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Aceruloplasminemia, an inherited disorder of iron metabolism

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Abstract

Ceruloplasmin, a multi-copper ferroxidase that affects the distribution of tissue iron, has antioxidant effects through the oxidation of ferrous iron to ferric iron. Aceruloplasminemia is an inherited disorder of iron metabolism due to the complete lack of ceruloplasmin ferroxidase activity caused by mutations in the ceruloplasmin gene. It is characterized by iron accumulation in the brain as well as visceral organs. Clinically, the disease consists of the triad of retinal degeneration, diabetes mellitus, and neurological disease, which include ataxia, involuntary movements, and dementia. These symptoms reflect the sites of iron deposition. The unique involvement of the central nervous system distinguishes aceruloplasminemia from other inherited and acquired iron storage disorders. Twenty-one mutations in the ceruloplasmin gene have been reported in 24 families worldwide. In Japan, the incidence was estimated to be approximately one per 2,000,000 in the case of non-consanguineous marriages. Excess iron functions as a potent catalyst of biologic oxidation. Previously we showed that an increased iron concentration is associated with increased levels of lipid peroxidation in the serum, cerebrospinal fluid, and erythrocyte membranes. The levels of malondialdehyde and 4-hydroxynonenals, indicators of lipid peroxidation, were also elevated in the basal ganglia and cerebral cortex. Positron emission tomography showed diminished brain metabolism of glucose and oxygen. Enzyme activities in the mitochondrial respiratory chain of the basal ganglia were reduced to approximate 45% and 42%, respectively, for complexes I and IV. These findings suggest that iron-mediated free radicals causes neuronal cell damage through lipid peroxidation and mitochondrial dysfunction in aceruloplasminemia brains.

Introduction

Ceruloplasmin is a blue copper oxidase that carries more than 95% of the plasma copper content in vertebrates. It is an α2-glycoprotein which is synthesized mainly in the liver (Frieden 1986). This protein plays an important role in iron mobilization from the tissues as a ferroxidase (Osaki *et al.*. 1966). A trinuclear copper cluster in the carboxyl-terminal domain is essential for ferroxidase activity. Ceruloplasmin is a key enzyme in copper and iron homeostasis.

Ceruloplasmin deficiency is a characteristic feature in copper metabolic disorders, including Wilson's disease and Menkes' disease. In Wilson's disease, an inability to transfer copper into the ceruloplasmin precursor protein, apoceruloplasmin, and a decrease in biliary copper excretion results in serum ceruloplasmin deficiency and excess copper accumulation. In Menkes' disease, copper absorption from the intestine is decreased, leading to copper and ceruloplasmin deficiencies in the body (Figure 1). In contrast, aceruloplasminemia, originally called familial apoceruloplasmin deficiency (Miyajima *et al.* 1987), is an iron metabolic disorder where ceruloplasmin deficiency is caused by a lack of apoceruloplasmin biosynthesis and copper metabolism is not disturbed (Miyajima *et al.* 1987) (Figure 1). This disease is characterized by marked iron accumulation in the brain as well as visceral tissues despite low serum iron levels (Logan *et al*. 1994; Morita *et al.* 1995; Miyajima *et al.* 1996a). These findings distinguish aceruloplasminemia clearly from hereditary haemochromatosis, the

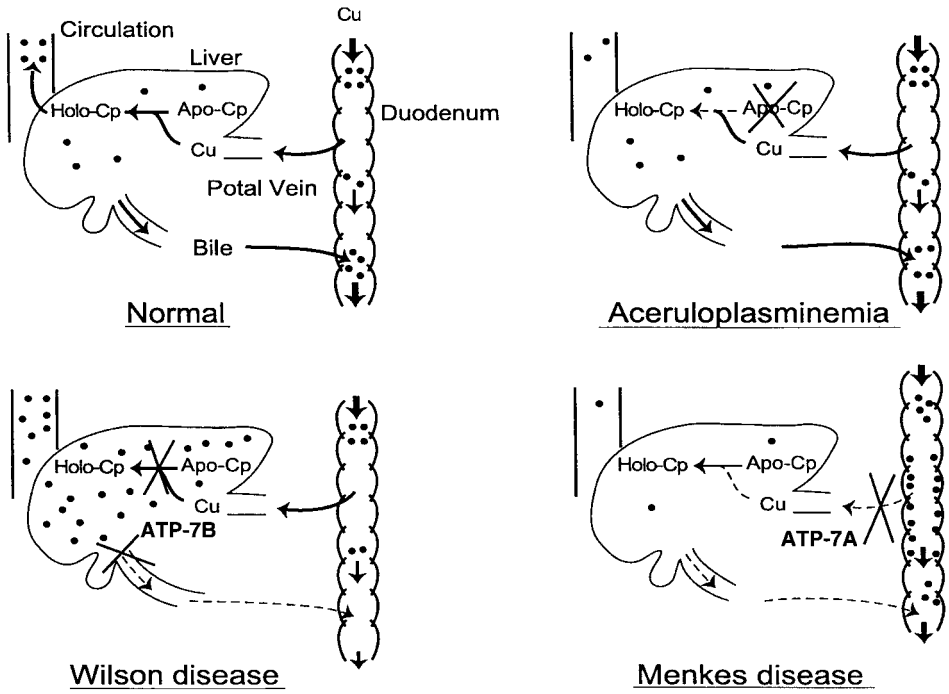


Fig. 1. Ceruloplasmin biosynthesis in the liver and copper absorption and excretion from and into the intestine. Primarily the liver synthesizes ceruloplasmin (Cp), and the six copper ions (• Cu) incorporated into apoceruloplasmin (Apo-Cp), a precursor protein of ceruloplasmin, during biosynthesis are mandatory for ferroxidase activity of the holoprotein (Holo-Cp). Wilson's disease and Menkes' disease are inherited disorders of copper metabolism resulting from the absence or dysfunction (X) of homologous copper-transporting ATPases (ATP-7B and ATP-7A). Striking differences in the clinical presentation of these diseases are entirely the result of the tissue-specific expression of each protein. The Wilson's disease ATPase (ATP-7B) is expressed in the liver and transports copper into the hepatocyte secretory pathway for subsequent incorporation into ceruloplasmin and excretion into bile. The Menkes' disease protein (ATP-7A) transports copper across the gasterointestinal tract, the placenta, and the blood-brain barrier. Ceruloplasmin deficiency is a characteristic feature in Wilson's disease and Menkes' disease. In aceruloplasminemia, ceruloplasmin deficiency is caused by a lack of apoceruloplasmin biosynthesis due to mutations in the ceruloplasmin gene or abnormal apoprotein that is unable to incorporate copper during biosynthesis.

most common iron metabolic disorder. Mutations in the causative gene, *HFE*, are responsible for most cases of hereditary haemochromatosis (Feder *et al.* 1996). In Wilson's disease and Menkes' disease, several gene mutations in the copper-transporting ATPases (ATP-7B and ATP-7A) have been found within the last eight years (Bull *et al.* 1993; Petrukhin *et al.* 1993; Tanzi *et al.* 1993; Yamaguchi *et al.* 1993; Chelly *et al.* 1993; Mercer *et al.* 1993; Vulpe *et al.* 1993). Aceruloplasminemia is characterized by mutations in the ceruloplasmin gene itself (Yoshida *et al.* 1995; Harris *et al.* 1995).

Clinical manifestations of aceruloplasminemia are the triad of retinal degeneration, diabetes mellitus (DM), and neurological symptoms, which include ataxia, involuntary movements, and dementia, reflect the sites of iron deposition (Miyajima *et al.* 1987; Logan *et al.* 1994; Morita *et al.* 1995; Miyajima *et al.*. 1996a). The actual pathogenesis of aceruloplasminemia is not yet clear, but iron-mediated, free-radical stress is speculated to contribute to tissue injury and neuronal cell death. We earlier reported a marked increase in lipid peroxidation in the plasma (Miyajima *et al.* 1996b), cerebrospinal fluid (Miyajima *et al.* 1998a), and erythrocyte membranes (Miyajima *et al.* 1998b) of patients with aceruloplasminemia. Ceruloplasmin is expressed in the central nervous system as well as in visceral organs and functions in brain iron metabolism. It plays an essential role for neuronal survival in the central nervous system (Klomp & Gitlin 1996). We hypothesize that oxidative stress caused by the accumulation of iron causes neuronal cell damage through lipid peroxidation and mitochondrial dysfunction in aceruloplasminemia and have examined brains of affected patients at autopsy for iron contents and activities of the mitochondrial respiratory chain as well as the levels of malondialdehyde (MDA)

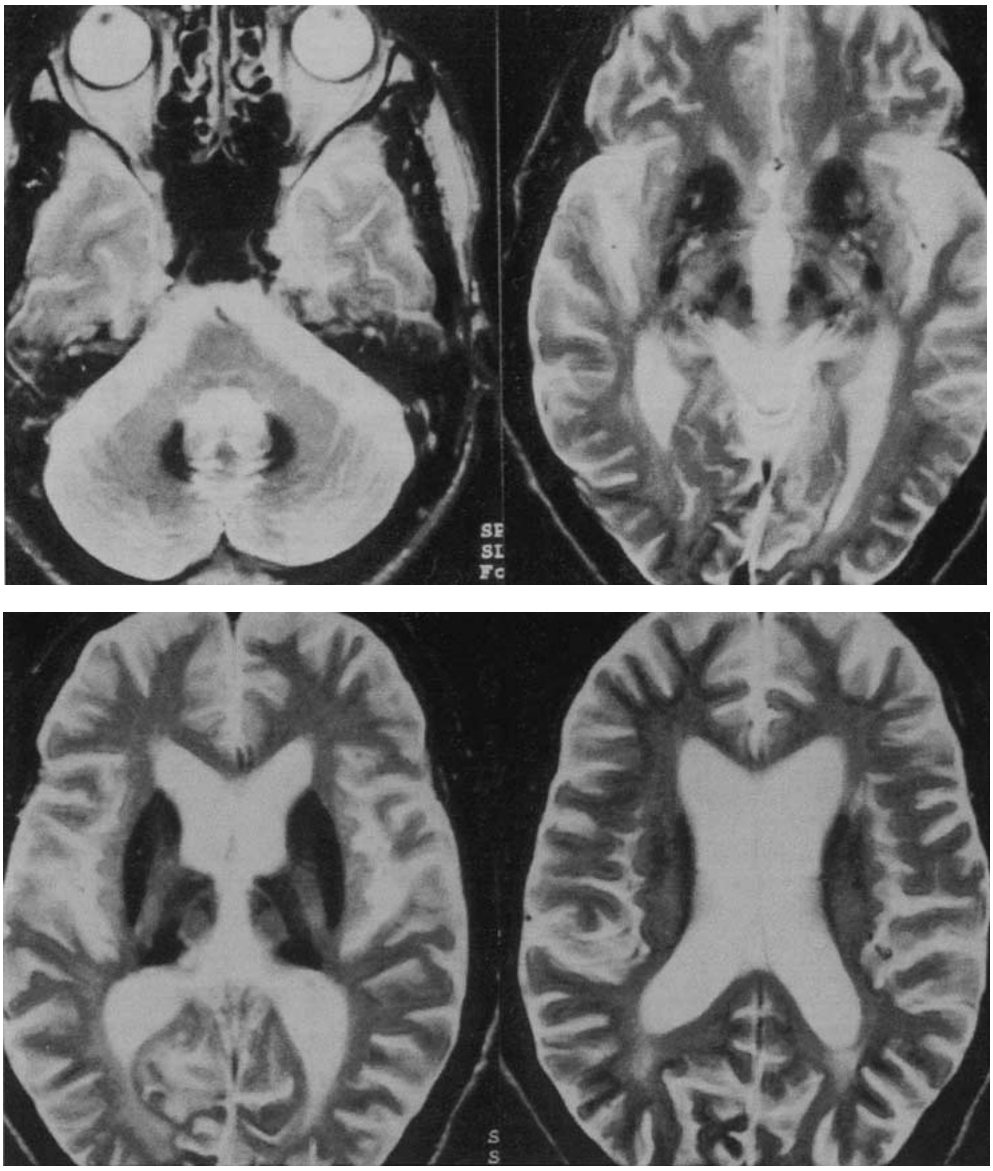


Fig. 2. Brain magnetic resonance images of patient 1 at age 65. T2-weighted magnetic resonance images (1.5 Tesla, TR/TE 2,500/80) reveals an increased iron content in basal ganglia, thalamus, and dentate nucleus.

and 4-hydroxynonenals (4-HNE), indicators of lipid peroxidation.

Patients

Patient 1, whose case originally was reported in 1987 (Miyajima *et al.* 1987), was a 66-year-old woman who suffered blepharospasm and retinal degeneration for the last 15 years of her life. She gradually had developed extrapyramidal symptoms of grimacing and rigidity, as well as ataxia. From age 59, she had suffered from DM. Her visual acuity and color vision were not disturbed. Several small yellowish opacities were scattered over the grayish atrophy of the retinal pigment epithelium. Fluorescein angiography demonstrated window defects corresponding to the yellowish opacities. Neurological examination at age 65 found bilateral blepharospasm synchronized with perioral spasm and neck dystonia. Her speech was scanning and gait slightly ataxic. Deep reflexes in the extremities were increased. There was no sen-

sory disturbance. Serum iron levels were decreased even though serum ferritin, a marker of iron deposits, was increased. The amount of iron on serum ferritin had increased with age along with the hepatic iron concentration. Treatment with the iron chelator desferrioxamine decreased serum ferritin levels as well as brain and liver iron stores, prevented progression of the neurological symptoms, and reduced plasma lipid peroxidation (Miyajima *et al.* 1997). Serum transferrin and the total iron-binding capacity were within the normal ranges. The liver had low signal intensities on both the T1- and T2-weighted magnetic resonance (MR) images. The insulin response to oral glucose loading was decreased, and islet cell antibodies were negative. Her full-scale IQ was 80; verbal IQ, 82; and performance IQ, 76 on the Wechsler Adult Intelligence Scale (WAIS). Mini mental state examination (MMSE) score was 25. Abnormal low intensities in the striatum, thalamus, and dentate nucleus on both T1- and T2- weighted MR images were indicative of iron accumulation (Figure 2). Positron emission tomography (PET) images showed a marked decrease in oxygen ($CMRO_2$) and glucose (CMRGlc) metabolisms in the basal ganglia and thalamus as well as cerebral and cerebellar cortices (Figure 3). The levels of CMGlc and $CMRO_2$ in the basal ganglia and thalamus of the patient were markedly decreased about 48% of the control values, and those in the cerebral and cerebellar cortices were reduced to 60% of the control values. Cerebral blood flow values did not differ from the control values. Reductions in glucose and oxygen metabolism along with preservation of perfusion were found diffusely throughout the brain. Most critically, there was mutation with a 5-base insertion at amino acid 410 in the ceruloplasmin gene, resulting in a frame-shift mutation and premature termination (Harris *et al.* 1995). She died from pancreatic cancer at age 66.

Patient 2. A 67-year-old woman, a younger sister of patient 1, who was asymptomatic except for retinal degeneration at the original presentation despite undetectable ceruloplasmin, had suffered from DM for the past 16 years (Miyajima *et al.* 1996a). Insulin therapy was begun at age 53. She developed blepharospasm and gait ataxia from age 56. Her MMSE score was 29. Cerebellar testing detected scanning speech, as well as gait ataxia, but she could walk unassisted. She had no motor deficits or sensory loss. She showed the absence of serum ceruloplasmin in association with mild anemia, low serum iron, and elevated serum ferritin. There also was no biochemical evidence of hepatic injury. MRI studies showed abnormally low intensities in the striatum, thalamus, dentate nucleus, and liver in both the T1- and T2-weighted images. She died from pneumonia at age 67.

Patient 3. A 60-year-old man had had DM and insulin therapy for 25 years and scanning speech and forgetfulness from age 56 (Okamoto *et al.* 1996). He had retinal degeneration and bilateral hearing disturbance. Facial grimacing and choreic involuntary movements of the upper extremities occurred during speech or voluntary movement of the extremities. Both his trunk and extremities showed cerebellar ataxia, and his gait was ataxic. His full-scale IQ was 68; verbal IQ, 70 and performance IQ, 62 on the WAIS. MMSE score was 15. His laboratory findings were more severe than those of patients 1 and 2. Serum ceruloplasmin was absent because of a mutation of the ceruloplasmin gene, the insertion of adenine in exon 3 producing a premature stop codon. He had had liver dysfunction from age 57 and died from cardiac failure at age 60.

Methods

Brains were obtained at autopsy within 6 h of death from the three patients with aceruloplasminemia and from six control subjects, mean age 63 years (three men and three women, 60–67 years old). The control subjects had arteriosclerosis and asymptomatic cerebral lacunae infarctions, and the cause of their deaths was acute myocardial infarction. The basal ganglia, thalamus, cerebral and cerebellar cortices were separated from the brain less than 1 h after autopsy. Samples of each brain area were cut immediately into small sections that then were frozen at -70 °C for biochemical examination.

Iron and copper concentrations

Brain tissue was dried in a microwave digestion unit (MLS-1200 MEGA, Milestone General) until a constant weight (10–15 mg) was obtained. The dried powder was weighed, and 0.7-ml samples of the ashed solutions were prepared with 0.1 N HCl. Sample solutions were analyzed directly in a flame atomic absorption spectrophotometer (Hitachi Z-6100) as described previously (Miyajima *et al.* 2001). Data was calculated on a dry weight basis because tissue density was not uniform in all the brain areas.

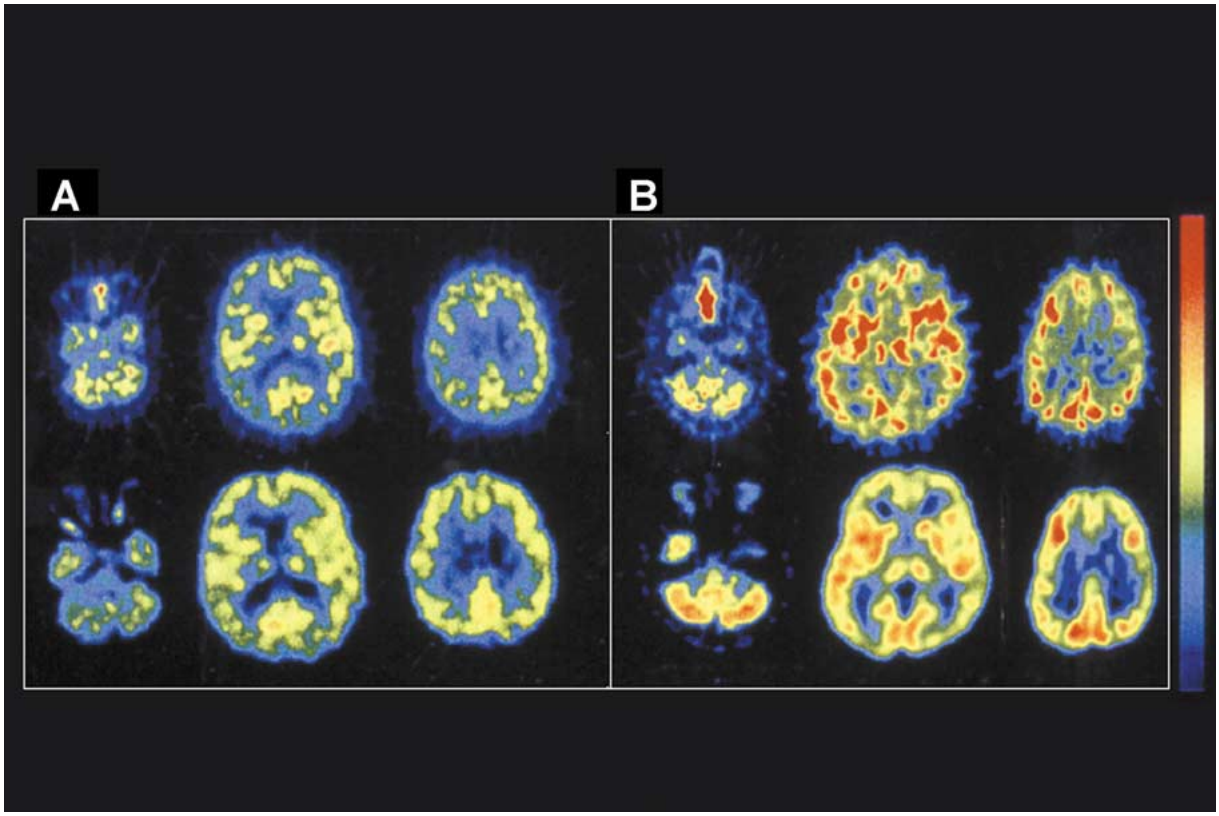


Fig. 3. Positron emission tomography images of oxygen and glucose metabolisms. The upper row indicates brain oxygen metabolism ($CMRO_2$), and the bottom row indicates brain glucose metabolism (CMRGlc). The panel A indicates the patient 1 at age 65, while the panel B indicates a 63-year-old healthy volunteer. Left lane, cerebellum level; center lane, basal ganglia level; right lane, level of centrum semiovale. Positron emission tomography images showed a marked decrease in CMGlc and $CMRO_2$ in the basal ganglia and thalamus as well as cerebral and cerebellar cortices of the aceruloplasminemia patient.

Mitochondrial enzyme assay

Approximately 200 mg of the brain tissue in the frontal cortex and basal ganglia, striatum, was taken from each of the patients and 6 controls. Each brain sample was removed from liquid nitrogen storage, weighed, and homogenized in a glass homogenizer in 9 volumes of ice-cold medium (32 mM sucrose, 1 mM EDTA, 10 mM Tris, pH 7.4). After three cycles of freezing and thawing, mitochondrial enzymes were assayed. NADH-ubiquinone oxidoreductase (complex I), succinate cytchrome c reductase (complex II + III) and cytochrome c oxidase (complex IV) were assayed by the method of Bowling and associates (Bowling *et al.* 1993).

Lipid peroxidation products

The levels of MDA+4-HNE were measured with a BIOXYTECH LPO-586^{TM} (OXIS International, Inc., Portland, USA). Briefly, pieces of frontal cortex and putamen were homogenized in 10 ml of 20 mM Tris buffer (pH 7.4) containing 5 mM butylated hydroxytoluene to prevent sample oxidation. After large particles were removed from the homogenate by centrifugation, the samples were subjected to the LPO-586 assay as described previously (Yoshida *et al.* 2000).

Results

Iron concentrations in the aceruloplasminemia patients were markedly increased in the brain and visceral organs as compared with the control subjects (Table 1). The distribution in order of iron level in both the aceruloplasminemia and control brains was globus pallidus > putamen > cerebral cortex, cerebellar cortex. Iron contents were markedly elevated in the basal ganglia of the aceruloplasminemia patients. The content in the livers was greater than in the brain.

Table 1. Comparison of Fe and Cu concentrations in eight regions in aceruloplasminemia patients 1, 2, and 3 and six control subjects.

	Iron				Copper			
Patient	1	2	3	Controls	1	2	3	Controls
Brain								
Putamen	3,868	2,240	5,607	653±64	30.5	31.7	33.0	25.2±4.3
Globus pallidus	4,372	3,486	7,207	735±54	33.2	34.6	43.2	24.1±2.2
Thalamus	2,430	1,785	3,363	394±36	29.0	29.4	33.5	27.3±2.2
Hippocampus	1,278	798	2,337	272±41	37.7	35.2	44.1	34.5±3.1
Cerebellar cortex	1,670	1,063	3,085	218±38	45.9	40.8	41.4	33.7±3.6
Liver	8,032	6,845	17,790	761±90	36.3	35.2	35.2	30.3±4.0
Pancreas	1,365	1,188	3,571	108±42	7.9	8.3	9.8	6.3±1.0
Heart	1,733	2,286	3,134	338±40	18.9	19.5	22.6	17.0±1.8

Results are expressed as means ± S.E. in μg g dry weight.

Table 2. Mitochondrial respiratory chain activities in the brain of aceruloplasminemia patients and control subjects ($n = 6$).

	Frontal cortex				Striatum			
Patient	1	2	3	Controls	1	2	3	Controls
Complex I	26.5*	29.6*	25.3*	42.8 ± 9.0	17.2*	23.0*	15.3*	38.0 ± 8.2
Complex II + III	196.5	204.3	180.8	225.4 ± 42.2	142.7	154.8	138.9	173.6 ± 30.5
Complex IV	345.2*	361.7*	320.0*	495.6 ± 63.6	183.5*	198.5*	170.6*	452.2 ± 65.8

Results are expressed as means ± S.D. in nmol/min/mg protein for mitochondrial enzyme activities. Complex I represents NADH-ubiquinone oxidoreductase; complex II+III, succinate cytochrome c reductase; complex IV, cytochrome c oxidase. Significance was determined by the Mann-Whitney U-test (* $P < 0.05$).

Copper levels, unlike iron, were evenly elevated in all the regions examined in the patients with aceruloplasminemia, suggesting that ceruloplasmin is not essential for copper transport and distribution.

Table 2 shows enzyme activities in the frontal cortex and striatum. Highly significant decreases in complex I activities in the brains were found for the aceruloplasminemia patients, whereas complex II + III activities were within the control range. Complex I activities in the basal ganglia of our patients were decreased less than 45% of the control values. Complex IV activities also were significantly reduced to about 42% (basal ganglia) and 68% (cerebral cortex) of the control values.

The levels of MDA + 4-HNE, which are good indicators of lipid peroxidation because they are generated in the process of oxidation of polyunsaturated fatty acids, were significantly elevated both in the frontal lobe and putamen (Figure 4). Those levels in aceruloplasminemia patients were higher in the putamen than in the frontal cortex.

Discussion

Aceruloplasminemia is an autosomal recessive disorder that affects iron metabolism and is characterized by mutations in the ceruloplasmin gene, the absence of serum ceruloplasmin, low serum iron, and iron accumulation in the brain, liver, and other tissues. A summary of clinical manifestations in 45 Japanese patients is shown Table 3. The symptoms, in order of frequency were retinal degeneration, diabetes, anemia, and neurological symptoms. These neurological symptoms reflected regions of iron accumulation, and included ataxia, involuntary movement, parkinsonism, and cognitive dysfunction. Table 4 shows a summary of mutations in the ceruloplasmin gene in aceruloplasminemia (Yoshida *et al.* 1995; Harris *et al.* 1995; Daimon *et al.* 1995; Takahashi *et al.* 1996; Okamoto *et al.* 1996; Harris *et al.* 1996; Yazaki *et al.* 1998; Miyajima *et al.* 1999; Kohno *et al.* 1999; Daimon *et al.* 2000; Hellman *et al.* 2000; Roetto *et al.* 2001). 16 truncation mutations and 5 missense mutations have been reported in 24 families worldwide. The relationship between genotype and phenotype has not

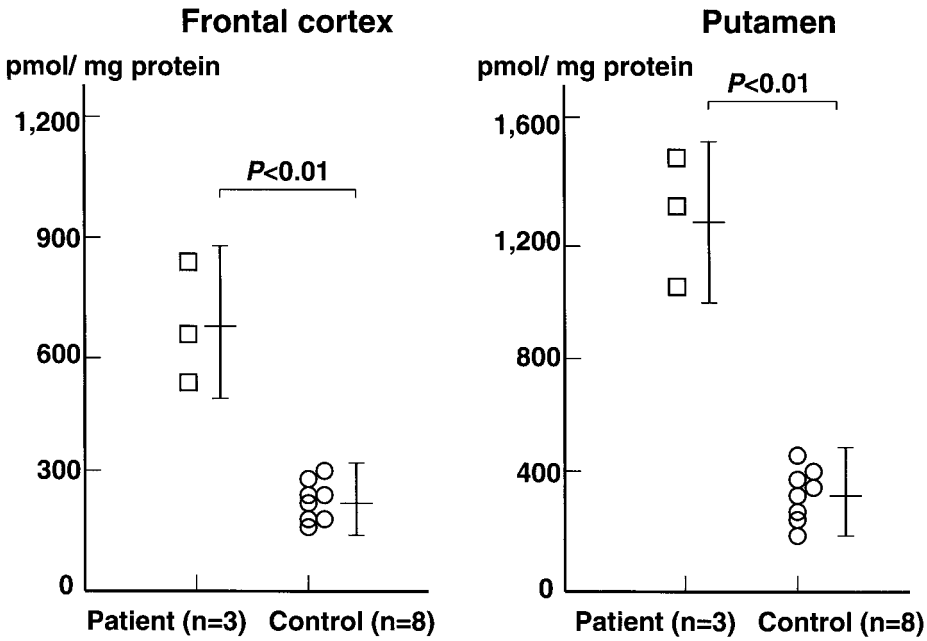


Fig. 4. Levels of malondialdehyde and 4-hydroxynonenals in the frontal cortex and putamen. Results are expressed as pmol/mg of protein. Each value represents the mean of triplicate determination. The solid bars represent the mean ± S.D. The protein concentration of the samples was measured by the method of Bradford with bovine serum albumin (BioRad, Tokyo, Japan) as the standard. $P < 0.01$ compared to the controls (Student's *t*-test).

been addressed. There is no hot spot for the mutation, but a nonsense mutation in exon 15 is relatively frequent in Japanese patients. In Japan, 17 families have been reported up to this year. We screened the serum ceruloplasmin concentrations of 5,000 adult individuals, and the incidence of aceruloplasminemia was estimated to be approximately 1 per 2,000,000 in the case of non-consanguineous marriages in Japan (Miyajima *et al.* 1999). Aceruloplasminemia should be considered in patients with features of the diseases hypoceruloplasminemia and hemosiderosis.

Almost all the energy used by the brain's cells is supplied by glucose metabolized by the mitochondrial respiratory chain and oxidative phosphorylation system. PET studies showed markedly decreased CMRGlc and $CMRO_2$ in a patient with aceruloplasminemia, suggestive of mitochondrial dysfunction. We speculated that diffuse brain energy hypometabolism is secondary to mitochondrial deficit. This speculation was partly supported by the mitochondrial enzyme assay showing significantly deficient mitochondrial activities in complexes I and IV in the aceruloplasminemia brains. Defects in oxidative phosphorylation and oxidative damage may be important in a variety of neurodegenerative diseases, including Parkinson's disease, Huntington's disease, Friedrich's ataxia, and Alzheimer disease (Bowling & Beal 1995; Schapira 1999). The progressive course and age-related increase in the incidences of these disorders may be due

Table 3. Clinical manifestations and onset in 45 Japanese patients with aceruloplasminemia

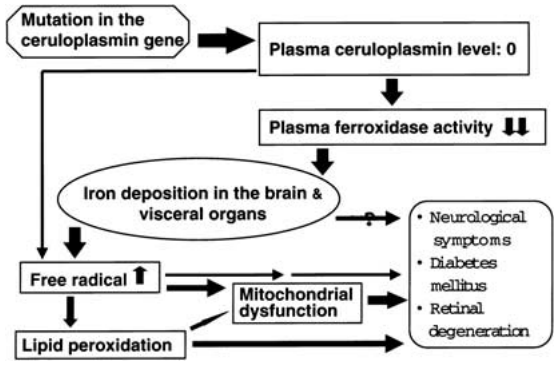
1. Clinical manifestations
Retinal degeneration (93%)
Diabetes mellitus (89%)
Anemia (80%)
Neurological symptoms (73%)
Ataxia (86%): dysarthria (scanning speech, slurred speech), gait ataxia, limb ataxia, nystagmus
Involuntary movement (60%): dystonia (blepharospasm, grimacing, neck dystonia), tremor, chorea
Parkinsonism (41%): rigidity, akinesia
Cognitive dysfunction and dementia (25%)
2. Onset
Diabetes mellitus
20–29 years old, 25%; 30–39 years old, 40%; 40–49 years old, 23%; ≥50 years old, 12%
Neurological symptoms
30–39 years old, 2%; 40–49 years old, 71%; 50–59 years old, 23%; ≥60 years old, 4%

Table 4. Mutations in the ceruloplasmin gene in aceruloplasminemia

Japan (Hamamatsu)	5-bp insertion	nt 1287, frame shift, Exon 7
Japan (Matsumoto)	g→ a transition	nt 3019-1, 3′ splice acceptor site, Intron 17
Japan (Nagasaki, Yamagata, Kumamoto, Shizuoka, Hamamatsu, Nagoya, Yaizu)	G→ A transition	nt 2630, nonsense, Exon 15
Japan (Osaka)	1-bp (A) insertion	nt 607, frame shift, Exon 3
UK (Belfast)	1-bp (G) deletion	nt 2389, frame shift, Exon 13
Japan (Hiroshima)	a→ g transition	nt 1209-2, 3′ splice acceptor site, Intron 6
Japan (Kakegawa)	1-bp (G) deletion	nt 2482, frame shift, Exon 14
Japan (Toyama)	1-bp (G) deletion	nt 2068, frame shift, Exon 11
Japan (Kakegawa)	C→ G transition	nt 587, missense, Pro177Arg, Exon 4
Japan (Osaka)	C→ T transition	nt 2701, nonsense, Exon 16
	T→ G transition	nt 2991, missense, His978Gln, Exon 17
Japan (Yamagata)	A→T transition	nt 139, missense, Ile28Phe, Exon 1
Germany (Heidelberg)	1-bp (T) insertion	nt 2510, frame shift, Exon 14
Italy	1-bp (A) insertion	nt 2974, frame shift, Exon 17
	G→ G transition	nt 550, missense, Gln165Glu, Exon 3

Novel five truncation and one missense mutations have been recognized in France*, Canada*, the Netherlands, America, and Japan up to September 2001. (*Personal communication from Dr. Yoshida K, Shinshu Univ. Sch. of Med., Matsumoto, Japan)

to the interaction between oxidative damage and defects in the mitochondrial respiratory chain. Studies with isolated submitochondrial particles showed that complexes I and IV are the respiratory chain component most sensitive to oxidative damage (Schewe *et al.* 1981; Narabayashi *et al.* 1982). The finding that the levels of MDA+4-HNE were elevated in brain homogenates obtained from aceruloplasminemia patients provides direct evidence that lipid peroxidation is enhanced in the brain with this disease. Mitochondrial enzyme complexes are membrane-bound and sensitive to the lipid environment. Change in the lipid environment, such as increased lipid peroxidation, could result in inhibition of the respiratory chain function (Fry & Green 1981). Lipid peroxidation products associated with iron accumulated were higher in the aceruloplasminemia brains than in the control brains. Central nervous system damage caused by excess iron through its ability to donate an electron and to promote oxygen free radical formation reflects the site and iron content deposited, as well as the process of iron deposition in the brain. The amounts of iron accumulated in our patient 3, who showed progressive involuntary movement, ataxia, and dementia, were greater than those in patients 1and 2, whose symptoms were mild. An inverse relationship was shown between the amounts of iron accumulated and the levels of mitochondrial enzyme activities in all the brain regions examined. The antioxidant activity of ceruloplasmin can be mainly ascribed to its ferroxidase activity which effectively inhibits ferrous ion-stimulated lipid peroxidation and ferrous ion-dependent formation of hydroxyl radicals in the Fenton reaction. Ceruloplasmin is not a ferroxidase but also a scavenger of reactive oxygen species (Gutteridge, 1983). In aceruloplasminemia, lipid peroxidation and mitochondrial dysfunction caused by free radicals, due to a lack of ceruloplasmin and excessive iron may be a part of the pathogenesis (Scheme 1).



Scheme 1. Putative mechanisms of tissue injury and neuronal degeneration in patients with aceruloplasminemia.

References

Bowling AC, Beal MF. 1995 Bioenergetic and oxidative stress in neurodegenerative diseases. *Life Sci* **56**, 1151–1171.

Bowling AC, Mutisya EM, Walker LC *et al.* 1993 Age-dependent impairment of mitochondrial function in primate brain. *J Neurochem* **60**, 1964–1967.

Bull PC, Thomas GR, Rommens JM *et al.* 1993 The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* **5**, 327–337.

Chelly J, Tumer Z, Tonnesen T *et al.* 1993 Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. *Nat Genet* **3**, 14–19.

Daimon M, Kato T, Kawanami T *et al.* 1995 A nonsense mutation of the ceruloplasmin gene in hereditary ceruloplasmin deficiency with diabetes mellitus. *Biochem Biophys Res Commun* **217**, 89–95.

Daimon M, Suga S, Ohizumi T *et al.* 2000 A novel mutation of the ceruloplasmin gene in a patient with heteroallelic ceruloplasmin gene mutation (HypoCPGM). *Tohoku J Exp Med* **191**, 119–125.

Feder JN, Gnirke A, Thomas W *et al.*. 1996 A novel HMC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* **13**, 399–408.

Frieden E. 1986 Perspectives on copper biochemistry. *Clin Physiol Biochem* **4**, 11–19

Fry M, Green DE. 1981 Cardiolipin requirement for electron transfer in complex I and III of the mitochondrial respiratory chain. *J Biol Chem* **256**, 1874–1880.

Gutteridge JMC. 1983 Antioxidant properties of caeruloplasmin towards iron- and copper-dependent oxygen radical formation. *FEBS Lett* **157**, 37–40.

Harris ZL, Takahashi Y, Miyajima H *et al.* 1995 Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc Natl Acad Sci USA* **92**, 2539–2543.

Harris ZL, Migas MC, Hughes AE *et al.* 1996 Familial dementia due to a frameshift mutation in the caeruloplasmin gene. *QJM* **89**, 355–359.

Hellman NE, Schaefer M, Gehrke S *et al.* 2000 Hepatic iron overload in aceruloplasminemia. *Gut* **47**, 858–860.

Klomp LWJ, Gitlin JD. 1996 Expression of the ceruloplasmin gene in the human retina and brain: implications for a pathogenetic model in aceruloplasminemia. *Hum Mol Genet* **5**, 1989–1996.

Kohno S, Miyajima H, Takahashi Y, Inoue Y. 1999 Aceruloplasminemia with a novel mutation associated with parkinsonism. *Neurogenet* **2**, 237–238.

Logan JI, Harveyson KB, Wisdom GB, Hughes AE, Archbold GPR. 1994 Hereditary caeruloplasmin deficiency, dementia and diabetes mellitus. *QJM* **87**, 663–670.

Mercer JF, Livingston J, Hall B *et al.* 1993 Isolation of a partial candidate gene for Menkes disease by positional cloning. *Nat Genet* **3**, 20–25.

Miyajima H, Nishimura Y, Mizoguchi K *et al.* 1987 Familial apoceruloplasmin deficiency associated with blepharospasm and retinal degeneration. *Neurology* **37**, 761–767.

Miyajima H, Takahashi Y, Shimizu H *et al.* 1996a Late onset diabetes mellitus in patients with hereditary aceruloplasminemia. *Int Med* **35**, 641–645.

Miyajima H, Takahashi Y, Serizawa M, Kaneko E, Gitlin JD. 1996b Increased plasma lipid peroxidation in patients with aceruloplasminemia. *Free Radic Biol Med* **20**, 757–760.

Miyajima H, Takahashi Y, Kamata T *et al.* 1997 Use of desferrioxamine in the treatment of aceruloplasminemia. *Ann Neurol* **41**, 404–407.

Miyajima H, Fujimoto M, Kohno S, Kaneko E, Gitlin JD. 1998a CSF abnormalities in patients with aceruloplasminemia. *Neurology* 51, 1188–1190.

Miyajima H, Adachi J, Tatsuno Y *et al.* 1998b Increased very-long-chain fatty acids in erythrocyte membranes of patients with aceruloplasminemia. *Neurology* **50**, 130–136.

Miyajima H, Kohno S, Takahashi Y, Yonekawa O, Kanno T. 1999 Estimation of the gene frequency of aceruloplasminemia in Japan. *Neurology* **53**, 617–619.

Miyajima H, Adachi J, Kohno S *et al.* 2001 Increased oxysterols associated with iron accumulation in the brains and visceral organs of aceruloplasminemia patients. *QJM* **94**, 417–422.

Morita H, Ikeda S, Yamamoto K *et al.* 1995 Hereditary ceruloplasmin deficiency with hemosiderosis: A clinicopathological study of a Japanese family. *Ann Neurol* **137**, 646–656.

Narabayashi H, Takeshige K, Minakami S. 1982 Alteration of inner-membrane components and damage to electron-transfer activities of bovine heart submitochondrial particles induced by NADPH-dependent lipid peroxidation. *Biochem J* **202**, 97–105.

Okamoto N, Wada S, Oga T *et al.* 1996 Hereditary ceruloplasmin deficiency with hemosiderosis. *Hum Genet* **97**, 755–758.

Osaki S, Johnson DA, Frieden E. 1966 The possible significance of the ferrous oxidase activity of ceruloplasmin in normal human serum. *J Biol Chem* **241**, 2746–2751.

Petrukhin K, Fischer SG, Pirastu M *et al.*. 1993 Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nat Genet* **5**, 338–342.

Roetto A, Bosio S, Gobbi MDe *et al.* 2001 Molecular characterization of aceruloplasminemia in an Italian patient. In: Powell LW *et al.* (eds), Bioiron 2001 (abstract) P26.

Schapira AHV. 1999 Mitochondrial involvement in Parkinson's disease, Huntington's disease, hereditary spastic paraplegia and Friedreich's ataxia. *Biochim Biophys Acta* **1410**, 159–170.

Schewe T, Albracht SPJ, Ludwig P. 1981 On the site of action of the inhibition of the mitochondrial respiratory chain by lipoxygenase. *Biochim Biophys Acta* **636**, 210–217.

Takahashi Y, Miyajima H, Shirabe S *et al.* 1996 Characterization of a nonsense mutation in the ceruloplasmin gene resulting in diabetes and neurodegenerative disease. *Hum Mol Genet* **5**, 81–84.

Tanzi RE, Petrukhin K, Chernov L *et al.* 1993 The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* **5**, 344–350.

Vulpe C, Levinson B, Whitney S *et al.* 1993 Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper transporting ATPase. *Nat Genet* **3**, 7–13.

Yamaguchi Y, Heiny ME, Gitlin JD. 1993 Isolation and characterization of a human liver cDNA as a candidate gene for Wilson disease. *Biochem Biophys Res Commun* **197**, 271–277.

Yazaki M, Yoshida K, Nakamura A *et al.* 1998 A novel splicing mutation in the ceruloplasmin gene responsible for hereditary ceruloplasmin deficiency with hemosiderosis. *J Neurol Sci* **156**, 30–34.

Yoshida K, Furihata K, Takeda S *et al.* 1995 A mutation in the ceruloplasmin gene is associated with systemic hemosiderosis in humans. *Nature Genet* **9**, 267–272.

Yoshida K, Kaneko K, Miyajima H *et al.* 2000 Increased lipid peroxidation in the brains of aceruloplasminemia patients. *J Neurol Sci* **175**, 91–95.