*Turk J Gastroenterol 2007; 18 (1): 53-57*

# Definition of C282Y mutation in a hereditary hemochromatosis family from Turkey

## Türkiye'de C282Y mutasyonu saptanan bir herediter hemokromatoz ailesi

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*Hereditary hemochromatosis is an autosomal recessive disorder associated with the mutation of the HFE gene. C282Y and H63D mutations in this gene have been described. Hereditary hemochromatosis is primarily associated with the C282Y mutation; the importance of H63D is not well known. In previously reported studies, the C282Y mutation was not detected in Turkey. We herein present a family in which the C282Y mutation was detected. A consanguineous marriage produced 10 children. A 33-year-old man (index case) was diagnosed with hemochromatosis (transferrin saturation rate 80%, ferritin 514 ng/ml, liver biopsy showed +3 iron accumulation, liver involvement in MRI), and genetic analysis showed homozygous C282Y mutation. With family screening, another brother was also diagnosed with hemochromatosis. Transferrin saturation rate was high (>45%) in seven healthy brothers and the father. Genetic analysis revealed two cases with C282Y homozygous mutations, three with C282Y/H63D compound heterozygous mutations, one C282Y heterozygous and three H63D heterozygous among the family members. This is the first family in Turkey in which the C282Y mutation has been detected.*

**Key words:** Hemochromatosis, HFE, C282Y, Turkey, mutation

*Herediter hemokromatoz otozomal resesif kalıtsal bir hastalıktır ve HFE gen mutasyonu ile ilişkilidir. Bu gende C282Y ve H63D mutasyonları tanımlanmıştır. Herediter hemokromatoz çoğunlukla C282Y mutasyonu ile ilişkili bulunmuştur. Saptanan H63D mutasyonunun ise önemi bilinmemektedir. Ülkemizde, önceki çalışmalarda C282Y mutasyonu bulunmamıştır. Bu yazıda Hemokromatoz tanısı alan indeks vakanın araştırılması ile Türkiye'de ilk kez C282Y mutasyonu saptanan bir hemokromatoz ailesi bildirilmiştir. Toplam 10 çocuklu olan ailede, anne ve baba teyze çocukları idi. 33 yaşında erkek hastanın (indeks vaka), yapılan rutin tetkiklerinde Ts: %80, ferritin: 514 ng/ml saptanması üzerine yapılan incelemelerde (Batın MRG'de karaciğerde hemokromatoz ile uyumlu tutulum, karaciğer biyopsisinde hepatositlerde 3(+) demir birikimi) hemokromatoz tanısı konuldu. Genetik analizde C282Y homozigot mutasyonu saptandı. Bunun üzerine incelenen aile bireylerinde bir erkek kardeşe daha hemokromatoz tanısı konuldu. Sağlıklı olan diğer kardeşlerin 7'sinde ve babada transferrin saturasyonu >%45, 1 tanesinde ise transferrin saturasyonu <%45 olarak saptandı. Genetik analizde 2 kişide C282Y homozigotluğu, 3 kişide C282Y/H63D bileşik heterozigotluğu, 1 kişide C282Y heterozigotluğu ve 3 kişide H63D heterozigotluğu saptandı. Böylece, Türkiye'de ilk kez C282Y mutasyonu bulunan hemokromatoz ailesi tanımlandı.*

**Anahtar kelimeler:** Hemokromatoz, HFE, C282Y, Türkiye, mutasyon

## INTRODUCTION

Hereditary hemochromatosis (HH) is the most frequent (2-5/1000) genetic liver disease in Caucasians (1). Therefore, screening for the HFE mutation is important in these countries. In HH, the most important causes of death are complications of cirrhosis and hepatocellular carcinoma (HCC). Phlebotomy and deferoxamine therapy can prevent liver cirrhosis and HCC, thus early diagnosis is very important in hemochromatosis and secondary iron overload disease (2). Before the HFE gene mutation was defined, transferrin saturation rate (TSR) had been used as a screening test. Detection of HFE gene mutation confirms the diagnosis and also helps in the diagnosis of hemochromatosis before clinical symptoms and signs appear (3). Thus, screening with TSR and HFE gene mu-

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**Manuscript received:** 08.06.2006 **Accepted:** 19.10.2006

tation analysis is important for early diagnosis in the relatives of hemochromatosis patients. In Turkey, the C282Y mutation was not previously detected in healthy blood donors and hemochromatosis patients. The impact of the detected H63D mutation is not clear (4-7). We herein present an HH family in which the C282Y mutation was detected.

## MATERIALS AND METHODS

In all family members, whole blood count and biochemical tests [iron, total iron binding capacity (TIBC), ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactic dehydrogenase (LDH), glucose, total protein, albumin, gamma globulin, prothrombin time (PT), blood urea nitrogen (BUN), creatinine, bilirubin, cholesterol, and triglyceride] were analyzed.

### DNA Isolation

Five ml of peripheral blood samples were collected from each patient and DNA isolation was performed with High Pure PCR Template Preparation Kit (Roche Diagnostic, Germany) according to manufacturer's instructions.

### Mutation Detection

A total volume of 40 μL was used for the PCR reactions. Each sample was amplified by two sets of primers *(C282Y F5'-tggcaagggtaaacagatcc, C282YR 5'-ctcaggcactcctctcaacc and H63DF5'-acatggttaaggcctgttgc, H63DR5'- gccacatctggcttgaaatt)* (8). A negative control was always used to avoid contamination. The annealing temperature was 61˚C for C282Y and 58˚C for H63D. After the confirmation of PCR products by 2% agarose gel electrophoresis, the PCR products were digested at 37˚C by RsaI enzyme for the detection of C282Y mutation. Genotyping was performed on 4% agarose gel electrophoresis. Amplification product of C282Y was 389 bp, and after the digestion, the samples which produced two fragments of 249 bp and 140 bp were genotyped as wild type; 249bp, 140bp, 111bp and 29bp were genotyped as heterozygotes; and 249bp, 111bp and 29bp were genotyped as homozygote mutant. For the genotyping of H63D mutation, the amplification product of 208bp was digested at 55˚C by BclI enzyme. Wild type genotypes were digested to 138bp and 70 bp fragments, whereas heterozygotes were digested to 208bp, 138bp and 70 bp; in the case of homozygote mutation, no digestion was observed.

### Case 1

Hs-A, a 33-year-old man, was referred to our department for further analyses because of high TSR 80% (N:<60%), Fe 173 ug/dl (50-175), TIBC 214 ug/dl (250-410) and serum ferritin level 514 ng/ml (21-274). He consumed alcohol for 13 years (160 g/day). Physical examination was normal. Serum AST and ALT levels were 14 IU/L (5-42) and 10 IU/L (5-45), respectively. Magnetic resonance imaging (MRI) revealed iron overload in liver. Liver biopsy showed 3+ iron overload. Hepatitis B surface antigen and antibody to hepatitis C virus were negative. C282Y homozygote mutation was detected by genetic analysis. Phlebotomy was performed twice per week (total 22 sessions), and then maintenance phlebotomy continued (last serum ferritin level 9 ng/ml, Hb 14 g/dl).

### Case 2

İ-A, his 47-year-old brother, had skin rash, pruritus, fatigue and hyperpigmentation. He had arthritis in his medical history. Hepatomegaly (2 cm below the costal margin) was found in his physical examination. Laboratory analyses were as follows: Fe 235 ug/dl, TIBC 245 ug/dl, TSR 95%, ferritin 6691 ng/ml, AST 63 IU/L, and ALT 76 IU/L. Viral serology was also negative. Iron overload was detected in liver by MRI. Liver biopsy revealed focal nodularity and +3 iron overload. C282Y homozygous mutation was detected by genetic analysis. Phlebotomy was performed twice per week (total 36 sessions), then maintenance phlebotomy was continued (last serum ferritin level 44 ng/ml, Hb 11 g/dl).

### Case 3

Hay-A, a 45-year-old sister, was admitted for further analyses after her two brothers had been diagnosed with hemochromatosis. She was suffering from fatigue and infertility. She had menopause for four years and was diagnosed with osteoporosis. She had received hormone replacement therapy for three years. Physical examination was normal. Laboratory analyses were as follows: Fe 151 ug/dl, TIBC 216 ug/dl, TSR 69%, ferritin 491 ng/ml, AST 20 IU/L, and ALT 19 IU/L. Since DNA could not be isolated, mutation analyses were not performed. Phlebotomy was performed once a month.

### Case 4

Hac-A, a 33-year-old sister, was also admitted for further analysis because of family history. She

was also suffering from infertility like Hay-A. Hs-A and Hac-A were twins. Physical examination was normal. Laboratory analyses were as follows: Fe 165 ug/dl, TIBC 262 ug/dl, TSR 62%, ferritin 180 ng/ml, AST 15 IU/L, and ALT 11 IU/L. H63D heterozygous mutation was detected by genetic analysis. Phlebotomy was performed twice a week, and then maintenance phlebotomy was continued (last serum ferritin level 116 ng/ml, Hb 12 g/dl).

**Case 5**

Hur-A, a 44-year-old sister, was admitted because of her family history. Physical examination and laboratory analysis were normal (Fe 87 ug/dl, TIBC 326 ug/dl, TSR 26%, ferritin 26 ng/ml, AST 18 IU/L, and ALT 17 IU/L ) and no mutation was detected by genetic analysis.

**Case 6**

Ab-A, their 78-year-old father, was also examined because of family history. Physical examination was normal and laboratory analysis results were as follows: Fe 227 ug/dl, TIBC 343 ug/dl, TSR 66%, ferritin 79 ng/ml, AST 23 IU/L, and ALT 39 IU/L. Genetic analysis showed C282Y/H63D as compound heterozygous.

**Case 7**

Hnf-A, a 50-year-old sister, was screened because of her family history and she had no complaint. Laboratory analyses were as follows: Fe 250 ug/dl, TIBC 375 ug/dl, TSR 66%, ferritin 24 ng/ml, AST 22 IU/L, and ALT 16 IU/L. C282Y/H63D compound heterozygous mutation was detected by genetic analysis.

**Case 8**

Hu-A, a 42-year-old sister, was also screened because of her family history. Hepatomegaly was found in her physical examination. Laboratory analyses were as follows: Fe 149 ug/dl, TIBC 249 ug/dl, TSR 59%, ferritin 69 ng/ml, AST 22 IU/L, and ALT 19 IU/L. H63D mutation was detected in one allele.

**Case 9**

M-A, a 41-year-old brother, was admitted because of his family history. Physical examination was normal. Laboratory analyses were as follows: Fe 123 ug/dl, TIBC 183 ug/dl, TSR 67%, ferritin 146 ng/ml, AST 24 IU/L, and ALT 25 IU/L. Genetic analysis revealed H63D mutation in one allele.

**Case 10**

Ah-A, a 53-year-old brother, had a normal physical examination. Laboratory results were as follows: Fe 151 ug/dl, TIBC 251 ug/dl, TSR 60%, ferritin 245 ng/ml, AST 20 IU/L, and ALT 15 IU/L. C282Y heterozygous mutation was detected.

**Case 11**

E-A, a 51-year-old brother, was admitted because of family history. He had five-year history of alcohol consumption (160 g/day twice per week). Laboratory results were as follows: Fe 207 ug/dl, TIBC 238 ug/dl, TSR 86%, ferritin 267 ng/ml, AST 28 IU/L, and ALT 30 IU/L. Genetic analysis showed C282Y/H63D compound heterozygous mutations.

**Case 12**

Hur-A, a 44-year-old sister, had normal physical examination and biochemical analysis (Fe 87

**Table 1.** Demographic and biochemical findings of hemochromatosis family

| | Age | Gender | Fe (50-175) (ug/dl) | TIBC (ug/dl) (250-410) | TSR (%) | Ferritin (ng/ml) | AST (5-42) | ALT (5-45) | MRI | Liver histology | H63 D | C282 Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hs-A | 33 | M | 173 | 214 | 80 | 514 | 14 | 10 | Liver | 3 (+) iron | (-) | + / + |
| İ-A | 47 | M | 235 | 245 | 95 | 6691 | 63 | 76 | Liver | Focal nodular 3 (+) iron | (-) | + / + |
| Ab-A | 78 | M | 227 | 343 | 66 | 79 | 23 | 39 | | | + / - | + / - |
| Hnf-A | 50 | F | 250 | 375 | 66 | 24 | 22 | 16 | | | + / - | + / - |
| Hü-A | 42 | F | 149 | 249 | 59 | 69 | 22 | 19 | | | + / - | (-) |
| M-A | 40 | M | 123 | 183 | 67 | 146 | 24 | 35 | | | + / - | (-) |
| Ah-A | 53 | M | 151 | 251 | 60 | 245 | 20 | 15 | | | (-) | + / - |
| E-A | 51 | M | 207 | 238 | 86 | 267 | 28 | 30 | | | + / - | + / - |
| Hur-A | 44 | F | 87 | 326 | 26 | 26 | 18 | 17 | | | (-) | (-) |
| Hay-A | 45 | F | 151 | 216 | 69 | 491 | 20 | 19 | | | | |
| Hac-A | 33 | F | 165 | 262 | 62 | 180 | 15 | 11 | | | + / - | (-) |

TSR: Transferrin saturation rate [female (N:<50%), male (N:<60%)], Ferritin: Female (N: 4.6-204), Male (N: 21-274)

ug/dl, TIBC 326 ug/dl, TSR 26%, ferritin 26 ng/ml, AST 18 IU/L, ALT: 17 IU/L), and no mutation was detected by genetic analysis.

In total, two brothers were diagnosed with hemochromatosis in a family from Giresun (Tirebolu) (Table 1). TSR was high (>45) in seven healthy children and the father, and normal (<45) in one. Demographic and biochemical findings are shown in Table 1.

## DISCUSSION

Survival rate in HH is similar with that in a healthy population if therapy begins before cirrhosis and diabetes occur. Therefore, early diagnosis is very important (2). TSR and serum ferritin levels were used for screening before gene mutations were defined. However, TSR can be normal in early decades in HH. Specificity, sensitivity and predictivity rates of TSR were 93%, 92%, 86%, respectively, in women (TSR >50%) and men (TSR >60%). If TSR baseline limit is accepted as 45% for diagnosis, specificity and predictivity will decrease (8).

The responsible gene for HH was defined in 1996 and named as HFE. C282Y and H63D mutations have been described in the HFE gene (9). These mutations were detected in 65-100% of cases that had clinical features of HH. The C282Y mutation is the more important one for HH. Determination of the HFE gene mutation confirms the diagnosis and also facilitates disease diagnosis in the asymptomatic period. Thus, HFE gene mutation screening and TSR analysis are important for early diagnosis in the relatives of patients diagnosed with hemochromatosis. The relationship between the H63D gene mutation and iron overload has not been clear in previous studies (l0). H63D phenotype expression is complex. It is suggested that the H63D mutation is a predisposing factor in iron overload with the other genetic mutations (for example: C282Y heterozygote) and environmental factors (11).

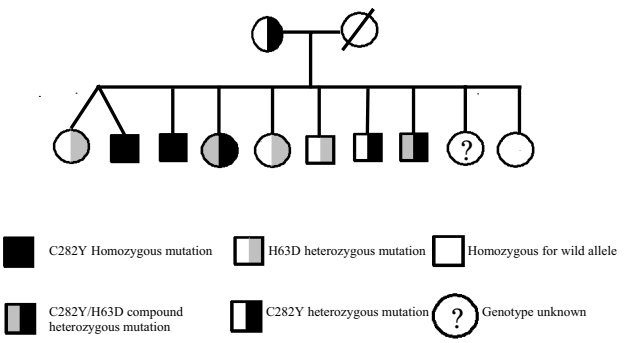


**Figure 1.** Distribution of C282Y and H63D mutations in hemochromatosis family pedigree

In our country, there have been limited studies about HFE gene mutations. H63D mutation was detected in healthy donors and hemochromatosis patients but not C282Y in the limited studies in Turkey (4-7). The impact of the H63D mutation found in our country is not known.

In this reported family, we first detected the HFE gene mutation in one of the brothers, and then performed further analyses in the other family members. TSR was high (>45%) in seven healthy children and the father and normal (<45%) in one. Genetic analysis revealed two patients with C282Y homozygous mutation, three sibs with C282Y/H63D compound heterozygous mutation, one with C282Y heterozygous and three with H63D heterozygous mutations in the whole family. DNA could not be isolated in only one member of the family (Figure 1). Detection of the C282Y mutation at the Black Sea coast in Turkey can be accepted as a finger print of Celts migration to this area, as the HFE gene mutation was generally detected in Celtics (12) and it is known that Celtics migrated to northern Turkey (13).

In conclusion, our study presents the first detection of C282Y in Turkey. Further epidemiologic investigations must be performed in the Black Sea region.

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