REVIEWS

To the centenary of N. V. Konovalov

Biological Functions of Ceruloplasmin and Their Deficiency Caused by Mutation in Genes Regulating Copper and Iron Metabolism

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Ceruloplasmin, a multicopper ferroxidase, is involved in iron and copper homeostasis and integrates these metabolic pathways. Impaired biosynthesis of ceruloplasmin caused by gene mutations disturbs iron metabolism with iron deposition in different organs, especially in the basal ganglia, and severe neuronal damage. Dysfunction of ATP7B, a copper-transporting ATPase leads to the development of Wilson's disease, *i.e.*, multiple abnormalities in copper metabolism associated with reduced synthesis of holoceruloplasmin and biliary copper excretion controlled by both proteins. The lowest content of serum ceruloplasmin is observed in the most grave early neurological form of Wilson's disease (according to N. V. Konovalov's classification), which confirms the important role of ceruloplasmin in the striatal metabolism of catecholamines.

Key Words: *ceruloplasmin; copper; iron; Wilson's disease*

Being discovered in 1948, ceruloplasmin (CP) was intensely studied since 1952, when considerable reduction of CP plasma concentration in patients with Wilson's disease (WD) was demonstrated. However, its biological functions was difficult to investigate. CP is a high-molecular-weight protein with single polypeptide chain. There are several isoforms of CP characterized by different tissue distribution and multiple cooperative interactions with copper and iron metabolism [2,11,12]. The new understanding of its biological functions came with recent insights into the structure and function of its gene, discovery of copper-transporting ATPases with their peculiar tissue-specific expression, and with clarification of metabolic abnormalities impairing functions of the brain and other organs.

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Molecular forms of ceruloplasmin

CP, a copper-containing ferroxidase (ferro:O_2-oxidoreductase, EC 1.16.3.1) binds over 95% total serum copper. CP molecule consists of 1046 amino acid residues, contains about 8% carbohydrate residues and 6-7 Cu atoms of all three types [12]. Spatial organization and catalytic properties of CP are determined by copper ions.

CP gene is located in chromosome 3θ25. Apart from the liver, CP mRNA was found in the cerebral cortex, cerebellum, hypothalamus, chorioid plexus,

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intestine, kidney, heart, splenic reticuloendothelial system, and bronchial epithelium [3,36,37]. Thus, CP provides copper binding and transport in many tissues.

The molecular weight of CP is 132±4 kD [11]. At the same time, SDS-PAAG electrophoresis demonstrated that human serum (from adults and newborns) contains three CP-immunoreactive polypeptides with apparent molecular weights of 200, 135, and 115 kD; their concentrations were proportional to total oxidase activity [49]. Lysates of hepatic HepG2 cells contained two types of newly synthesized polypeptides with molecular weights of 200 and 135 kD, which were secreted into the medium. It was hypothesized, that CP with a molecular weight of 200 kD is a dimer of the 135 kD-polypeptide, a product of posttranslational modification, and that secretion of CP with different molecular weights is physiologically significant.

Hepatocytes synthesize three CP isoforms: two secretory (serum CP and 200 kD CP) and one nonsecretory CP-like protein with a molecular weight of 50 kD [13-15]. Their synthesis is programmed by three mRNAs consisting of 4100, 3400, and 1800 nucleotides, respectively [18].

Serum CP isoforms with varying molecular weight were revealed in other studies [23,25]. SDS-electrophoresis and Western-blot analysis revealed two bands corresponding to 132 and 125 kD, respectively. CP with a molecular weight of 125 kD was found also in the bile.

The role of ceruloplasmin in iron homeostasis

The functional role of CP in mammals remains unclear. CP catalyzing redox processes oxidizes Fe^{2+} to Fe^{3+} providing a gradient for Fe^{2+} efflux [44]. The oxidized iron ions released from intracellular stores participate in the transferrin transport cycle and are transported into neurons, hepatocytes and developing reticulocytes [12,32].

Similar ferroxidase mechanisms determine activities of other multicopper oxidases such as *Fet3p*, a homologous yeast copper oxidase [26,46]. However, in contrast to CP, these enzymes are integrated into membranes. In this connection, Z. Harris *et al.* [33] pay special attention to the glycosylphosphatidylinositol-anchored form of CP. They also note that our current knowledge does not allow to conclude which form of CP (soluble or membrane-bound) possesses physiological activity.

The important role of CP in iron homeostasis was confirmed by the analysis of specific mutations in the CP gene associated with the complete absence of CP in the blood, aceruloplasminemia. This autosomal recessive genetic disorder is associated with iron deposition in the basal ganglia and retina [33,42] causing neurodegeneration.

It was shown that iron imbalance in mice with homoallelic CP gene mutation ($CP^{-/-}$) is due to reduced release of Fe^{2+} from intracellular stores [33]. At the initial stages of aceruloplasminemia the rate of iron release is sufficient for erythropoiesis, but gradual decrease in its serum concentration results in microcytic anemia followed by tissue damage and death. External administration of CP restores blood iron concentration.

Patients with aceruloplasminemia have an elevated content of LPO products in the cerebrospinal fluid and an increased iron concentration 3-fold exceeding the normal [41]. Their neurons, therefore, suffer from both the excess of reactive oxygen species produced by Fe^{2+}-overloaded glial cells and deficit of Fe^{3+} involved in the transferrin transport cycle [32]. Thus, the importance of CP for iron metabolism and redistribution in human body is of no doubts [31,42].

Involvement of ceruloplasmin in copper homeostasis

Homeostasis of copper as a vitally important but toxic trace element is strictly controlled by biological mechanisms. In contrast to other cations, copper balance is provided by the liver [48]. Some molecular mechanisms of hepatocyte homeostatic function in copper metabolism were clarified after discovery of ATP7B, a copper-transporting P-type ATPase, protein product of WD gene [60].

ATP7B performs the ATP-dependent selective transport of Cu^{2+} across the cell membrane. In the liver, this transporter is localized in hepatocytes being integrated into the membranes of the Golgi apparatus (its terminal compartments) and cytoplasmic vesicular compartments (the post-Golgi structures, located near or within the canalicular membranes) [51,52]. This localization determines the double transport function of ATP7B [26]: it transfers Cu^{2+} from cytosol to the trans-Golgi network participating in holo-CP biosynthesis and from the cytosol to bile participating in biliary copper excretion through post-Golgi vesicular compartments [43,56]. ATP7B translocations within the cell and its predominant function are determined by copper levels [51].

Irrespective of other functions of holo-CP, the binding of Cu^{2+} with the formation of active complex is an integral part of a copper homeostatic mechanism. Copper comes to the liver from the intestine with serum albumin and returns to the blood as a component of holo-CP [27,28]. Apo-CP incorporates copper during its biosynthesis in the trans-Golgi network of hepatocytes. The biosynthesis and secretion are indepen-

dent processes [28], and when CP is synthesized under conditions of copper deficit, unstable apo-CP, devoid of oxidase activity is secreted into blood.

CP can serve as a source of copper for other cells [60]. CP receptors which can promote copper accumulation by mammalian cells were revealed in the plasma membranes of erythrocytes, liver endothelium, cardiac cells, and lymphocytes [60]. High-affinity binding of CP was found in cells monkey kidney cultured bound CP was transferred to the cells and transformed into low-molecular peptides [14].

After CP binding to its receptors, Cu^{2+} ions are released, reduced, and enter the cell, while the CP protein remains outside. Apo-CP re-uptake is performed by hepatocytes: the half-life period of apo-CP in the blood is less than 4 h, and that of CP is 4.2 days [32].

Copper balance is regulated by hepatic ATP7B which participates in copper excretion from hepatocytes with both serum CP and bile [56]. CP seems to be involved in both these mechanisms, since bile contains a copper fraction packed into CP or CP-like (cross-immunoreactive) protein preventing copper reabsorption in the intestine [35]. This copper fraction (regulatory copper) is thought to be removed from the circulation with bile. However, considerable amount of Cu^{2+} secreted into the gastrointestinal tract with digestive juices, is reabsorbed and recycled [39]. Therefore, CP holds the central position in copper metabolic pathways.

The purified canalicular membranes of human liver contain protein with a molecular weight of 189-200 kD which can be either CP-like or CP-binding protein [22].This protein can serve as a membrane receptor or transporter for biliary copper/CP excretion. It was shown that bile contains CP with a molecular weight of 125 kD which can be a copper transporter [23,25].

Three CP isoforms were proposed for the role of billiary CP [13,18,59]. Two of them were revealed by affinity chromatography in the bile of healthy subjects [59]: one protein corresponds to 132 kD serum CP and possesses oxidase activity, another protein with a molecular weight of 80 kD shows no enzymatic activity. The latter is thought to be similar to the CP-like serum protein revealed in WD gene carriers [45]. The third isoform can be CP with a molecular weight of 200 kD synthesized in the liver [13,18].

Thus, CP binds and stores copper in a nontoxic form and can transport it to different tissues keeping the necessary concentration of copper ions. As a component of bile, CP can bind copper secreted by other tissue (regulatory fraction) preventing its reabsorption.

The role of CP as a tissue copper transporter is put into doubt by recent data on metabolic kinetics of CP protein and incorporated copper and especially by the absence of abnormalities in copper metabolism in patients with aceruloplasminemia [32]. Therefore, the hypothesis on the copper-transporting function of CP brought up in 1964 [2,12] still needs experimental support.

The role of ceruloplasmin in copper-iron metabolic interactions

ATP7B (its gene is located in chromosome 13q14.2) is classified to the integral membrane proteins-transporters, which transfer cations through the cell membranes of such diverse organisms as humans and bacteria. Humans have one more ATPase of this kind, ATP7A. Defects in its gene located in X-chromosome cause the development of Menkes disease which is characterized by reduced levels of ceruloplasmin and other copper-containing proteins. ATP7A and ATP7B are homologous by 65%, but have opposite distribution: ATP7A is expressed in most tissues except the liver, while ATP7B is abundantly expressed in the liver [21]. One of these ATPases is present in the trans-Golgi network of all cells [50,56].

The two ATPases have similar functions and regulate cell homeostasis by common biochemical mechanisms [16,50]. Conservation of these proteins during evolution suggests that copper transport from the placenta and gastrointestinal tract to the blood, from cerebral endothelium to the nervous tissue, and from hepatocytes to bile is provided by the same mechanism [32]. This mechanism operates also in prokaryotes which makes it possible to study the level of functional activity of the gene of interest (for instance, ATP7B gene in patients with (Wilson disease) by means of exogenous expression [26]. This approach allows to understand biological functions of CP in iron metabolism.

Interaction of copper and iron metabolic proteins *Fet3p* and *Ccc2p* in yeast *S. cerevisiae* is considered as a model of interactions between CP and copper-transporting ATPases. The *CCC2* gene of yeast is a homolog of ATP7B, and its protein product *Ccc2p* is involved in high-affinity iron uptake. Copper is transported though the plasma membrane by *Ctr1p* protein, then chaperone protein *Atx1p* translocates it to *Ccc2p*, which transfer copper through the membrane of the post-Golgi vesicular compartment to multicopper oxidase *Fet3p,* homologous to CP [26]. *Fet3p* together with *Ftr1p*, a high-affinity iron transporter, provide the inward transport of iron ions. When *Ccc2p* function is impaired by gene mutation, copper is not incorporated into *Fet3p* and yeast cells loose the ability to grow on iron-deficient medium. In this case, the introduction of normal ATP7B gene can compensate *Ccc2p* dysfunction and restore their growth. This ele-

gant functional assay makes it possible to distinguish the normal and pathological ATP7B variants without application of harmful reagents.

The analysis of cooperative effects of CP and ATP7B on iron metabolism in multicell organisms is a much more complex problem. Individuals with patological homoallelic mutations of ATP7B gene suffer from WD. Serum concentrations of CP and copper are dramatically reduced, which is accompanied by a significant decrease in iron ($p<0.001$) and transferrin concentrations ($p<0.02$) [7,55]. Transferrin saturation with iron ions remains normal (34.8% *vs.* 36.8% in healthy individuals). The low level of transferrin could be explained by hepatic dysfunction, however, similar decrease was found in lateral amyotrophic sclerosis, severe neurological disease, which is known to be associated with dysfunction of the copper-containing enzyme superoxide dismutase [4]. However, the total iron concentration in lateral amyotrophic sclerosis remains within the normal range, while transferrin saturation dramatically (by 45.1%) increases and CP oxidase activity surpasses the normal (Table 1). Therefore, it can be suggested that reduced ferroxidase activity of CP contributes to iron imbalance in patients with defective ATP7B genes.

The autopsy analysis of iron concentrations in the liver, basal ganglia, and cerebral cortex of a WD patient revealed no abnormalities, although the level of some other trace elements deviated from the normal [7]. At the same time, the liver of Long-Evans Cinnamon (LEC) rats, which are an authentic animal model of WD, accumulates considerable amount of iron and copper, which toxic effects accelerate and aggravate the course of hepatitis [53].

ATP7B dysfunction and serum ceruloplasmin

In addition to the facts discussed above, close functional relationships between the two copper-containing enyzmes, free-circulating CP and membrane-bound ATP7B, in copper balance regulation are evidenced by a number of other facts. Thus, the CP and sATP7B genes are expressed in a highly coordinated manner during fetal development in sheeps [40]. CP/ATP7B interactions are clearly manifested under conditions of copper imbalance caused by partial or complete dysfunction of ATP7B occurring in WD. Due to impaired copper transport from hepatocyte cytosol to extracellular space copper is accumulated in the liver, brain, and kidney. Toxic effects of copper cause hepatolenticular degeneration or hepatocerebral dystrophy by N. V. Konovalov [5,6,11,34,54]. Low concentrations of serum CP in WD patients can be revealed by measuring both oxidase activity and immunoreactive CP, but in some cases CP concentration can be normal [9,60]. CP deficiency in this disease directly depends on the primary genetic defect and manifests itself in the early childhood, long before the appearance of WD symptoms [21]. LEC rats [60] and mice with toxic milk [58], which are experimental models of WD also have very low concentrations of serum CP.

Impaired cholepoietic function of ATP7B plays a crucial role in WD pathogenesis. The process of bile production clearly reveals close relationships between ATP7B and CP: the deficit of biliary CP in WD patients was repeatedly reported [22,25,35]. A decrease in serum CP can result from impaired biosynthetic function of ATP7B. The liver of WD patients was found to contain a considerable amount of holo-CP [22]; however, it does not mean that its level meets all body demands.

CP characteristics in WD patients were studied in detail by S. A. Neifakh *et al.* [11,12]. Routine examination reveals only minor difference between CP from healthy individuals and WD patients. [10]. Thorough analysis shows that specific oxidase activities of serum CP in patients are slightly (no more than by 20%) lower than in healthy individuals. Considerable decrease was observed only in one of 10 examined patients.

It was reported that apart from normal CP, a CP-like protein differing from apo-CP by electrophoretic mobility is present in the serum of individuals with the defective ATP7B gene (both homo- and heterozygotes) [45]. This protein isolated by affinity chromatography had a molecular weight about 80 kD, showed no enzymatic activity, and differed from CP by immunological characteristics. The ratio of these molecular forms in homo- and heterozygotes for the WD gene is thought to reflect the proportion of mutant genes and can be a useful index in the diagnosis of WD and heterozygous carriage. However, according to our unpublished data this index is nonspecific: in our study the CP-like protein was revealed not only in the serum of all WD patients' relatives, but also in control patients.

In this study, we also determined CP oxidase activity and immunoreactivity and calculated CP specific activity as the ratio of oxidase activity to immunoreactive CP in WD patients and their relatives. CP specific activity in patients turned out to be lower than in their healthy heterozygous parents (about 65%, $p<0.02$). It directly correlated with the absolute value of CP oxidase activity ($r=0.689$ at $p<0.001$). In other words, reduction in the concentration of enzymatically active CP was accompanied by a proportional increase in the content of immunoreactive CP-like protein without enzymatic activity (Table 2).

Another immunoreactive CP-like protein with a molecular weight of 60 kD isolated from pooled se-

TABLE 1. Serum Concentrations of Transferrin (Measured by Iron-Binding Capacity), CP, and Trace Elements in Patients with Neurodegenerative Diseases (μg/100 ml, *M±m.*) (from [8] with Modifications)

Index	Control	WD	LAMS	GSD	HC	DMD	FD
CP	31.1±4.0 (33)	7.4±5.5* (178)	38.1±6.0* (23)	33.0±5.6 (7)	33.5±6.2 (21)	—	—
Copper	117±14 (65)	42±18* (44)	132±23** (33)	111±23 (7)	120.0±19.5 (23)	118±19 (39)	—
Transferrin	326±43 (27)	287±61*** (22)	262±69*** (12)	374±51 (5)	334±96 (10)	350±53 (5)	
Iron	120±37 (88)	100±30* (44)	118±41 (24)	105±29 (6)	105±31 (16)	113±26 (32)	132±63 (14)
Transferrin saturation with iron, %	36.8	34.8	45.1	28.2	30.1	35.5	37.7

Note. WD: Wilson's diseease; LAMS: lateral amyotrophic sclerosis; GSD: Gallerworden-Spatz disease; HC: Huntington's chorea; DMD: dystonia musculorum deformans; FD: Fridreich' disease. *$p<0.001$, **$p<0.01$, ***$p<0.02$ in comparison with the control. Here and in Tables 2 and 3 the number of patients is given in parentheses.

rum of WD patients also showed no ferroxidase activity but could bind to CP receptors on erythrocytes protecting them from copper-induced lysis [18].

Native PAAG-electrophoresis reveals high concentration of apo-CP in the blood of BW patients and LEC rats exceeding the concentration of holo-CP [38]. Since in patients with abnormal ATP7B apo-CP synthesis is not affected, this protein is secreted into the blood instead of holo-CP and react with CP antibodies. This fact additionally testifies to the abnormal copper incorporation with dysfunctional ATP7B. At the same time, no abnormalities were found in the transcription and translation of CP gene in the liver of LEC rats.

Inherited deficiency of CP functional activity can also take place. It was reported about two families of WD patients with half of normal CP specific activity [30]. Single case of sharply reduced oxidase activity reported by S. A. Neifakh *et al.* [11] can also be explained by hereditary factors. Especially high deficit of CP function at its moderate decrease in one of the parents was revealed in a patient with early and severe WD, one of the three WD forms determined by different alleles of the ATP7B gene [24].

Backcross of LEC rats with the analysis of cosegregation of traits in the offsping was performed to investigate the correlation between the dysfunctional *Atp7b* gene and abnormal cooper incorporation into CP with resulting predominance of apo-CP. Apo-CP turned out to be a predominant isoform in 8 out of 11 rats with the pair of defective *Atp7b* and in none of 19 normal rats [38].

In LEC rats, the *in vivo* synthesis of holo-CP was recovered after infusion of recombinant adenovirus carrying ATP7B cDNA. After transgenic expression, ATP7B was located to the trans-Golgi network, blood plasma showed the presence of holo-CP, in liver lysosomes and bile the content of copper increased which indicates that ATP7B participates in biliary copper excretion [57].

Despite tissue accumulation of copper, some WD patients show no decrease in the blood concentration of CP. According to our data, the normal level of CP was observed only in 2.98% of 335 examined patients occurring more frequently at the preneurological stage of the disease (8.82%) and less frequently after the appearance of neurological symptoms (2.33%) [9]. WD patients and control patients with other inherited diseases revealed similar variability of individual CP concentrations with their similar distributions (Fig. 1), but these distributions overlapped only in the region of abnormally high CP concentrations in WD patients and abnormally low CP concentrations in the control group.

Considerable variations in CP concentrations can be explained by differences in the type and extent of

APT7B dysfunction after gene mutation. For instance, ATP7B loosing the ability to translocate from the trans-Goldgi network to vesicular compartments with the increase in copper concentration cannot participate in biliary copper excretion, but retains its function in holo-CP synthesis [26]. It is suggested that normal CP concentrations in some patients can be attributed to a specific stage of the disease characterized by simultaneous increase in both synthesis and secretion of apo-CP as an acute phase protein [32]. The studies on LEC rats indicate that in extrahepatic tissues the Menkes ATPase (ATP7A) can catalyze copper incorporation into apo-CP. This mechanism could be responsible for normal level of holo-CP in patients with dysfunctional ATP7B.

A variety of ATP7B gene mutations, their location in different DNA sites, and great lenght of ATP7B mRNA limit current possibilities to reveal the disease at its preclinical stage. Until the development of the efficient methods of DNA analysis and WD gene screening, the measurements of CP enzyme activity and other biochemical and clinical indices remain the only methods of the diagnosis of this disease [20]. Some new approaches were proposed for the measurement of enzymatically active CP: detection of holo-CP with monospecific antibodies [61] and detection of its isoforms [23].

No clear correlation was found between a diverse manifestations of WD and specific ATP7B mutations, which suggests that some extragenetic or environmental factors can significantly contribute to its pathogenesis [20,26]. It remains unknown, in particular, whether the cerebral accumulation of copper is specific for WD. Usually, it is considered to be secondary with respect to copper accumulation in the liver. The interaction between the ATP7B and CP genes during the development of pathological process also remains unclear. Clarification of this question would allow to disclose the relationships between the decrease in CP concentration and clinical types of the disease or its major neurological forms according to N. V. Konovalov. These forms are distinguished by specific cerebral localization of the pathology and the rate and mechanisms of its progressive development.

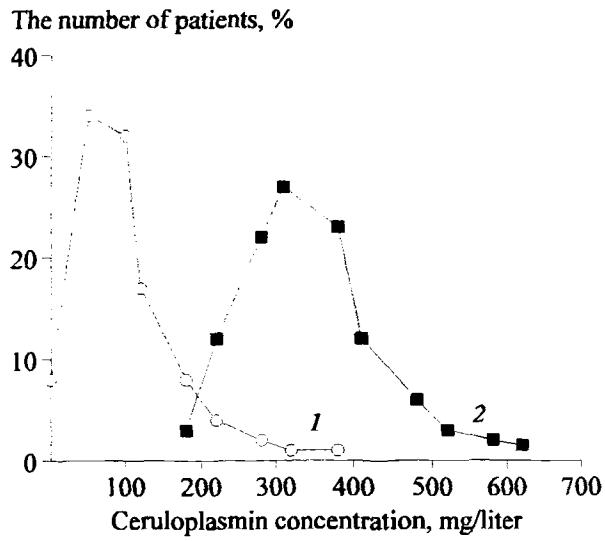

Fig.1. Distribution of CP concentrations in 335 patients with Wilson's disease *(1)* and 827 patients *(2)* with inherited extrapyramidal and cerebellar disorders without copper imbalance (from [10] with modifications).

It is suggested that different neurological forms of WD are genetically determined and therefore associated with diverse independent mutations of the ATP7B gene. This suggestion does not exclude, however, some heterogeneity of the early form of WD [19].

The extent of CP deficit in different neurological forms of WD has not been compared yet. It was shown, however, that the lowest blood levels of copper are typical of the early and very severe (rigid-arhythmo-hyperkinetic) form of the disease [10], which is usually diagnosed in a juvenile period, when the serum concentration of CP is minimal both in norm and under conditions of ATP7B dysfunction [9]. Our analysis of factors affecting blood level of CP in WD patients (the stage of WD, age and gender of patients) confirmed that this form is characterized by a more pronounced decrease in serum CP than other forms and the pre-neurological stage of WD served as the control (Table 3). This conclusion is based on a large biochemical

TABLE 2. Specific Activity of CP as a Function of Serum Concentration of Enzymatically Active and Immunoreactive CP in WD Patients *(M±m)*

Index, % of healthy parent value	Concentration of CP with enzymatic activity, mg/liter				
	7.0-24.9 (*n*=23)	24.0-74.9 (*n*=14)	75.0-149.0 (*n*=7)	150.0-329.0 (*n*=11)	total: 75.3±10.6 (*n*=55)
Oxidase activity	6.2±0.5	19.4±2.0	36.3±4.2	73.2±7.5	26.8±3.8
Immunoreactivity	21.3±1.7	33.7±3.4	40.0±3.3	72.8±7.6	37.2±3.2
Specific activity	33.8±3.8*	63.4±8.0**	92.6±11.6	104.7±8.2	63.0±5.1

Note. *$p<0.01$, **$p<0.05$ compared to the group with the highest CP concentration

TABLE 3. Serum Concentration of CP with Enzyme Activity (mg/liter) in Patients with Different Neurological Forms of WD *(M±m)*

WD form, age (years)		Men	Women	Total
Early form (rigid-arhythmohyperkinetic)				
	before 9	—	109.4 (1)	109.4 (1)
	10-14	65.8±21.1 (7)	41.8±16.4 (5)	55.7±13.9 (12)
	15-19	21.0±5.7 (18)	61.4±14.0^{++} (16)	40.0±7.9 (34)
	20-24	15.9±5.4 (11)	49.8±17.3 (9)	31.1±9.0 (20)
	average 16.9±0.5	30.7±6.3 (37)	56.4±9.2^{+++} (31)	42.4±5.6 (68)
Tremor-rigid form	15-19	43.9±4.7^{++} (5)	87.2±13.5$^{\infty}$ (9)	71.7±10.4^{+++} (14)
	20-24	78.1±13.5^{+} (18)	76.2±10.0 (9)	77.5±9.5^{+} (27)
	25-29	89.9±20.0 (7)	85.5±15.6 (13)	87.1±12.0 (20)
	30 and older	49.9±11.2 (4)	145.2±19.4^{x} (7)	111.4±19.3 (11)
	average 23.5±0.7	72.2±8.6 (34)*	94.9±8.3** (38)	84.2±6.1* (72)
Tremor form	10-14	31.5±10.5 (2)	—	31.5±10.5 (2)
	15-19	87.2±47.9 (3)	39.1±12.6 (6)	55.2±18.0 (9)
	20-24	46.2±9.0^{+++} (12)	90.4±13.3$^{\circ\circ xx}$ (20)	73.8±9.6^{++} (32)
	25-29	*68.5±10.4 (27)*	*126.0±18.0$^{\circ x}$ (15)*	*89.1±10.1 (42)*
	30 and older	83.5±11.0 (30)	115.3±18.5$^{\circ\circ}$ (17)	95.0±9.8 (47)
	average 27.1±0.6	70.7±6.4* (74)	101.6±9.4**^{x} (58)	84.3±5.5* (132)
Control (preneurological stage)	до 9	69.0±13.3 (3)	24.7±4.7 (2)	51.3±16.8 (5)
	10-14	72.5±45.2 (3)	93.3±48.5 (5)	85.8±36.8 (8)
	15-19	94.2±25.7^{+++} (6)	90.4±26.3 (8)	92.0±17.9^{+++} (14)
	20-24	71.8±34.3 (3)	139.5±69.5 (2)	98.8±33.3 (5)
	25-29	—	149.0 (1)	149.0 (1)
	30 and older	—	64.0 (1)	64.0 (1)
	average 15.7±1.0	80.3±14.3** (15)	91.1±18.2 (19)	86.3±11.9** (34)

Note. *$p<0.001$, $p<0.01$ in comparison with the early form group of the same gender; $^{+}p<0.001$, $^{++}p<0.01$, $^{+++}p<0.05$ in comparison with the early form group of the same age; $^{\circ}p<0.001$, $^{\circ\circ}p<0.02$ in comparison with the same form group at the age of 15-19, $^{x}p<0.01$, $^{xx}p<0.02$, $^{xxx}p<0.05$ in comparison with men.

material collected from 272 WD patients with neurological symptoms [9].

Thus, the most severe among the neurological forms of WD is accompanied by the most considerable reduction of serum CP and its development can be determined by a specific allele of the ATP7B gene. These data indirectly support the hypothesis on the genetic origin of WD neurological forms [19] and suggest that cerebral pathology associated with the dysfunction of ATP7B is not the secondary process. The correlation between the level of CP deficit and neurological manifestations of WD confirms the data on close interactions between ATP7B and CP in the pathogenesis of this disease and the important role of CP in the metabolism of neurotransmitters in the basal ganglia.

CP gene expression in astrocytes is an important factor for CNS iron and copper homeostasis and the survival of basal ganglia and retinal neurons [29,36]. Normally, the basal ganglia contain relatively high concentrations of iron, copper [8], and catecholamines. Dopaminergic neurons in the basal ganglia are concentrated in the substantia nigra. It is known that neuromelanin-rich neurons are less resistant to reactive oxygen species than neuromelanin-poor cells [1]. In this connection, the high CP gene expression in the specific population of glial cells located near brain microvessels surrounding the dopaminergic neuromelanin-containing neurons of the substantia nigra (as shown by *in situ* hybridization with CP cDNA) is of special importance [36]. CP with its high oxidase activity with respect to different compounds can protect neurons in this region from oxygen radicals, promote redistribution of Fe ions, and participate in the metabolism of catecholamines. Disturbances in this complex system in aceruloplasminemia lead to severe neurodegeneration and neurological disorders not characteristic of other inherited or acquired abnormalities of iron metabolism [29,36].

In contrast to the hepatic tissue containing the whole-size ATP7B, the cerebral tissue contains its shorter variant resulting from alternative splicing. The brain ATP7B in not a membrane-bound, but water-soluble protein floating free in the cytosol [62]. Therefore, it should have different functions in copper transport compared to the hepatic ATP7B. In the brain, ATP7B was revealed the hippocampus, olfactory bulbs, cerebellum, cerebral cortex, and brainstem nuclei [47], where it probably participates in the synthesis of CP or regulation of its functions. Copper deposition in the brain caused by ATP7B gene mutations primarily damages the neurons of the basal ganglia. Since this structure expresses a high level of CP, it can be suggested that dysfunction of this protein determines the neurological manifestations associated with the defective ATP7B gene, which are very similar to the symptoms of aceruloplasminemia.

CP still remains an enigmatic protein [12]. Despite its important role in tissue copper transfer is sufficiently obvious, there are some arguments against this function: aceruloplasminemia (genetically determined absence of serum CP) is not always accompanied by changes in copper metabolism, but is characterized by severe disturbances in iron homeostasis damaging CNS functions. It can be concluded, that the reduced activity of blood CP is important for WD diagnosis, although not directly results from WD gene mutations.

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