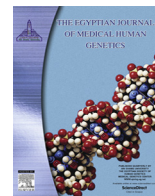


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## Case Report

## Asymptomatic hemochromatosis case with HFE c.1007–47G&gt;A, c.340+4T&gt;C heterozygous mutations and alpha globin –3.7 kb deletion

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## ABSTRACT

**Background:** Hereditary hemochromatosis is a disease associated with iron deposition which is caused by the mutations in “hereditary Fe (iron)” (HFE) gene.

**Case:** The 16-year-old male patient was diagnosed with hereditary hemochromatosis after c.1007–47G>A heterozygous c.340+4 T>C heterozygous mutations were detected in HFE gene analysis after a suspicion of hemochromatosis due to increase of hemoglobin value from 14.8 g/dL to 16.8 g/dL and the level of ferritin from 68 ng/ml to 300 ng/ml in routine check-up controls in two-years period. In addition, due to low mean corpuscular volume (MCV) (76 fL), and mean corpuscular hemoglobin (MCH) (26 pg) levels, gene mutation analysis was carried out and the patient was also shown to carry  $\alpha$  thalassemia –3.7 deletions.

**Conclusion:** Early diagnosis of hemochromatosis is important in terms of prognosis and morbidity. We aimed to emphasize that we can easily diagnose the disease by performing genetic analysis in cases with suspected hemochromatosis even they have no complaints.

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## 1. Introduction

Hemochromatosis is the abnormal accumulation of iron in parenchymal organs, leading to organ toxicity. It is the most common autosomal recessive genetic disorder and the most common cause of severe iron overload [1]. Hemochromatosis is classified as primary and secondary. In some diseases with deposition of iron, there is no secondary disorder that causes the disease and they are classified as primary hemochromatosis. Secondary hemochromatosis develops secondary to another disease such as thalassemia with ineffective erythropoiesis [2].

Hereditary hemochromatosis (HH) is a disorder of iron metabolism with genetic and environmental factors involved in pathogenesis and causes progressive iron accumulation in various parenchymal organs. In 1996, Feder et al. identified hereditary Fe (iron) gene mutations (HFE) responsible for HH. With the detection of these mutations, a great deal of improvement has been made in the identification of the disease [1]. The most common type of HH seen due to changes in the HFE gene is called HFE-associated HH.

Along with the identification of new mutations, other subgroups of the disease have also been identified and they are referred to as non-HFE related HH (non-HFE).

The HFE gene encodes a 343-amino acid HFE protein which is a major histocompatibility complex (MHC) class 1 molecule and found in the HLA-A locus on chromosome 6. HFE protein is a transmembrane protein with intracellular and extracellular components and plays an important role in the regulation of iron metabolism [3]. Two important mutations in the HFE gene have been identified. C282Y mutation takes place with tyrosine substitution instead of the cysteine in the codon 282 whereas H63D mutation is formed by aspartate passage instead of histidine in the codon 63. The biological effect of the first mutation is stronger than the H63D mutation. In their study, Feder et al. found that 83% of the HH cases were homozygous (C282Y) and 8% were heterozygous (C282Y/H63D). Conformational structure of the protein is impaired and the iron absorption increases after HFE gene mutation. HH type 1 Classical hereditary hemochromatosis is characterized by HFE gene mutation [4]. Juvenile hemochromatosis, defined as type 2; type 2A if a hemojuvelin (HJV) mutation is present, or type 2B if a hepsin (HAMP) mutation is present [5]. Type 3 hemochromatosis occurs as a result of transferrin receptor 2 (TfR-2) mutation. Type 4 haemochromatosis occurs due to ferroporphyrin mutation [6].

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The most practical, easy and feasible method for the screening in hereditary hemochromatosis is the measurement of ferritin and transferrin saturation values. The analysis of genetic mutations in susceptible cases is important in diagnosis [7]. Direct genetic screening should be performed in the first degree relatives of individuals who are defined with hereditary hemochromatosis [8].

**2. Case report**

A 14-year-old male patient was admitted to the Pediatrics Clinic for routine check-up. His physical examination was normal and blood analysis revealed: erythrocyte (RBC) count  $6.07 \times 10^6/\mu\text{L}$  ( $4.1\text{--}5.1 \times 10^6/\mu\text{L}$ ), hemoglobin (Hb) 14.6 g/dL (13–16 g/dL), hematocrit (Hct)%43.1 (%37–49), mean corpuscular volume (MCV) 71 fL (73–95 fL), mean corpuscular hemoglobin (MCH) 24.1 pg (26–30 pg), ferritin 68 ng/ml (13–68 ng/ml). On peripheral blood smear, mild hypochromia and anisocytosis observed. His family history was clear. There was no significant finding in his or family's history.

After 2 years, at the age of 16, he was admitted to the Pediatrics clinic for a routine check up once more. His physical examination was normal. However, it was found that RBC count was  $6.07 \times 10^6/\mu\text{L}$ , Hb was 14.6 g/dL, Hct was 43.1%, MCV and MCH were 71 fL and 24.1 pg, respectively, ferritin level was 300 ng/ml. The patient had no changes in his nutrition status or was not on medication in that period. Serum iron, iron binding capacity, and other acute phase reactants were normal. Due to low levels of MCV and MCH, he was suspected as having thalassemia triad, however, no abnormal hemoglobin was detected in his hemoglobin electrophoresis (Hb A2%2.1). He was diagnosed as alpha thalassemia due to detected  $-\alpha 3.7, -\alpha 4.2$  single gene deletion in "Multiplex Ligation-Dependent Probe Amplification" (MLPA) method (Fig. 1). Erythropoietin levels analysed for polycythemia were normal (7 IU/L) and a clinically insignificant wild type (normal) genomic sequence involving JAK2-V617F mutation was detected in DNA point mutation mini sequence analysis. With the pre-diagnosis of hereditary hemochromatosis, c.1007–47G>A heterozygous c.340+4 T>C heterozygous mutations were detected in HFE gene mutation analysis (Fig. 2). Previously, c.1007–47G>A mutation has been reported to be associated with iron overload and c.340+4 T>C mutation is defined as the polymorphism associated with hemochromatosis [9].

**3. Discussion**

For suspected hemochromatosis cases, ferritin level and fasting transferrin saturation should be measured at first [10]. HFE gene mutation analysis was performed in our case because of an increase in ferritin levels in two-years period, and the patient was diagnosed as hereditary hemochromatosis due to the detected heterozygous mutations for  $-47\text{G}>\text{A}/+4\text{T}>\text{C}$ . According to our knowledge, these mutations are reported in Turkish population for the first time.

Ferritin increases in neoplasms and infections as an acute phase reactant. Alcoholic liver disease, Hepatitis C infections, and non-alcoholic steatohepatitis may also elevate ferritin levels [11]. Therefore, ferritin is not a specific marker for hemochromatosis and transferrin saturation should be evaluated concurrently. In case of normal levels of both ferritin and transferrin saturation, hemochromatosis should be ruled out [12]. Transferrin saturation levels above 45 are valuable for the diagnosis and HFE mutation should be analysed. Prior to 1996 the diagnosis of hemochromatosis was made by liver biopsy. However, after the detection of HFE gene mutations, genetic analysis substitute liver biopsy [13,14]. Our case was diagnosed as hereditary hemochromatosis based on unaccompanied additional complaints, negative acute phase reactants, increase in ferritin levels in two-years period, and detection of HFE gene mutation.

Today, C282Y and H63D mutations are detected in whole blood by polymerase chain reaction (PCR). Accumulation of iron in the body and detection of homozygous C282Y mutation is sufficient for the diagnosis of the disease [15]. C282Y homozygosity is the most common mutation for hemochromatosis and proceeds with maximum iron accumulation. While the patients with both C282Y/H63D heterozygosity show moderate iron accumulation, H63D homozygotes and C282Y heterozygotes are usually normal [16]. These two mutations are previously defined in Turkish population [7]. Genetic analysis may be useful for diagnosis of hemochromatosis. However, only for patients with hepatic fibrosis and cirrhosis liver biopsy is supportive [17].

Alpha-thalassemia is a hereditary anemia that results from a defective synthesis of alpha-globin. Revised alpha-thalassemia can be inherited or acquired and is originated by defects or deletions in one or more genes of the four alpha-globin genes. Hypochromic and microcytic cells characterize alpha-thalassemia [18].

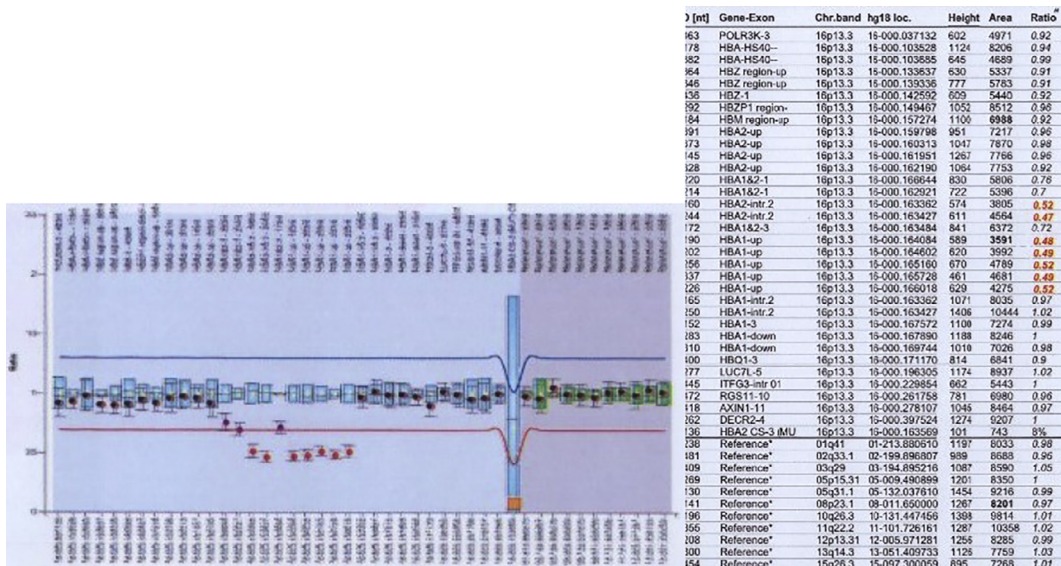


Fig. 1. (A and B) Heterozygous  $\alpha$  thalassemia  $-3.7$  deletion determined by MLPA method (SALSA MLPA P140 HBA probemix, MRC Holland).

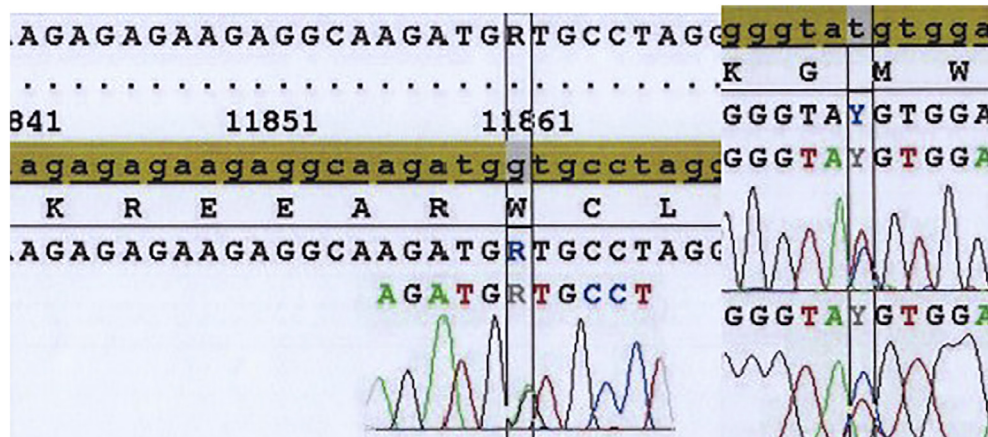


Fig. 2. (A) Heterozigot c.1007–47G>A mutation, (B) Heterozigot c.340+4T>C mutation.

Agostinho et al. showed that the allelic frequencies of H63D mutation in the alpha-thalassemia for Caucasians was 13.70%. and for the C282Y mutation the frequency observed 2.38% [19]. Besides, Pietrangelo et al. found compound heterozygous patients (H63D/C282Y) in the alpha-thalassemic patients [20].

In a study carried out in Hong Kong, iron overload in alpha-thalassemia was not related to hemochromatosis mutations [21]. However, more data about the role of *HFE* mutations in alpha-thalassemic patients are still necessary.

#### 4. Conclusion

Although there are no complaints or symptoms, hemochromatosis can be detected incidentally by routine pediatric examination and blood analysis, and its diagnosis can be made easily by mutation analysis.

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