. The importance of mantaining copper homeostasis is demonstrated by the existence of two well characterized hereditary disorders in humans, Menkes disease due to copper deficiency and Wilson's disease due to copper overload [1]. Wilson's disease (WD) is an autosomal recessive disorder of copper transport resulting from the defective function of a copper transporting P-type ATPase (ATP7B) [2]. Biosynthetic studies of WD protein indicate that it is synthesized as a single-chain polypeptide with a molecular mass of 195 KD and is localized to the trans-Golgi network of the cell [3]. In this location under low copper conditions, it transports copper into the secretory pathway for incorporation into ceruloplasmin. In elevated intracellular copper levels there is a trafficking

of WD protein to a cytoplasmic vesicular compartment adjacent to the canalicular membrane, or according to other authors to the plasma membrane [3]. As copper is transported to this compartment, the intracellular copper concentration falls and this protein is recycled back to the trans-Golgi network while copper is exported [3]. This copper-dependent trafficking of WD protein is rapid and represents a novel post-translational mechanism allowing for restoration of cellular copper homeostasis.

Epidemiology and clinical presentation

WD incidence is approximately 1/50–10,000 live births worldwide, while in some small populations such as the Sardinian, it is approximately 1/3,000 live births which is one of the highest worldwide [4, 5]. The gene frequency in the healthy population is about one in 90. Clinical manifestations of WD may be of any kind, but usually the symptoms of presentation are hepatic or neuropsychiatric, with a vast range of disturbances for both groups of symptoms (Table 1). In children, however, clinical symptoms may be absent, and typical features, such as the K-F rings, are rarely seen before the age of 7 years, making the diagnosis of the disease more difficult than in adults [6, 7].

Table 1

Signs and symptoms of Wilson's disease

Hepatic	Subclinical	Abnormal liver function test
	Acute	Mild, self-limiting hepatitis
		Acute/fulminant liver failure with/without haemolysis
	Chronic	Chronic active hepatitis with progressive fibrosis
		Liver cirrhosis
Neurologic	Movement	Tremor
	and coordination	Dysarthria,dysphagia
		Ataxia, dystonia, choreathetosis
		Rigidity, bradykinesia, hypomimia, parkinsonism
		Micro-/macrographia
	Other	Concentration and cognitive impairment
		Hypersalivation, drooling
		Epilepsy
Psychiatric	Generally	Personality change (aggressive behavior, impairment of emotional control etc.)
		Disorders of mood
		Sleeplessness, anxiety etc.
	Psychotic	Hallucinations,
		Catatonia
		Delusions
Other organs	Eyes	Sunflower cataract; Kayser-fleischer ring
	Fertility	Amenorrhoea, ovarian dysfunction, infertility, abortion
	Musculoskeletal	Stiffness, back pain, osteoarthritis, osteoporosis
	Kidneys	Tubular dysfuncion, aminoaciduria
	Heart	Cardiomyopathy, arrhythmias
	Haematologic	Anaemia, thrombocytopaenia
	Other	Gall stones, endocrine disturbances

Hepatic manifestations may range from asymptomatic minor biochemical disturbances to, but mostly chronic, hepatitis, cirrhosis or severe fulminant hepatic failure [6, 7].

The spectrum of neurological manifestations is wide, including tremor, hypersalivation, Dysarthria, coordination defects, dystonia and ataxia [6, 7]. The spectrum of psychiatric manifestations is considerable and may include different disturbances such as altered working performance, anxiety, depression and antisocial behaviour [6, 7]. Wilson's disease can manifest with an impressive spectrum of neurological, behavioural or psychiatric disorders, which may be its first clinical manifestation, appearing simultaneously with hepatic signs, or some years later.

The clinical hallmark of Wilson's disease is the Kayser-Fleischer ring, which is present in 95% of patients with neurological symptoms and somewhat over half of those without neurological symptoms [6, 7]. They are not entirely specific for Wilson's disease, since they may be found in patients with chronic cholestatic diseases including children with neonatal cholestasis.

Diagnosis

The diagnosis of WD may be made readily when the classic symptoms such as liver disease, neurological sign Kayser-Fleischer rings and some laboratory data such as low serum ceruloplasmin levels and elevated urinary copper excretion are present. However, since not all the symptoms are always present it is difficult to establish the diagnosis, expecially in children [6, 7]. No single test can confirm the diagnosis with 100 percent accuracy since all routine tests for WD diagnosis can give false positive and false negative results (Table 2). It is the combination of clinical and family history, physical examination, and certain key laboratory tests that collectively establish the diagnosis. Wilson's disease should be considered in cases of liver abnormalities of unknown origin and/or unexplained neurolgical symptoms particularly in young individuals [6, 7]. To obtain a more reliable diagnosis of WD, the Leipzig scoring system was proposed by an international consensus of experts (Table 3). The Wilson's disease scoring system provides a good diagnostic accuracy [8]. Molecular testing for *ATP7B* mutations is an important criterion of the Leipzig scoring system and has therefore become an important advance in the diagnostic armamentarium of clinicians, especially when routine testing is equivocal for WD.

Table 2

Routine tests for diagnosis of Wilson's disease

Test	Typical finding	False "negative"	False "positive"
Serum eruloplasmine	Decreased by 50% of lower normal value	Normal levels in patients with marked hepatic inflammation Overestimation by immunologic assay Pregnancy, estrogen therapy	Low levels in: – malabsorption – aceruloplasminemia – heterozygotes
24-hour urinary copper	> 1.6 µmol/24 h > 4 µmol/24 h in children	Normal: – incorrect collection – children without liver disease	Increased: – hepatocellular necrosis – cholestasis – contamination
Serum "free" copper	> 1.6 µmol/L	Normal if ceruloplasmin overestimated by immunologic assay	
Hepatic copper	> 4 µmol/g dry weight	Due to regional variation – in patients with active liver disease – in patients with regenerative nodules	Cholestatic syndromes
Kayser-Fleischer rings By slit lamp examination	Present	Absent – in up to 50% of patients with hepatic Wilson's disease – in most asymptomatic siblings	Primary biliary cirrhosis

Table 3

Scoring system developed at the 8th International Meeting on Wilson's disease, Leipzig 2001

Typical clinical symptoms and signs		Other tests	
KF rings		Liver copper (in the absence of cholestasis)	
Present	2	> 5 × ULN (> 4 µmol/g)	2
Absent	0	0.8–4 µmol/g	1
Neurologic symptoms**		Normal (< 0.8 µmol/g)	-1
Severe	2	Rhodanine-positive granules*	
Mild	1	Urinary copper (in the absence of acute hepatitis)	
Absent	0	Normal	0
Serum ceruloplasmin		1–2 × ULN	1
Normal (> 0.2 g/L)	0	> 2 × ULN	2
0.1–0.2 g/L	1	Normal, but > 5 × ULN after D-penicillamine	2
< 0.1 g/L	2	Mutation analysis	
Coombs-negative hemolytic anemia		On both chromosomes detected	4
Present	1	On 1 chromosomes detected	1
Absent	0	No mutations detected	0

TOTAL SCORE	Evaluation:
4 or more	Diagnosis established
3	Diagnosis possible, more tests needed
2 or less	Diagnosis very unlikely

* If no quantitative liver copper available, ** or typical abnormalities at brain magnetic resonance imaging. KF, Kayser-Fleischer; ULN, upper limit of normal

Treatment

All Wilson's disease patients – even in the presymptomatic stage – need lifelong drug therapy. In general pharmacological treatment alternatives include copper chelators and zinc salts. Essential for long-term success of pharmacological treatment for Wilson's disease is the patients' adherence [6, 7].

Currently only liver transplantation is able to cure the disease (hepatic form). However, transplantation is only mandatory in some special situations [1, 6, 7]. Wilson's disease copper overload is treated with chelating agents such as penicillamine, trientine and tetrathiomolymbdate. Zinc is used mostly for maintenance therapy or the treatment of asymptomatic WD patients. Zinc acts by inducing metallothionein production that reduces or blocks copper absorption in the intestine preventing the copper overload.

Molecular basis of Wilson's disease. The experience of a single center.

In recent years we have carried out mutational analysis of the ATP7B gene in approximately 700 Wilson's disease families with the presence of at least one affected member, originating mostly from Mediterranean countries (Sardinia, Italy, Turkey, Greece, Serbia, Portugal, Albania, Spain, Saudi Arabia, Slovenia and Columbia). We have characterized 87% of the analyzed chromosomes and identified 175 mutations, a few of which have been described by others in non-Mediterranean populations [5, 9–17]. There is a high allelic heterogeneity in WD with the presence of some relatively frequent and a large number of rare mutations. These data, in general, make difficult use of DNA analysis in the diagnosis of WD and also the genotype-phenotype correlation studies. We observed that for each analysed population there is a small number of mutations that are prevalent among the analysed chromosomes and a large number of rare mutations. In the majority of cases mutations reside in 12 exons of the ATP7B gene that code for Transmembrane regions and the large cytoplasmatic ATP loop. These data suggest that Wilson's disease in the Mediterranean population seems to result from a limited number of relatively common mutations and from a large number of rare ones and permit the development of specific strategies and methods for molecular diagnosis of Wilson's disease in each analysed population. In the Italian population we have analyzed 285 Wilson's disease families and detected 115 disease-causing mutations suggesting a high allelic heterogeneity for WD in this population. There are not frequent mutations in this population since the most common mutations account for 43% of the total. In the population of the Island of Sardinia the molecular analysis of 152 WD families permitted the characterization of 97% of the analyzed alleles and the identification of 24 different mutations [5, 13]. The six most common mutations in this population account for approximately 85% of the total, while one of them, -441/-427del, singularly accounts for 65% of the analysed chromosomes and that suggests the presence of a founder effect. Searching for the presence of the 15nt deletion in 5290 random Sardinian DNA samples we detected 122 heterozygotes, that means 3.8% of carrier frequency [5]. These data suggest that the search for mutation in the Sardinian population can be used not only for single diagnostic tests but probably also for a systematic mass screening.

Table 4

*Most frequent mutations detected in 285 Wilson's disease families of Italian origin**

Mutation	N° Chromosomes	Exon	Domain	%
p.H1069Q	85	14	SEHPL	14.8
c.2532delA	28	10	Tm4	4.9
p.R1319X	24	19	Tm8	4.2
p. G591D	24	5	Cu5	4.2
p.R969Q	22	13	Tm6	3.8
c.2304-2305insC	20	8	Tm4	3.5
p.G626A	11	6	Cu6	1.9
c.3648-3653del	11	17	Tm6	1.9
c.-441/-427del	11	5' UTR	Promoter	1.9
p.T977M	11	13	Tm5	1.9

*Not included families originating from Sardinia, see table 5.

Table 5

Mutations detected in 152 families of Sardinian origin

Mutation	N° Chromosomes	Exon	Domain	%
-441_427del	196	5'UTR	Promoter	64.9
p.V1146M	24	16	ATP loop	7.94
c.2463delC	22	10	Td	7.28
c.213-214delAT	7	2	Cu1	2.37
p.A1018V	6	13	ATPloop	1.98
p.R778W	6	8	Tm4	1.98
c.1512-1513insT	4	3	Cu5	1.32
p.G1000R	4	13	Ch/Tm6	1.32
p.H1069Q	4	14	SEHPL	1.32
c.2304-2305insC	3	8	Tm4	0.99
c.2035delC	2	7	Tm1-Tm2	0.66
p.G869R	2	11	Td	0.66
p.S921Q	2	12	Tm5	0.66
p.T993M	2	13	Ch/Tm6	0.66
c.1285+5G->T	1	2^{VI}	Cu4	0.33
c.2122-8 T->G	1	8	Tm3	0.33
p.I747F	1	8	Tm3	0.33
p.V890M	1	11	A-domain	0.33
p.R919W	1	12	Tm5	0.33
p.G943S	1	12	Tm5	0.33
p.L1043P	1	14	ATP loop	0.33
p.G1089V	1	15	ATP loop	0.33
p.R1151C	1	16	ATP loop	0.33
p.N1270S	1	18	ATP hinge	0.33
c.3852-3875del24	1	18	ATP hinge	0.33
Unknown	8	–	–	2.64

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Резиме

ВИЛСОНОВА БОЛЕСТ

Георгиос Лудијанис[1], Марија Барбара Лепори[2], Ева Мамели[2], Валентина Деси[2], Антониета Запу[2]

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Вилсонова болест (ВБ) е нарушување во транспортот на бакар, кое е резултат на дефектна функција на бакарниот транспортер P-type ATP-ase, ATP7B. Инциденцата на ВБ изнесува 1/50–10.000 раѓања. Клиничките манифестации на ВБ се најразновидни, но обично презентирачките симптоми се хепатални или невропсихијатриски, со широк дијапазон на симптоми. Меѓутоа, кај деца клиничките симптоми можат да бидат отсутни, правејќи ја дијагнозата многу потешка отколку кај адултите. Хепаталните манифестации рангираат од асимптомни минорни биохемиски нарушувања до акутен, но почесто хроничен хепатитис, цироза или тешка фулминантна хепатална инсуфициенција. Спектарот на невролошките нарушувања е широк и вклучува тремор, хиперсаливација, дизартрија, дефекти во координација, дистонија, атаксија. Спектрумот на психијатриски манифестации е значаен и вклучува различни нарушувања, како што се нарушена работна способност, анксиозност, депресија и асоцијално поведение. Kayser-Fleischerov прстен (KF) е присутен кај 95% пациенти со невролошки симптоми, а кај оние без невролошки симптоми во повеќе од половина. Кај деца што се презентираат со хепатална болест, KF е обично отсутен. За да се постигне сигурна дијагноза на ВБ, од страна на интернационален консензус на експерти е воведен Leipzig скоринг-систем. Оптоварувањето со бакар кај ВБ се третира со хелирачки агенси, како што се пеницилламин, триентин и тетратиомолибдат. Цинкот се користи најмногу како терапија на одржување или за третман на асимптоматски ВБ-пациенти.

Клучни зборови: Вилсонова болест, бакар, цироза, деца.