

# **H63D But Not C282Y Mutation of HFE Gene Is Contributed In Acute Myocardial Infarction**

**Shahrokh Iravani**, AJA Cancer Research Center (ACRC), AJA University of Medical Sciences, Tehran, Iran; E. mail: aja.imedres@gmail.com

**Zahra Shaker**, AJA Cancer Research Center (ACRC), AJA University of Medical Sciences, Tehran, Iran; E. mail: aja.imedres@gmail.com

**Amin Ayatipour**, AJA Cancer Research Center (ACRC), AJA University of Medical Sciences, Tehran, Iran; E. mail: aja.imedres@gmail.com

**Hamed Naghoosi\***, Biotechnology Department, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran; E. mail: h.naghoosi@gmail.com

**Pedram Azimzadeh**, Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; E. mail: azimzadeh.pedram@gmail.com

\* **Correspondence to:** Hamed Naghoosi, Biotechnology department, Iranian research organization for science and technology (IROST), Tehran, Iran; E. mail: h.naghoosi@gmail.com

Received date: 27 June 2014; accepted date: 22 October 2014

Academic editor: Kotze, Maritha J

## **Abstract**

HFE mutation related hemochromatosis is one of the most common causes of iron overload in north European populations. The aim of present study was to investigate the association of HFE gene mutations in susceptibility to Acute Myocardial Infarction (AMI) among a sample of Iranian population. We analyzed two major mutations of HFE gene, H63D at exon2 and C282Y located in exon4 in 131 patients with AMI and 130 healthy controls. There were a significant difference in the distribution of Codon 63 mutation of the HFE gene among AMI cases and controls but there was no relationship between C282Y mutation and risk of AMI. Our results suggest that the H63D mutation of HFE gene is associated with susceptibility to AMI in Iranian population.

**Keywords:** Hemochromatosis, HFE gene, Mutation, Acute Myocardial Infarction

## **Introduction**

Hereditary Hemochromatosis is an iron metabolism disorder that causes accumulation of iron in liver, thyroid gland, pancreas and pituitary gland. Hemochromatosis is usually categorized in early steps, which is genetically inherited in families, and secondary forms. Hereditary hemochromatosis is one of the most frequent genetic disorders with an incidence of 0.2 to 0.5% in northern European people (Bacon, 2001). Formerly, diagnosis of such disease was based on detection of excess of iron in liver tissue and serum levels of iron and transferrin (Scheuer et al., 1962, Bassett et al., 1986). By discovery of HFE gene and its mutations, diagnostic procedures have been moved toward the genetic tests. It has been shown that HFE gene encodes a MHC-I like protein that normally interacts with  $\beta_2$ -microglobulin (Van Der et al., 2006). Previously published data showed that more than eighty percent of patients with clinically confirmed hemochromatosis status are homozygous for the C282Y mutation (Beutler, 1997) that substitutes cysteine residue on position 282 by a tyrosine residue and distorts the association of HFE and  $\beta_2$ -microglobulin (which is normally via disulfide bond) and results in higher uptake of iron by enterocytes leading to systemic iron overload (Van Der et al., 2006).

H63D is another common mutation of HFE gene that leads a histidine substitution by aspartic acid, and does not influence on HFE/ $\beta_2$ -microglobulin interaction. Although its biochemical mechanism of action is not clear, however it is shown that H63D mutation is in association with increased serum transferrin saturation but not results in iron overload (Gochee et al., 2002, Van Der et al., 2006).

Despite all previous studies, the relationship between HFE gene mutations with iron overload is not completely clear. Siroaska et al. in 2011 assayed the hypothesis and found no significant association between iron disorders and HFE mutations (Sikorska et al., 2011). Nassar et al. in 1998 revealed that there was a higher iron value in male subjects suffering from coronary artery diseases but this relationship was not contributed to the C282Y or H63D mutations of HFE gene. So there could be another mechanism for effect of iron overload on liver or heart diseases (Nassar et al., 1998).

HFE gene is located on chromosome 6:37.p10 and encodes Human hemochromatosis protein. Two major genetic variations in the HFE gene sequence are single point mutations including 845A (Codon 282) which leads cysteine to tyrosine change and 187G in which a histidine substitutes by aspartic acid (Codon 63). The aim of this study was to find out the association of two major HFE gene mutations (H63D and C282Y) with the risk of occurrence of AMI among Iranian patients.

## **Materials and Methods**

### **Study population**

A total of 261 individuals with same ethnic basis were included in the study. We conducted a case-controlled study on 131 Iranian patients suffering from AMI and 130 ethnic matched unrelated healthy control subjects who had no history of heart diseases. Serum Ferritin level, Triglycerides and Total Cholesterol were measured in all subjects.

## DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood using standard phenol-chloroform protocol (Sambrook, 2001). DNA amplification was performed by the Polymerase Chain Reaction (PCR) using oligonucleotide primers, followed by Restriction fragment length polymorphism for genotype determination. In brief, for detection of H63D mutation, PCR amplification of exon 2 of HFE gene was conducted by following primers: Forward, 5'-GCCACATCTGGCTTGAAATT-3' Reverse, 5'- CAGCCCATCCCCTAACCAAAG-3' and the PCR product was digested with BclI restriction enzyme (Fermentas, Lithuania). For detection of C282Y mutation, PCR amplification of exon 4 of HFE gene was conducted using following primers: Forward, 5'-CAGCCCATCCCCTAACCAAAG-3', Reverse 5'-CCTTCCTCCAACCTATAGAA-3' and the PCR product was digested with RsaI restriction enzyme (Fermentas, Lithuania).

## Statistical Analysis

Statistical analysis was performed by SPSS software version 20 (IBM SPSS Statistics; SPSS, Chicago, IL) and P values calculated lower than 0.05 were considered as significant. To compare two included groups (AMI Cases and Healthy individuals) for genotype status we conducted Chi-squared test and for comparison of the quantitative variables such as age we used Students T test. The Hardy-Weinberg equilibrium was also calculated by Chi-squared test using Court Lab software.

## Results

Mean age was  $56.97 \pm 11.44$  years in patients group and  $55.77 \pm 12.53$  years in healthy control individuals. There was no significant difference between case and control groups according to their age and gender status. Total cholesterol was significantly higher in AMI cases as compared to control group ( $P=0.014$ ). Serum Ferritin level in AMI patients was significantly higher than control individuals ( $P=0.005$ ). **Table-1** lists the basic information and laboratory characteristics of two studied groups. Thirty (22.9%) of AMI patients and 24 (18.5%) controls were C282Y heterozygote, whereas there were 7 (5.3%) AMI cases and 4 (3.1%) controls with C282Y homozygous genotype. Among 131 AMI patients, 48 (36.6%) had the heterozygous genotype for H63D mutation compared to 18 (13.8%) of 130 healthy control subjects. Frequency of H63D homozygous mutation was 9 (6.9%) in AMI patients and 5 (3.8%) in controls.

There was a significant difference among individuals with one or two mutations in contrast to non-mutated individuals between case and control group ( $p=0.000$ ). By omitting individuals with no mutation, no significant difference was seen between individuals with one or two mutations in case and control groups ( $p=0.056$ ).

The summary of results and distribution of C282Y and H63D mutations among two studied groups is shown in **Table-2**.

Genotype and allele distribution were also in Hardy-Weinberg equilibrium in one and two mutation groups in both cases and control individuals ( $p > 0.05$ ).

## Discussion

In the present study, we tried to investigate the relationship between two major mutations in HFE gene (H63D and C282Y) and susceptibility to acute myocardial infarction in a group of Iranian population. Our results show that H63D mutation is significantly associated with higher susceptibility to AMI, however, there is not a significant correlation between carrying of one or two mutations and risk of AMI development.

Hemochromatosis is known as a hereditary (autosomal recessive) trait that causes disorders in metabolism, absorption, and storage of iron in several tissues of body. HFE Exon4 C282Y mutation is evidently related with hereditary hemochromatosis but the role of H63D mutation in development of iron overload disorders is controversial. Hence, it encourages researchers to study the relationship between these genetic variations and risk of AMI among our population. Present case-controlled study was aimed to compare the prevalence of HFE gene mutations (H63D and C282Y) among Iranian patients with AMI and healthy control subjects. Tuomainen et al. performed a prospective cohort study in men in Finland and found that there is a strong association between C282Y mutation of HFE gene and increased risk of AMI. They also declared that men carriers of this mutation are at 2 fold risk for first AMI in comparison with subjects without mutation (Tuomainen et al., 1999). Hetet et al. reviewed the results of three studies previously performed on HFE gene mutations and cardiovascular diseases; they concluded that there is no sufficient evidence for this relationship (Hetet et al., 2001). Davis et al. in 2008 examined the association of iron overload, HFE gene mutations, and type2 diabetes. They reported no significant relationship between C282Y / H63D mutations or clinical features and outcomes of type2 diabetes (Davis et al., 2008).

Several research groups have studied the HFE mutations and patients' susceptibility to hereditary hemochromatosis and other disorders among Iranian population but to our knowledge the association of these mutations with Iranian patients suffering from AMI has not been previously investigated. Bonkovsky et al. in their study on Hepatitis B (HBV) patients declared that the C282Y mutation is in association with an increase of the possibility of persistence of viral infection and probable development of liver disorders (Sendi et al., 2005). Ghaziani et al. also tested the relationship of these mutations with HBV infection and Serum iron status. they concluded that H63D has significant effect on progression of hepatic fibrosis (Ghaziani et al., 2007). HFE gene mutations are also studied in Iranian minor beta-thalassaemia patients and the results showed that C282Y and H63D mutations are more common in patients rather than healthy controls (Jazayeri et al., 2003). Jowkar et al assessed the presence of H63D and C282Y mutations among Iranian patients with cryptogenic cirrhosis and showed that HFE mutation mediated hemochromatosis is not a major cause for this kind of liver cirrhosis. However there is some doubt in their patient selection criteria, so their results are not completely reliable (Sendi and Mehrab-Mohseni, 2012, Jowkar et al., 2011). The results of our study revealed that the frequency of H63D mutation in studied patients with AMI is higher compared to healthy control subjects. We also analyzed the effects of carrying one or two mutations simultaneously, and no significant difference in susceptibility to AMI was seen between individuals carrying just one mutation (H63D) and carriers of both mutations (H63D and C282Y).

In conclusion, the present data suggest that carriers of H63D mutation of HFE gene are at higher risk for acute myocardial infarction in comparison with non-carriers, so alteration in codon 63 of HFE protein has significant association with patients' susceptibility to AMI.

### **Acknowledgements:**

The work presented in this article was supported by AJA University of Medical Sciences, Tehran, Iran. The authors are grateful to Shahid Beheshti university research center lab staff for their valuable technical assistance.

### **References:**

1. Bacon, B. R. (2001) "Hemochromatosis: diagnosis and management" *Gastroenterology*, 120, 718-25.
2. Bassett, M. L., Halliday, J. W. and Powell, L. W. (1986) "Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis" *Hepatology*, 6, 24-9.
3. Beutler, E. (1997) "Genetic irony beyond haemochromatosis: clinical effects of HLA-H mutations" *Lancet*, 349, 296-7.
4. Davis, T. M., Beilby, J., Davis, W. A., Olynyk, J. K., Jeffrey, G. P., Rossi, E., Boyder, C. and Bruce, D. G. (2008) "Prevalence, characteristics, and prognostic significance of HFE gene mutations in type 2 diabetes: the Fremantle Diabetes Study" *Diabetes Care*, 31, 1795-801.
5. Ghaziani, T., Alavian, S.-M., Zali, M. R., Shahraz, S., Agah, M., Jensen, K. P., Ansari, S., Sendi, H., Lambrecht, R. W., Covault, J. and Bonkovsky, H. L. (2007) "Serum measures of iron status and HFE gene mutations in patients with hepatitis B virus infection" *Hepatology Research*, 37, 172-178.
6. Gochee, P. A., Powell, L. W., Cullen, D. J., Du Sart, D., Rossi, E. and Olynyk, J. K. (2002) "A population-based study of the biochemical and clinical expression of the H63D hemochromatosis mutation" *Gastroenterology*, 122, 646-651.
7. Hetet, G., Elbaz, A., Garipey, J., Nicaud, V., Arveiler, D., Morrison, C., Kee, F., Evans, A., Simon, A., Amarenco, P., Cambien, F. and Grandchamp, B. (2001) "Association studies between haemochromatosis gene mutations and the risk of cardiovascular diseases" *European Journal of Clinical Investigation*, 31, 382-8.
8. Jazayeri, M., Bakayev, V., Adibi, P., Haghghi Rad, F., Zakeri, H., Kalantar, E. and Zali, M. R. (2003) "Frequency of HFE gene mutations in Iranian beta-thalassaemia minor patients" *Eur J Haematol*, 71, 408-11.
9. Jowkar, Z., Geramizadeh, B. and Shariat, M. (2011) "Frequency of Two Common HFE Gene Mutations (C282Y and H63D) in a Group of Iranian Patients With Cryptogenic Cirrhosis" *Hepat Mon*, 11, 887-889.
10. Nassar, B. A., Zayed, E. M., Title, L. M., O'Neill, B. J., Bata, I. R., Kirkland, S. A., Dunn, J., Dempsey, G. I., Tan, M. H. and Johnstone, D. E. (1998) "Relation of HFE gene mutations, high iron stores and early onset coronary artery disease" *Canadian Journal of Cardiology*, 14, 215-20.
11. Sambrook, J. (2001) *Molecular cloning : a laboratory manual / Joseph Sambrook, David W. Russell*, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory.

12. Scheuer, P. J., Williams, R. and Muir, A. R. (1962) "Hepatic pathology in relatives of patients with haemochromatosis" *Journal of Pathology and Bacteriology*, 84, 53-64.
13. Sendi, H., Ghaziani, T., Zali, M. R., Adibi, P., Agah, M., Jazayeri, M., Shahraz, S., Magnius, L. and Bonkovsky, H. L. (2005) "Hemochromatosis mutations in Iranians with hepatitis B virus infection" *Clin Infect Dis*, 40, 29.
14. Sendi, H. and Mehrab-Mohseni, M. (2012) "HFE Gene Mutations in Cryptogenic Cirrhosis Patients" *Hepat Mon*, 12, 48-9.
15. Sikorska, K., Romanowski, T., Stalke, P., Izycka-Swieszewska, E. and Bielawski, K. P. (2011) "Iron overload and HFE gene mutations in Polish patients with liver cirrhosis" *Hepatobiliary Pancreat Dis Int*, 10, 270-5.
16. Tuomainen, T. P., Kontula, K., Nyysönen, K., Lakka, T. A., Helio, T. and Salonen, J. T. (1999) "Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation : a prospective cohort study in men in eastern Finland" *Circulation*, 100, 1274-9.
17. Van Der, A., Peeters, P., Grobbee, D., Roest, M., Marx, J., Voorbij, H. and Van Der Schouw, Y. (2006) "HFE mutations and risk of coronary heart disease in middle-aged women" *European journal of clinical investigation*, 36, 682-690.