CASE REPORT

Iron chelation therapy in aceruloplasminaemia: study of a patient with a novel missense mutation

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We describe a novel missense mutation of ceruloplasmin in a patient with aceruloplasminaemia causing the replacement of a neutral amino acid (phenylalanine) with a polar one (serine) at position 198, probably leading to abnormal folding and secretion of the protein. The patient showed mild microcytic anaemia, mild hepatic iron overload, and marked brain iron overload. Six months of therapy with deferiprone was ineffective in removing iron from the tissues. Deferoxamine was more efficient in removing excess iron from the liver but aggravated the disease related anaemia. After more than one year of chelation treatment, the brain magnetic resonance imaging signal did not change. Overall, these findings indicate that treatment of iron overload in aceruloplasminaemia is a difficult challenge and that new iron chelators, more efficient in crossing the blood-brain barrier, are needed.

Ceruloplasmin (Cp) has an essential role in normal iron homeostasis favouring cellular iron release and iron incorporation into apo-transferrin.[1][2] Aceruloplasminaemia is a rare autosomal recessive disorder due to mutations of the Cp gene characterised by iron overload in the brain, pancreas, retina, and liver due to cellular iron retention and increased iron absorption.[3] In adulthood, patients develop ataxia and dementia, diabetes, and retinal degeneration.[4][5] Mild microcytic anaemia, low serum iron, and increased serum ferritin are typical features and are related to the defective cellular iron release into plasma.[3][6][7] Plasma may be helpful but long term use has not been challenged and the risk of infections should be considered.[4] Phlebotomy is not helpful[8] and may worsen the disease related anaemia. Different outcomes have been reported using deferoxamine[7][9] whereas no data are available for the oral iron chelator deferiprone that can be an attractive therapeutic candidate due its stability, lipophilicity, and ability to penetrate the blood-brain barrier.[10]

We report the case of a young woman with aceruloplasminaemia due to a novel missense mutation of the Cp gene in whom we also evaluated the efficacy of both deferiprone and deferoxamine on iron removal and their effect on iron and haematological indices.

CASE REPORT

A 40 year old Italian woman was investigated for chronic asthenia and mild microcytic anaemia. Haemoglobin was 10.6 g/dl and mean corpuscular volume 74 fl, serum iron was 27 µg/l, transferrin saturation 10%, and serum ferritin level 471 µg/l in the absence of inflammatory alterations (C reactive protein 0.3). No alteration in glucose or lipid metabolism was found. Cp was undetectable on immuno-turbidimetric assay (Tina-quant; Roche, Mannheim, Germany) and at western blot analysis using human anticeruloplasmin antibody (DakoCytomation, Milan, Italy). Ferroxidase activity was not measured. General, neurological, and fundus oculi examinations were normal.

Imaging studies
Magnetic resonance imaging (MRI) of the proband's brain showed marked hypointensity in the dentate and red nuclei, thalamus, pulvinar, neostriatum, and putamen. Gradient echo images also showed a thin hypointense rim along most of the cerebellar and cerebral cortex. Magnetic susceptometry by superconductive quantum interference device (SQUID) analysis, performed according to Brittenham and colleagues,[11] showed a moderate hepatic iron overload (fig 1B).

Genetic investigation and family study
There was no history of consanguinity in the family although the grandparents originated from the same village in Southern Italy. Only the proband's younger brother and offsprings were available for clinical and laboratory analysis, and genetic study. Direct sequencing of the whole Cp gene, including exon 1 to 20 and intron-exon boundaries, was carried out by ABI Prism 3100 Avant DNA sequencer (PE Applied Biosystems, Foster City, California, USA). Sequences were compared with GenBank Accession Nos D45028-D45045 and HGMD No M13699. Four nucleotide changes were detected at the homozygous state in the proband: two intronic T→C transversions (IVS2 +20 and IVS15 −12), previously described in healthy subjects;[12] a never described G→A transversion (AAG→AAA) at nucleotide 2682 in exon 16 that does not cause an amino acid change (K894K) and that was also present in homozygosity in the other unaffected relatives; and a T→C transversion (TTT→TCT) at nucleotide 593 in exon 4 causing a phenylalanine to serine replacement in the protein (F198S). The F198S mutation was confirmed in the proband by restricted fragment length polymorphism (RFLP) analysis using Hinf I digestion and was not found in 50 healthy individuals. The Cp gene sequence and RFLP analysis were performed in all available relatives. The proband's family pedigree, together with the results of the molecular study, biochemical tests, and SQUID measurements are shown in fig 1A and 1B. A mild hepatic iron overload was observed in the proband's brother, daughter, and son at SQUID (fig 1B). MRI was normal in the proband's offspring whereas a mild T2 weighted hypointensity in the pulvinar and posterior and lateral putamen was found in the brother.

Iron chelation therapy
The patient's written informed consent and local ethics committee approval were obtained. Deferiprone (75 mg/kg/day) was administered for six months but was not effective

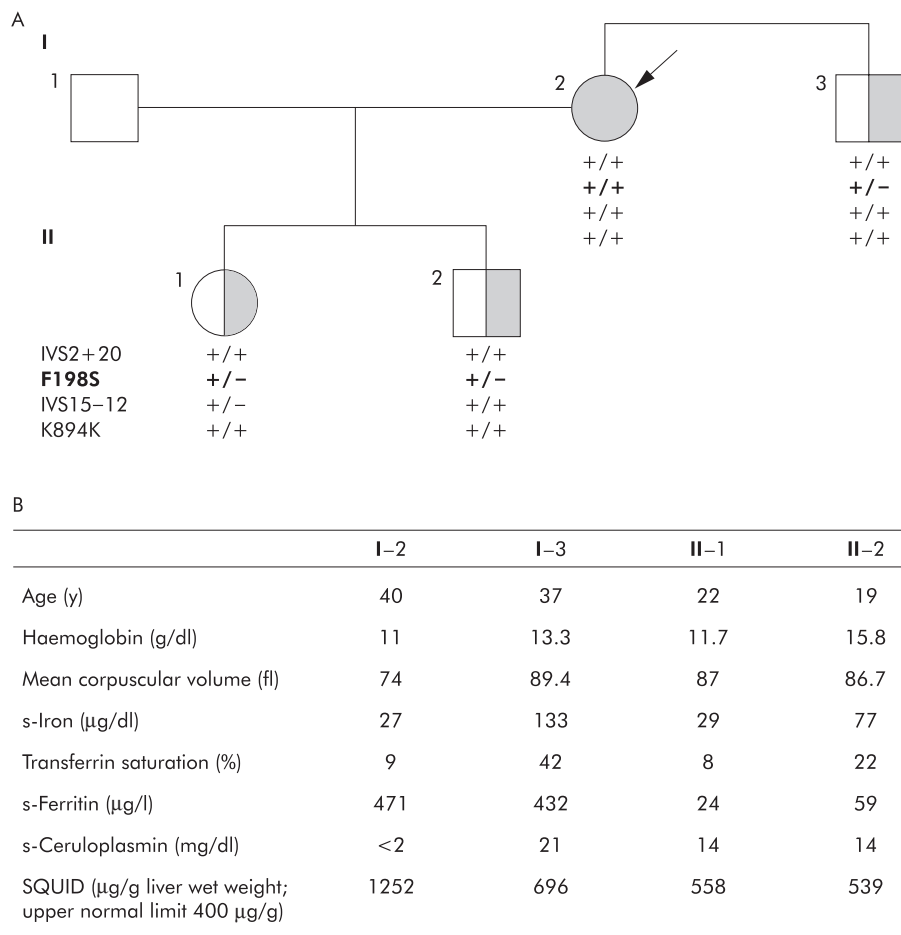..

Figure 1 (A) Family pedigree. (B) Age, iron, and biochemical data of the proband and relatives. SQUID, superconductive quantum interference device.

	I–2	I–3	II–1	II–2
Age (y)	40	37	22	19
Haemoglobin (g/dl)	11	13.3	11.7	15.8
Mean corpuscular volume (fl)	74	89.4	87	86.7
s-Iron (µg/dl)	27	133	29	77
Transferrin saturation (%)	9	42	8	22
s-Ferritin (µg/l)	471	432	24	59
s-Ceruloplasmin (mg/dl)	<2	21	14	14
SQUID (µg/g liver wet weight; upper normal limit 400 µg/g)	1252	696	558	539

(fig 2). Deferoxamine (20 mg/kg/day for five days a week) was administered by subcutaneous bolus injections for the next eight months (fig 2). Urinary iron excretion levels and haemoglobin, mean corpuscular volume, serum iron, and serum ferritin behaviour during the treatment periods are shown in fig 2A and B. A second brain MRI, performed after more than one year of chelation treatment, was unchanged.

DISCUSSION

We describe the case of a young woman with aceruloplasminaemia, homozygous for a new missense Cp gene mutation (F198S). The phenylalanine 198 residue is located just before Val[199] that forms together with other non-polar residues (Leu[74], Ile[77], Ile[159], and Phe[267]) a tightly packed hydrophobic pocket critical for correct folding of human Cp.[13] A proline residue at position 177 projects into that pocket and a mutation changing proline to arginine at this position (P177R), causing retention of the mutant human Cp in the endoplasmic reticulum and aceruloplasminaemia, was recently described.[13] We suggest that replacement of a neutral amino acid (phenylalanine) with a polar one (serine) at position 198 leads to incorrect folding of the protein and impaired cellular trafficking of Cp, as demonstrated for the P177R mutant.[13] The proband had a slight microcytic anaemia with low serum iron and a moderate hepatic iron overload not in the range capable of inducing hepatic damage. In contrast, brain MRI showed marked iron overload in the basal nuclei, and mild iron overload in the cerebellar and cerebral cortex.[3] Absence of neurological abnormalities in the patient is probably explained by her young age as most patients with aceruloplasminaemia develop neurological symptoms in their sixties,[7 8 12] indicating that brain iron overload requires several years before cellular damage becomes clinically manifest. The three heterozygous relatives showed slightly increased hepatic iron stores by SQUID and the oldest one showed a reduction in the brain MRI signal suggesting mild iron accumulation in the basal nuclei. Heterozygosity for aceruloplasminaemia is generally considered unable to affect iron homeostasis but recent reports noted neurological abnormalities in elderly individuals with a single Cp mutation showing brain iron deposition at MRI and autopsy.[14 15] Follow up studies are needed to understand whether iron overload increases with time and leads to clinical manifestations in heterozygous individuals.

At the time of the study the patient had no major sign or symptom of the disease but the amount of iron accumulation in the brain makes the development of neurological alterations likely in the future. For this reason we challenged the efficacy of the two iron chelators available for human therapy. Deferiprone was not effective in removing iron from tissues, as demonstrated by the very low urinary induced iron excretion and lack of a reduction in serum ferritin after five months of therapy. The patient was highly compliant with all diagnostic and therapeutic procedures, and hence we suggest that deferiprone failure may be explained by individual variability in deferiprone response and/or by the lower efficacy of deferiprone compared with deferoxamine.[10]
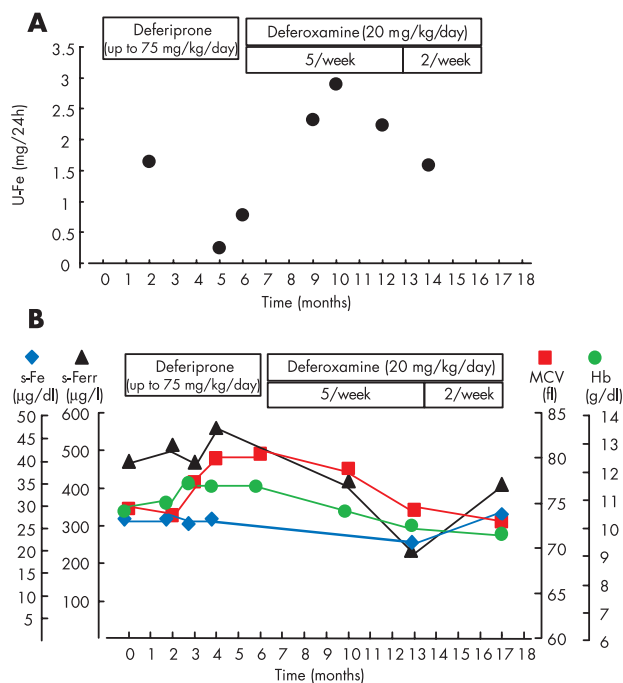
Figure 2 (A) Urinary iron excretion (U- Fe) by deferiprone and deferoxamine. (B) Serum iron (s-Fe), serum ferritin (s- Ferr), haemoglobin (Hb), and mean corpuscular volume (MCV) during iron chelation therapy.

Deferoxamine induced iron excretion was higher than that induced by deferiprone. Serum ferritin halved during the first four months of deferoxamine treatment but a concomitant decrease in haemoglobin and serum iron level was observed, suggesting that deferoxamine sequestered iron available for erythropoiesis. The brain MRI signal did not change in our patient after more than one year of iron chelation treatment although it is possible that minor changes were not detectable as MRI is a semiquantitative method of brain iron measurement. Lorèal and colleagues[7] reported normalisation of serum ferritin and hepatic iron concentration in a woman with aceruloplasminaemia after one year of deferoxamine plus vitamin C treatment together with aggravation of anaemia leading to discontinuation of treatment. Brain iron accumulation and neurological manifestations did not improve. In contrast, Miyajima and colleagues[9] showed slight improvement in neurological symptoms, decrease in brain iron stores by MRI, and no adverse effects in a 63 year old woman with aceruloplasminaemia after 10 months of a less intensive deferoxamine treatment that induced a partial decrease in hepatic iron overload and serum ferritin levels.

In conclusion, treatment of iron overload in aceruloplasminaemia remains a difficult challenge. Deferoxamine seems to be more efficient than deferiprone in removing excess iron from the liver but it should be administered cautiously to avoid aggravation of the disease related anaemia. Both deferoxamine and deferiprone seem unable to remove sufficient amounts of iron from the brain although it cannot

be excluded that they can prevent further iron accumulation and progression of neurological symptoms. Development of new iron chelators (capable of crossing the blood-brain barrier) and of more sensitive methods (capable of quantifying brain iron) could improve treatment and monitoring of therapeutic efficacy in brain iron overload disorders.

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REFERENCES

1 **Mukhopadhayay CK**, Attieh ZK, Fox PL. Role of ceruloplasmin in cellular iron uptake. *Science* 1998;**279**:714–17.
2 **Harris ZL**, Durley AP, Man TK, *et al.* Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proc Natl Acad Sci U S A* 1999;**96**:10812–17.
3 **Nittis T**, Gitlin JD. The copper-iron connection: hereditary aceruloplasminemia. *Semin Hematol* 2002;**39**:282–9.
4 **Yonekawa M**, Okabe T, Asamoto Y, *et al.* A case of hereditary ceruloplasmin deficiency with iron deposition in the brain associated with chorea, dementia, diabetes mellitus and retinal pigmentation: administration of fresh-frozen human plasma. *Eur Neurol* 1999;**42**:157–62.
5 **Morita H**, Ikeda S, Yamamoto K, *et al.* Hereditary ceruloplasmin deficiency with hemosiderosis: a clinicopathological study of a Japanese family. *Ann Neurol* 1995;**37**:646–56.
6 **Bosio S**, De Gobbi M, Roetto A, *et al.* Anemia and iron overload due to compound heterozygosity for novel ceruloplasmin mutations. *Blood* 2002;**100**:2246–8.
7 **Lorèal O**, Turlin B, Pigeon C, *et al.* Aceruloplasminemia: new clinical, pathophysiological and therapeutic insights. *J Hepatol* 2002;**36**:851–6.
8 **Hellman NE**, Schafer M, Gehrke S, *et al.* Hepatic iron overload in aceruloplasminemia. *Gut* 2000;**47**:858–60.
9 **Miyajima H**, Takahashi Y, Kamats T, *et al.* Use of desferrioxamine in the treatment of aceruloplasminemia. *Ann Neurol* 1997;**41**:404–7.
10 **Beutler EA**, Hoffbrand VA, Cook JD. Iron deficiency and overload. *Hematology* (*Am Soc Hematol Educ Program*), 2003:40–61.
11 **Brittenham GM**, Farrell DE, Harris JW, *et al.* Magnetic-susceptibility measurement of human iron stores. *N Engl J Med* 1982;**307**:1671–5.
12 **Lee PL**, Gelbart T, West C, *et al.* A study of genes that may modulate the expression of hereditary hemochromatosis: transferrin receptor-1, ferroportin, ceruloplasmin, ferritin light and heavy chain, iron regulatory proteins (IRP)-1 and −2, and hepcidin. *Blood Cells Mol Dis* 2001;**27**:783–802.
13 **Hellman NE**, Kono S, Miyajima H, *et al.* Biochemical analysis of a missense mutation in aceruloplasminemia. *J Biol Chem* 2002;**277**:1375–80.
14 **Daimon M**, Morial S, Susa A, *et al.* Hypocaeruloplasminemia with heteroallelic caeruloplasmin gene mutation: MRI of the brain. *Neuroradiology* 1999;**41**:185–7.
15 **Miyajima H**, Kono S, Takahashi Y, *et al.* Cerebellar ataxia associated with heteroallelic ceruloplasmin gene mutation. *Neurology* 2001;**57**:2205–10.