A Turkish Patient with Aceruloplasminemia Found to Have a Novel Pathogenic Variant Presenting with High Ferritin Level and Microcytic Anemia

Yüksek Ferritin Düzeyi ve Mikrositik Anemi ile Prezente Olan, Yeni Bir Patojenik Varyanta Sahip Olduğu Tespit Edilen Türk Aseruloplazminemi Olgusu

Hande Özkalaycı[1], Meral Uluköylü Mengüç[2], Naz Güleray Lafcı[3], Ayşegül Öztürk Kaymak[4]

[1]Bolu Abant İzzet Baysal Training and Research Hospital, Department of Medical Genetics, Bolu, Türkiye
[2]Bolu Abant İzzet Baysal Training and Research Hospital, Department of Hematology, Bolu, Türkiye
[3]Hacettepe University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye
[4]Düzen Laboratory, Department of Medical Genetics, Ankara, Türkiye

To the Editor,

Aceruloplasminemia is a rare autosomal recessively inherited iron accumulation disease that can manifest with neurological findings in the fourth and fifth decades of life, peripheral retinal degeneration, microcytic anemia, decreased serum iron and increased ferritin concentrations, diabetes, or abnormal glucose tolerance [1]. The incidence of this disorder is 1/2,000,000 in non-consanguineous marriages in Japan. Aceruloplasminemia is the only known disease that involves both the brain and systemic iron metabolism among the iron overload syndromes [2]. Here, a long-term undiagnosed case with microcytic anemia accompanied by high ferritin levels is presented.

A 34-year-old Turkish female patient followed by an external hospital with an undiagnosed iron metabolism disease applied to the hematology outpatient clinic. Anemia and high ferritin levels had been detected during a premarital screening test 7 years ago. She had no history of oral or parenteral iron replacement for the last 3 years. Bone marrow biopsy had been performed before admission and the results were reported as normocellular, with an increase in depository iron, together with alpha-thalassemia and *HFE* gene testing. The genetic test results for alpha-thalassemia were negative and *HFE* gene sequencing revealed a heterozygous p.His63Asp mutation. Moderate iron accumulation was observed in the liver by T2-weighted magnetic resonance imaging (MRI). She refused the recommended liver biopsy. Her ferritin level was over 1000 µg/L and an oral iron chelator was administered for a while. However, the drug was discontinued due to a grade 2-3 allergic reaction.

Her parents had fourth-degree consanguinity. There were no individuals with neurological findings or similar blood test results in her family history. The liver was palpable at 1 cm below the right costal margin and the spleen was not palpable. She did not have any neurological findings. Her full blood count was as follows: white blood cell count, 8160/mm^3; red blood cell count, 4,480,000/mm^3; hemoglobin, 10 g/dL; hematocrit, 33.2%; mean corpuscular volume, 74.1 fL; mean corpuscular hemoglobin, 22.3 pg; mean corpuscular hemoglobin concentration, 30.1 g/dL; red cell distribution width, 17.5% (normal range: 11.6-16%). The serum iron level (33 µg/dL; normal: 25-156 µg/dL) and total iron-binding capacity (317 µg/dL; normal: 240-450 µg/dL) were normal. C-reactive protein (CRP) was slightly increased (9 mg/L; normal: 0-5 mg/L). Transferrin saturation was low (10%; normal: 15-50%). The serum ferritin level was high (681 µg/L; normal: 10-204 µg/L). Liver function test results were within normal values. Inflammation markers were normal despite high ferritin levels that persisted during follow-up. Liver size was increased and consistent with moderate iron deposition, while the parenchymal signal was diffusely decreased in T2A-weighted upper abdomen MRI (iron load: 10.3 mg/g). Signal changes secondary to iron accumulation were detected in both kidneys. Due to a lack of evidence of any inflammatory state, we did not consider inflammation. Regarding the differential diagnosis of thalassemia, a peripheral smear and hemoglobin electrophoresis were performed, but neither was consistent with thalassemia and the alpha-thalassemia gene test had been negative. Although *HFE* gene analysis had been performed previously in another outpatient clinic, we did not suspect hemochromatosis due to low transferrin saturation.

After a comprehensive review of the literature on iron metabolism disorders, aceruloplasminemia was suspected based on the high ferritin levels, microcytic anemia, and iron accumulation in the liver and kidneys. Her serum ceruloplasmin level (<0.023 g/L; normal: 0.2-0.6 g/L) and copper level (57.7 µg/dL; normal: 80-

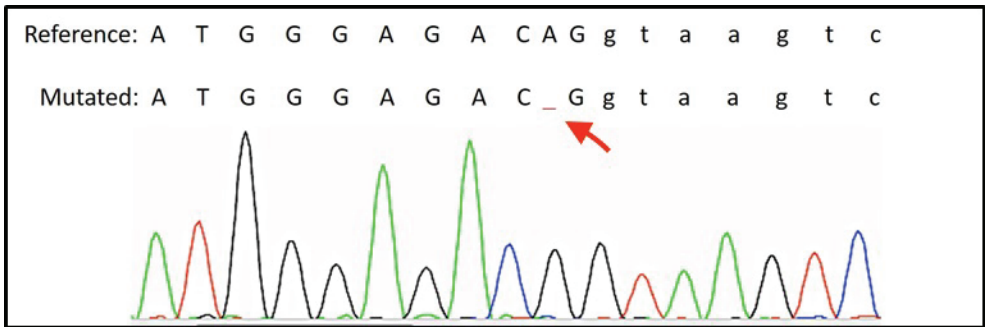

Figure 1. Chromatogram showing the novel pathogenic homozygous c.1712delA (p.Gln571ArgfsTer4) variant in exon 9 of the *CP* gene (NM_000096).

155 µg/L) were low and 24-hour urinary copper excretion was normal (6.65 µg/day; normal: 0-52 µg/day). Signal loss secondary to paramagnetic material accumulation in the bilateral basal ganglia and dentate nucleus was observed in a susceptibility-weighted imaging series by non-contrast brain MRI. As a result of an ophthalmological examination, due to borderline eye pressure and increased cup/disc ratio, visual field investigation was planned. A *CP* gene sequence analysis was performed. DNA isolated from EDTA-whole blood was amplified by polymerase chain reaction (PCR) with primers specific to the exons of interest. The purified PCR products were sequenced on an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). A novel c.1712delA (p.Gln571ArgfsTer4) homozygous pathogenic variant causing a frameshift and an early stop codon formation in exon 9 was detected in the *CP* gene (NM_000096) (Figure 1). Since the patient had a history of allergic reaction to deferasirox, iron chelation with deferasirox at the lowest dose of 15 mg/kg and oral zinc therapy were begun. Her serum ferritin level decreased from 939 µg/L to 405 µg/L on the 13th day of treatment. We also planned to draw blood from her siblings and parents to check their complete blood counts and ferritin levels and to perform physical examinations for them.

Ceruloplasmin has various functions, such as copper transport, oxidation of biological amines and ferrous iron, and antioxidant activities via preventing the formation of free radicals in serum [3]. Ceruloplasmin is mostly secreted into plasma by hepatocytes [4]. It has been shown that an isoform plays a role in the cell surface stabilization of ferroportin in the astrocytes and bone marrow-derived macrophages. It is suggested that iron accumulation in the brain and low serum iron levels in these patients may develop secondary to the deterioration of iron export from these cells [5]. Aceruloplasminemia should be kept in mind in cases of microcytic anemia and increased serum ferritin levels. Atransferrinemia, divalent metal transporter 1 deficiency, hemochromatosis, and ferroportin disease should also be considered while approaching systemic hereditary iron overload disorders. Transferrin saturation and anemia status are very important in the process of differential diagnosis [6]. Biochemical changes are usually the earliest manifestations of aceruloplasminemia and may appear decades before other clinical manifestations, and especially neurological complications, which are irreversible [7]. Therefore, it is important for hematologists to consider aceruloplasminemia in the differential diagnosis of microcytic anemia and hyperferritinemia.

Keywords: Hyperferritinemia, Ceruloplasmin, Mutation, Anemia

Anahtar Sözcükler: Hiperferritinemi, Seruloplazmin, Mutasyon, Anemi

Ethics

Informed Consent: Informed consent was obtained from this patient.

Authorship Contributions

Surgical and Medical Practices: H.Ö., M.U.M.; Concept: H.Ö., M.U.M., N.G.L.; Design: H.Ö., M.U.M., N.G.L.; Data Collection or Processing: H.Ö.; Analysis or Interpretation: N.G.L., A.Ö.K.; Literature Search: H.Ö., M.U.M.; Writing: H.Ö., M.U.M., N.G.L.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Miyajima H. Aceruloplasminemia. Neuropathology 2015;35:83-90.
2. Miyajima H, Takahashi Y, Kono S. Aceruloplasminemia, an inherited disorder of iron metabolism. Biometals 2003;16:205-213.
3. Floris G, Medda R, Padiglia A, Musci G. The physiopathological significance of ceruloplasmin. A possible therapeutic approach. Biochem Pharmacol 2000;60:1735-1741.
4. Patel BN, Dunn RJ, David S. Alternative RNA splicing generates a glycosylphosphatidylinositol-anchored form of ceruloplasmin in the mammalian brain. J Biol Chem 2000;275:4305-4310.
5. De Domenico I, Ward DM, di Patti MC, Jeong SY, David S, Musci G, Kaplan J. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. EMBO J 2007;26:2823-2831.

6. Piperno A, Pelucchi S, Mariani R. Inherited iron overload disorders. Transl Gastroenterol Hepatol 2020;5:25.

7. Vila Cuenca M, Marchi G, Barqué A, Esteban-Jurado C, Marchetto A, Giorgetti A, Chelban V, Houlden H, Wood NW, Piubelli C, Dorigatti Borges M, Martins de Albuquerque D, Yotsumoto Fertrin K, Jové-Buxeda E, Sanchez-Delgado J, Baena-Díez N, Burnyte B, Utkus A, Busti F, Kaubrys G, Suku E, Kowalczyk K, Karaszewski B, Porter JB, Pollard S, Eleftheriou P, Bignell P, Girelli D, Sanchez M. Genetic and clinical heterogeneity in thirteen new cases with aceruloplasminemia. atypical anemia as a clue for an early diagnosis. Int J Mol Sci 2020;21:2374.

Address for Correspondence/Yazışma Adresi: Hande Özkalaycı, M.D., Bolu Abant İzzet Baysal Training and Research Hospital, Department of Medical Genetics, Bolu, Türkiye
Phone : +90 536 291 38 68
E-mail : handememis90@gmail.com ORCID: orcid.org/0000-0002-8520-623X

Received/Geliş tarihi: July 7, 2023
Accepted/Kabul tarihi: September 8, 2023
DOI: 10.4274/tjh.galenos.2023.2023.0270