SHORT REPORT

A case of aceruloplasminaemia: abnormal serum ceruloplasmin protein without ferroxidase activity

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A 34 year old diabetic man with a complete deficiency of serum ferroxidase activity, regardless of the presence of serum ceruloplasmin (Cp), a multicopper ferroxidase protein, is described. The patient had had diabetes mellitus for 13 years, and was also found to have retinal degeneration accompanied by the development of a hearing disturbance of unknown aetiology. Laboratory examination showed markedly increased serum ferritin and low serum iron. Magnetic resonance imaging showed a pronounced hypointensity in the putamen, caudate, cerebellar dentate, and thalamus on T2 weighted images, and also disclosed a low level signal in the liver, suggesting the accumulation of some magnetic substances in the brain and liver. Liver biopsies histochemically identified iron deposition in the hepatocytes. Most of these findings were consistent with the newly established autosomal recessive disease "aceruloplasminaemia", except for the presence of serum Cp and the lack of apparent neurological symptoms. Interestingly, no ferroxidase activity was detected in the patient's serum, whereas suppressed ferroxidase activity was found in his mother's serum. A nucleotide sequence analysis of the Cp gene showed two mutations; a C to T substitution at nucleotide 2701 in exon 16, resulting in a nonsense mutation at amino acid 882 (Arg882ter), and a T to G substitution at nucleotide 2991 in exon 17, resulting in an amino acid alternation at amino acid 978 (His978Gln). The second mutation was also found in the patient's mother. The absence of serum ferroxidase activity despite the presence of serum Cp protein in this compound heterozygote was considered to be due to the production of a non-functional Cp harbouring no ferroxidase activity.

Ceruloplasmin (Cp) is a multicopper ferroxidase that plays an important part in copper and iron metabolism in vertebrates.[1] Aceruloplasminaemia is a newly recognised autosomal recessive disorder that affects iron metabolism through a complete deficiency of Cp ferroxidase activity.[2] Patients diagnosed with it have been described to lack serum Cp protein.[2–8] Herein, we report a case of absent ferroxidase activity in serum that resembled aceruloplasminaemia in clinical symptoms and laboratory findings, despite the presence of serum Cp.

CASE REPORT

A 34 year old man had had diabetes mellitus for several years, during which time he had insulin therapy. He was admitted to hospital because of a sudden hearing disturbance in his right ear on 2 July 1999. There was no known drug misuse, consanguinity, liver disease, or family history of neurological abnormalities. Height and weight were 177 cm and 70 kg, respectively, and blood pressure was 120/70 mm Hg. No abnormalities were found in the chest and abdomen. Neurological examination showed no abnormal findings such as nystagmus, speech disturbance, ataxia, motor disturbances, sensory disturbances, or involuntary movement. Neuropsychiatric examinations showed no abnormalities, with WAIS full scale results showing an IQ of 92, verbal IQ of 89, and performance IQ of 96. An audiogram disclosed a sensory disturbance in the hearing ability of his right ear. Magnetic resonance imaging disclosed a pronounced hypointensity in the putamen, caudate, cerebellar dentate, and thalamus on T2 weighted images (fig 1, left and middle), and also disclosed a low level signal in the liver (fig 1, right), suggesting the accumulation of some magnetic substances in the brain and liver. An ophthalmological examination disclosed retinal degeneration, but no Kayser-Fleischer rings were found. Laboratory findings suggested abnormal iron metabolism: red blood cells, $436\times10^4/\mu l$; haemoglobin, 12.3 g/dl; packed cell volume, 37.2%; serum iron, 38 μg/dl (normal 60–160); ferritin, 1127 ng/ml (normal 16–320); total iron binding capacity (TIBC), 273 μg/dl (normal 238–367); transferrin, 177 mg/dl (normal 190–300); serum copper, 12 μg/dl (normal 68–128); urinary copper, 8.7 μg/day (normal 4.2–33.0); Cp, 7 mg/dl (normal 21–37); fasting serum C peptide, 0.45 ng/ml (normal 1.0–2.7); and urinary C peptide, 12.8 μg/day (normal 35–70).

We considered that the clinical and laboratory manifestations, especially diabetes mellitus, retinal degeneration, and markedly increased ferritin concentrations, regardless of the low serum iron and normal TIBC, were consistent with a newly established disease entity known as "aceruloplasminaemia", even though serum Cp protein in the patient was detected at a low concentration. As Cp is the major source of plasma ferroxidase activity, we investigated the ferroxidase activity in serum by the method of Erel.[9] As shown in table 1, ferroxidase activity was determined to be 0 U/l in the patient, which was similar to the findings of others with aceruloplasminaemia. After obtaining consent from his mother, who manifested no clinical symptoms, we measured the concentration of Cp and ferroxidase activity in her serum. Serum ferroxidase activity was 218 U/l, which was about half the normal value, by contrast with a Cp concentration that was maintained at near the lower limit of a normal range (20 mg/dl).

We performed liver biopsies in the patient to identify the accumulated substances in the liver. A haematoxylin-eosin stained section showed a normal architecture; however, coarse granular deposits with brown pigmentation were present in the cytoplasm of many hepatocytes. Using a Berlin blue stained section, these pigments proved to be iron. No accumulation of copper was found histochemically.

Abbreviations: Cp, ceruloplasmin; TIBC, total iron binding capacity

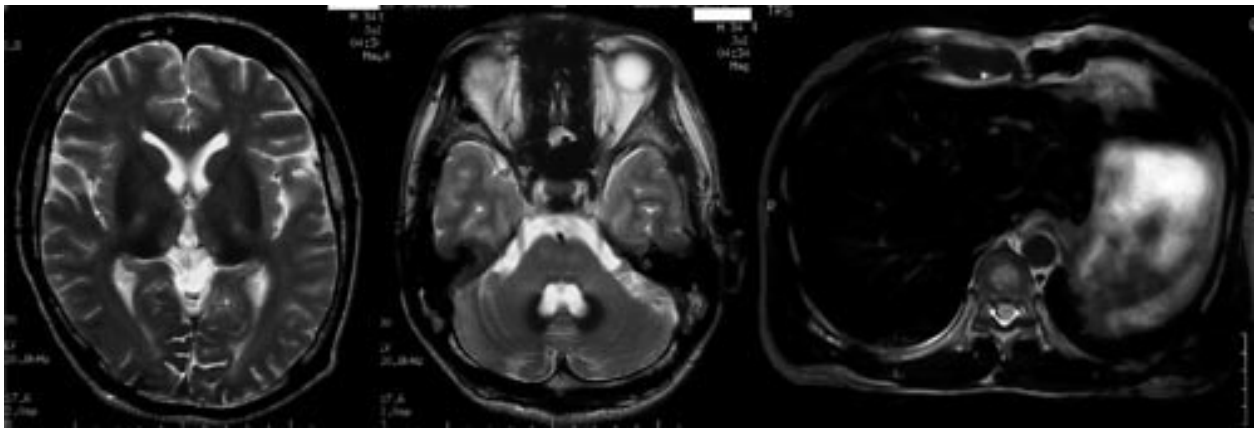

Figure 1 Brain and abdominal T2 weighted MR images showing a low signal intensity in the putamen and thalamus (left), the dentate nucleus of the cerebellum (middle), and the liver (right).

Table 1 Serum ferroxidase activity and ceruloplasmin (Cp)

	Ferroxidase activity (U/l)	Cp (mg/dl)
Healthy volunteers	591 (85)	28.5 (4.7)
Patient	0	7.0
Mother	218	20.0
Typical aceruloplasminenia	0	0

*Ten men and 10 women, aged 20-45 years, without neurological symptoms; data are presented as the mean (SD).

A nucleotide sequence analysis of the Cp gene showed two mutations; a C to T substitution at nucleotide 2701 in exon 16 and a T to G substitution at nucleotide 2991 in exon 17. The significance of these mutations is described in the following discussion.

DISCUSSION

Ceruloplasmin is a multicopper oxidase that catalyzes the oxidation of ferrous iron to ferric iron in plasma, and binds tightly to apotransferrin to form transferrin. Transferrin functions as a major iron transport protein, mobilising iron to the bone marrow from the gut, the site of absorption, and from the reticuloendothelial system, the site of storage. In patients with aceruloplasminaemia, marked iron accumulation is found in various tissues including the liver, pancreas, and brain. Accumulated iron stimulates the production of oxygen free radicals, which are considered to cause tissue damage. The ferroxidase activity of Cp decreases the availability of ferrous ion to participate in oxyradical damage and thereby leads to protection against neuronal damage. In the present patient, serum ferroxidase activity was absent, although only 5% of normal serum Cp ferroxidase activity is sufficient to prevent iron overload, as seen in Wilson's disease.[10] Therefore, we considered that the underlying pathogenesis in the present case was substantially identical to that of aceruloplasminaemia.

Three major clinical manifestations are known in aceruloplasminaemia; diabetes mellitus, retinal degeneration, and neurological manifestations. Our 34 year old patient had diabetes mellitus and retinal degeneration; however, he lacked such neurological symptoms as nystagmus, speech disturbance, ataxia, motor disturbances, sensory disturbances, and involuntary movement, except for sensory hearing disturbance. Among the three major manifestations, diabetes mellitus is often the first encountered and often arises between 20 and 30 years of age, before the development of apparent neurological symptoms. We could not deny the possibility that the hearing disturbance was the initial neurological manifestation related to the absent plasma ferroxidase activity, however, its sudden onset seemed unlikely to be a neurological manifestation in such a slow progressive disease. Apart from the inauthentic neurological symptom, the accumulation of some kind of magnetic substances in the brain was evident from MRI findings. In patients with aceruloplasminaemia, careful observation is necessary concerning the appearance of neurological symptoms during the follow up period. Further, the early introduction of iron chelating therapy may be effective to prevent insidious neuronal damage and delay the appearance of neurological manifestations.[11]

Magnetic resonance imaging has become indispensable to demonstrate accumulations of magnetic substances in various tissues. As a deficiency of serum ferroxidase causes iron deposition in the brain, MRI is useful for such diagnoses as aceruloplasminaemia, even when the patients are without apparent neurological manifestations, as seen in the present case. Marked hypointensity in both the basal ganglia and liver was evidence enough to speculate that the deposits in the liver were the same as those in the brain in this case. Those in the liver were identified as iron by histochemical examinations of biopsied liver specimens, clearly differentiating this patient from those with Wilson's disease, in whom copper accumulation is found in the liver and brain instead of iron. Haemochromatosis could also be excluded by the normally maintained liver lobule architecture and absence of plasma ferroxidase activity.

We recently performed a genetic analysis of the Cp gene in the present patient and found two mutations; a C to T substitution at nucleotide 2701 in exon 16, which introduced a premature stop codon at amino acid 882 (aa882 CGA;Arg-TGA;stop), and a T to G substitution at nucleotide 2991 in exon 17, which induced an amino acid alternation (aa978 CAT;His-CAG;Gln). The first mutation is considered to produce truncated Cp by premature termination in the translation, whereas the second, also found in his mother, is speculated to produce a non-functional Cp harbouring no ferroxidase activity, probably due to a disrupted trinuclear copper cluster by the single amino acid alternation. Further molecular and genetic analyses, including an in vitro reconstruction

of the mutant Cp and its functional assessment, as well as an RFLP study of the family pedigree, are in progress to evaluate how these mutations affected Cp production and its ferroxidase activity.

In conclusion, we investigated a case of abnormal ceruloplasmin protein without ferroxidase activity. This is the first known case in which the absence of serum ferroxidase activity was demonstrated, regardless of the presence of Cp in serum.

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