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HEREDITARY HEMOCHROMATOSIS GENE MUTATIONS IN PATIENTS WITH MYOCARDIAL INFARCTION

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Abstract: Hereditary hemochromatosis (HH) is a disorder of iron accumulation in tissues, which is related to coronary heart diseases. Free radicals and reactive oxygen species, created because of iron deposition, promote oxidation of LDL cholesterol and could lead to the development of atherosclerosis. Studies have shown that *HFE* gene mutation carriers might be at higher risk of developing cardiovascular diseases compared with non-carriers.
This study aimed to determine the frequency of *HFE* gene mutations in patients with myocardial infarction compared to a healthy group in eastern Slavonia.
A retrospective case-control study was carried out on a population of 400 participants. In the first group there were 200 patients (114 males and 86 females) with myocardial infarction. The second group consisted of 200 controls (103 males and 97 females) without a history of cardiovascular diseases.
All patients were genotyped for the three most common mutations of the HH in the HFE gene: C282Y, H63D, and S65C, by real-time PCR. The difference in the frequency of carriers of these mutations between the patients and the controls was not significant (C282Y: 4.5 vs. 8.1%; H63D: 19 vs. 24.5%; S65C: 3.5 versus 4%), and neither was the frequency and distribution of possible *HFE* gene genotypes and compound heterozygotes. There were no statistically significant associations of cardiovascular risk factors and *HFE* gene mutations in patients with myocardial infarction.
In this study, no association was found between the *HFE* gene mutation for HH and myocardial infarction in the population of eastern Slavonia.

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INTRODUCTION

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism marked by increased absorption and accumulation of iron in the parenchymal cells of the liver, pancreas, heart and some endocrine organs, which may lead to tissue injury and dysfunction.[1, 2] Based on clinical, biochemical, and genetic characteristics, five types of HH have been identified. The most common mutation is in the *HFE* gene on chromosome 6p21.3, and it is classic hemochromatosis (type 1).[2] In addition to the HFE gene, mutations in the genes that encode hemojuvelin (*HJV*, type 2A), hepcidin (*HAMP*, type 2B), transferrin receptor 2 (*TFR2*, type 3) and ferroportin (*SLC40A1*, type 4) have been associated with the regulation of iron homeostasis and the development of HH.[2, 3] If the transferrin saturation is higher than 45%, and ferritin exceeds local reference ranges, HFE mutations should be investigated.[4, 5]
The hemochromatosis *HFE* gene was discovered in 1996. The *HFE* gene encodes the major histocompatibility complex (MHC) class I-like protein HFE that binds β2-microglobulin. HFE influences iron absorption by modulating the expression of hepcidin, the primary regulator of iron metabolism.[6, 7] In the same year, 1996, two base-pair alterations of the *HFE* gene, termed C282Y and H63D, were identified in hereditary hemochromatosis.[8] The first was the missense mutation at position 282, where cysteine is replaced by tyrosine (p.Cys282Tyr, c.845G>A), the second mutation is the substitution of histidine for aspartic acid at position 63 (p.His63Asp, c.187C>G),

the third mutation is the substitution of cysteine for serine (p.Ser65Cys, c.193A>T).[2] Table 1 presents the data for the genotype frequency of the three most common mutations of the *HFE* gene with a clinical manifestation on iron status.

The penetrance of disease in C282Y homozygotes is incomplete,[9] and for C282Y homozygosity is 13.5%.[10] Penetrance is higher in males between 40 and 60 years of age than in female C282Y homozygotes (28% vs. 1%). 25–35% of C282Y homozygous individuals develop the disease.[9, 11] Only 1-2% of compound heterozygotes C282Y/H63D have a predisposition to develop the disease.[12] It is considered that homozygosity for H63D is not a sufficient genetic cause of iron overload, while S65C mutation may contribute to mild iron overload, with no clinical expression only when inherited in trans with the C282Y mutation.[10] Single heterozygosity had no risk for iron overload.[13]

The proposed mechanism for explaining HH was the disruption of a disulfide bond in the HFE protein that is critical for its binding to β2-microglobulin. The functional loss of HFE in humans has been shown to reduce hepcidin synthesis.[2, 14] The C282Y mutation disrupts a critical disulfide bond in the α3 domain of HFE, destroying the β2-microglobulin binding capacity of HFE and limiting its localization to the cytoplasm. All this leads to iron overload by decreasing the hepatic synthesis of hepcidin.[6, 15, 16] Mutations H63D and S65C affect the α1 binding groove, but do not prevent HFE presentation on cell surfaces.[6]

Iron-dependent cardiovascular disease risk might be related to increased oxidative stress and endothelial dysfunction.[1] An increased body iron store has been shown to be an independent risk factor for myocardial infarction (MI).[1, 17] Iron could promote the formation of reactive oxygen species and enhance lipid peroxidation, an essential step in atherosclerosis and myocardial infarction.[1, 18, 19] Oxygen free radicals presumably damage cells by oxidating various cellular components, and promote the damage that occurs during ischemia and reperfusion.[19, 20] Studies have shown that *HFE* gene mutation carriers might be at higher risk of developing cardiovascular diseases compared with non-carriers.[1]

This study aimed to determine the prevalence of *HFE* gene mutations in hereditary hemochromatosis among patients who had survived the myocardial infarction compared to a healthy group in eastern Slavonia.

MATERIAL AND METHODS

The study was conducted on patients with myocardial infarction at the Clinical Department of Cardiovascular Diseases and Intensive Care at the University Hospital Osijek, Croatia.

Thygesen et al. defined MI by the presence of at least two of the following: typical increase in the biochemical marker of myocardial necrosis - cardiac troponin T (above the 99th percentile), ischemic chest pain symptoms lasting for more than 30 minutes, and electrocardiography (ECG) changes indicative of ischemia (ST-segment elevation or depression).[21]

Systematic information on the past and present medical history was collected from all participants. Body mass index (BMI) was calculated as weight in kilograms divided by square of height in meters. Smokers were participants who currently smoked. All data given were checked in the patients' medical records.

Two hundred healthy sex- and age-matched participants were included in the control group. Their medical documentation did not show any history of cardiovascular diseases. The study was carried out according to the Declaration of Helsinki and its amendments. Written informed consent was obtained from all participants in the study.

In this study, the three most common mutations in the *HFE* gene were genotyped: Cys282Tyr (C282Y, 845G>A), His63Asp (H63D, 187C>G), and Ser65Cys (S65C, 193A>T).

Genomic DNA was obtained from peripheral blood lymphocytes using a QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany. Mutation genotyping was conducted by the real-time PCR method performed on the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) applying TaqMan SNP genotyping assays. The mutation analysis was done using SDS 7500 Software Version 2.3 (Applied Biosystems, Foster City, CA, USA).

Data were expressed as absolute frequencies with percentages or means with standard deviations (SD). The association between the genotypes and cardiovascular risk factors were tested using one-way

Table 1. Genotype frequency of the three most common HFE gene mutations

Genotype	Frequency	Disease manifestation
C282Y homozygote	90%	From no evidence to massive iron overload
C282Y/H63D compound heterozygote	5%	Mild to moderate iron overload
C282Y heterozygote	10%	Normal iron status
H63D homozygote	1%	Mild to moderate iron overload
H63D heterozygote	20%	Normal iron status
S65C homozygote	0.5%	Normal iron status or mild iron overload
C282Y/S65C compound heterozygote	<0.5%	Normal iron status or mild iron overload
S65C heterozygote	1-2%	Normal iron status
H63D/S65C compound heterozygote	<0.5%	Normal iron status or mild iron overload
no *HFE* mutation	<0.001%	Iron overload associated with mutations in other genes

analysis of variance (ANOVA), the Mann-Whitney U test, and Kruskal-Wallis test. Chi-square tests ($\chi 2$) on contingency tables were applied to compare allelic and genotype frequencies in controls and cases. The level of statistical significance was set at $P<0.05$ and all significant values were assessed after correction for the number of independent hypotheses tested. All analyses were adjusted by age and performed using Statistica 12 (StatSoft, Inc., version 12, Tulsa, OK, USA) system for Windows.

RESULTS

The prevalence of cardiovascular risk factors among all participants included in the study sample is summarized in Table 2. The mean age of the study population was 64±13 years, and 54.5% were males. A significant difference between two groups was found for hypertension and smoking. The association of the cardiovascular risk factors and *HFE* gene mutations in MI patients is shown in Table 3.

Table 2. Prevalence of cardiovascular risk factors among all participants included in the study by groups

Variables	MI patients (n=200)	Controls (n=200)	P value
Age year mean (SD)	66±12	62±13	0.086
Gender (males)	114 (57%)	104 (52%)	0.317
BMI* (kg/m^2)	28.7±4.7	26.9±4.3	0.327
Smokers	41 (20.5%)	39 (19.5%)	**<0.001**
Hypertension	107 (53.5%)	59 (29.5%)	**<0.001**
Dyslipidemia	26 (13%)	23 (11.5%)	0.648
Respiratory disease	2 (1.8%)	8 (4%)	0.055
Diabetes mellitus 2	44 (22%)	0	-
Kidney diseases	15 (7.5%)	12 (6%)	0.550
Liver disease	10 (5%)	9 (4.5%)	0.814

*BMI - body mass index; Numerical variables are presented as mean (SD), while categorical variables as number (percentage).

Table 3. The association of cardiovascular risk factors and HFE gene mutations in MI patients

Variables	C282Y	H63D	S65C
Age*	0.919	0.829	0.291
Gender	0.437	NA	0.434
BMI*	0.850	0.807	0.773
Smoking	0.373	0.221	0.489
Hypertension	0.055	0.923	0.179
Dyslipidemia	0.863	0.829	0.918
Respiratory diseases	0.758	0.463	0.787
Diabetes mellitus 2	0.987	0.620	0.176
Thyroid gland diseases	0.266	0.537	0.583
Kidney diseases	0.383	0.155	0.489
Liver diseases	0.482	0.405	0.538

Mann-Whitney U test P-value; * Kruskal-Wallis test P-value; BMI – body mass index; NA - not applicable

We did not find any association between the *HFE* gene mutations and previous MI. The frequency of carriers of mutations between MI patients and controls was not significant (C282Y: 4.5 vs. 8.1%; H63D: 19 vs. 24.5%; S65C: 3.5 versus 4%), and neither was the frequency and distribution of *HFE* genotypes and compound heterozygotes.

Table 4. Genotype frequency of the HFE mutation among study participants

HFE gene mutation	Genotype	Genotype frequency (%)		P
		MI patients	Controls	
C282Y	GG	95.5	92.0	0.148
	AG	4.5	8.0	
	AA	-	-	
H63D	CC	79.0	74.0	0.394
	CG	19.0	24.5	
	GG	2.0	1.5	
S65C	AA	96.5	96.0	0.779
	AT	3.5	4.0	
	TT	-	-	

The H63D mutation was the most common mutation of the three *HFE* gene mutations studied (Table 4). None of the participants were homozygotes for the C282Y mutation or the S65C mutation.

Among the nine C282Y heterozygous MI patients, there was one C282Y/H63D compound heterozygote. However, one C282Y/H63D compound heterozygote was also detected among the controls. Three H63D/S65C compound heterozygotes were found in the control group (Table 5).

Table 5. The prevalence of compound heterozygotes in the studied population

Compound heterozygote	MI patients (%)	Controls (%)
C282Y/H63D	0.5	0.5
C282Y/S65C	0	0
H63D/S6DC	0	1.5

DISCUSSION

There is no evidence for a higher prevalence of the *HFE* gene mutation in MI patients. The number of homozygotes was too small for statistical analysis. There was no significant association between a history of MI and mutations in the *HFE* gene, and our participants did not suffer from clinical HH. Also, there were no statistically significant associations of the 11 covariates and *HFE* gene mutations in patients with myocardial infarction (Table 3).

The most common mutation among our participants was H63D, which is similar to research done by Claeys and coworkers.[1] They showed that the H63D mutation is more common than the C282Y mutation in patients

with a history of cardiovascular diseases (26.5 vs. 12.4%),1 but the exact mechanism which would lead to HH is unknown.

Many studies have found an association of the C282CY mutation with an increased risk of myocardial infarction and coronary heart disease.[16, 18, 22] These studies suggest that the *HFE* gene mutation, especially C282Y, might promote cardiovascular diseases. Body iron status is involved in atherosclerotic cardiovascular disease. High iron status is linked with increased oxidation of LDL.[10, 23] Cells are shielded against iron-induced oxidative destruction by the generation of ferritin. The first study of the association between serum ferritin and cardiovascular diseases was done by Salonen et al.[24] The association between serum ferritin and risk for cardiovascular diseases has been investigated in 20 different studies. However, only in 3 of those studies a statistically significant association was found between ferritin levels and cardiovascular diseases.[25] A possible explanation is that heterozygotes are relatively common (15-20%), so heterozygotes would form a substantial pool of persons at increased risk for cardiovascular diseases.[25] In male Iranian cardiovascular patients, increased ferritin might be an independent predictor of MI.[23] Cardiac myocytes, like hepatocytes, have a high affinity for non-transferrin bound iron. In the heart, an iron overload occurs mainly in the ventricles, where it induces fibrosis and alteration of myocardial fibers. As iron overload severity increases, diastolic dysfunction of the left ventricle occurs, followed by impaired systolic dysfunction.[3] In the study by Gaenzer et al., it was shown that endothelial function is impaired in patients with hemochromatosis and profound iron overload, which could be a presentation of iron-induced oxidative stress.[26]

Carpenter et al. showed that dilated cardiomyopathy is associated with an increased frequency of the H63D mutation, but not the C282Y mutation. The H63D mutation has less effect on iron metabolism compared with C282Y.[27] The most common mutation among our participants was H63D. A higher prevalence of the H63D mutation was observed among MI patients than controls (2 vs. 1.5%). The frequency of the H63D mutation is higher than the C282Y mutation in populations of north-western European origin (40 vs. 20%).[1] Steinberg et al. found that the H63D mutation is more common in white individuals in the USA (15-40%) than C282Y. The prevalence of the C282Y mutation is estimated to be 5.4%, while the H63D mutation is seen in 13.5% of the total US population.[28] The H63D mutation is cosmopolitan, but its frequency is highest in whites of European descent, although the multicentric origin of this mutation, especially in Asia, cannot be excluded.[6]

Differences in levels of stored iron could explain the difference in the incidence of heart disease between men and women.[19] Men eat more red meat and have a higher intake of heme, which could lead to an increased risk of myocardial infarction, while exercise is associated with reduced mortality from cardiovascular disease.[19] A positive association of the *HFE* gene mutation and a higher risk from cardiovascular disease was found in women.[22] However, women have a lower incidence of coronary artery disease due to lower iron levels from menstruation, and increased incidence of cardiovascular diseases.[27] Male carriers of the C282Y mutation are at a higher risk from first myocardial infarction compared with non-carriers.[16] De-ironing therapy might prevent the potential tissue damage, such as cardiomyopathy of iron overload, by phlebotomy.[4, 29]

The effect of hemochromatosis on cardiovascular disease incidence is most pronounced in younger individuals.[16] If we assume that iron plays an important role in cardiovascular diseases, other genetic factors that determine iron status may lead to confusion or reduce the risk of hemochromatosis in specific populations. This problem might become more important when research involves elderly participants who usually have more iron replete.[1] In this study, the average age of participants was 65, and it is possible that the observed prevalence rate of homozygous and, perhaps of heterozygous, for the *HFE* mutations is underestimated due to survival bias.[16]

The majority of prospective studies have not shown the association of serum ferritin or other iron measurements with cardiovascular disease.[30] The effects of HFE on serum ferritin are lower compared to effects on serum iron and the saturation of serum transferrin with iron. Other unknown genes have an effect on iron stores that are considerably higher than those of *HFE*.[31] Differences in study designs and ethnic backgrounds, with different gene to gene or genes with the environment interactions, might be reasonable explanations for the opposite results. Genetic polymorphisms on other locus and non-genetic differences probably play a significant role in determining the iron status. If iron is involved, other factors can easily lead to confusion about the association between cardiovascular risk and heterozygosity of the *HFE* mutation in different studies.[30] The *HFE* gene is located in a gene-rich region where the most important is the *HLA* gene. The increased risk for MI could be modified by mutations of some other genes that are not related to iron metabolism but are coinherited with *HFE* gene mutations.[16] Finally, in the *HLA* gene region there are many other genes that encode for proteins involved in immune-inflammatory responses.[30]

We should consider some limitations of the study. The sample size is relatively small and included patients who had survived MI. Our sample had the advantage of being relatively homogenous for demographic variables such as age, ethnicity, and social environment, as well as cardiovascular risk factors. The first bias is selective mortality; only MI survivors are included. Surviving MI patients had endothelial dysfunction and/or atherosclerosis. The second bias is that serum ferritin levels were not measured as the

biochemical marker for HH, but it is known that ferritin levels in blood increase after MI. Another limitation of the study was lack of the measurement of the biomarkers of oxidative stress.

In conclusion, the C282Y, H63D, and S65C mutations in the *HFE* gene did not enhance the risk of MI in our patients with a history of myocardial infarction. The most exciting finding is that the most common mutation in this study was H63D. Our results, showing that predisposition to MI is not linked to *HFE* mutations, will require confirmation in a larger cohort.

REFERENCES

1. Claeys D, Walting M, Julmy F, Wuillemin WA, Meyer BJ. Haemochromatosis mutations and ferritin in myocardial infarction: a case-control study. Eur J Clin Invest. 2002;32:3-8.
2. Santos PCJL, Krieger JE, Pereira AC. Molecular diagnostic and pathogenesis of hereditary hemochromatosis. Int J Mol Sci. 2012;13:1497-1511.
3. Bardou-Jacquet E, Brissot P. Diagnostic Evaluation of Hereditary Hemochromatosis (HFE and Non-HFE). Hematol Oncol Clin North Am. 2014;28:625-635.
4. Swinkels DW, Jorna ATM, Raymakers RAP. Synopsis of the Dutch multidisciplinary guideline for the diagnosis and treatment of hereditary haemochromatosis. Neth J Med. 2007;65:452-455.
5. Zoller H, Cox TM. Hemochromatosis: genetic testing and clinical practice. Clin Gastroenterol Hepatol. 2005;3:945-958.
6. Barton JC, Edwards CQ, Acton RT. HFE gene: Structure, function, mutations, and associated iron abnormalities. Gene. 2015;574:179-192.
7. Sood R, Bakashi R, Hegade VS, Kelly SM. Diagnosis and management of hereditary haemochromatosis. Br J Gen Pract. 2013;63:331-332.
8. Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2006;145:209-223.
9. Porto G, Brissot P, Swinkels DW, Zoller H, Kamarainen O, Patton S, et al. EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis (HH). Eur J Hum Genet. 2016;24:479-495.
10. European Association For The Study Of The Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol.;53:3-22.
11. Mohamed M, Phillips J. Hereditary haemochromatosis. BMJ. 2016;353:i3128.
12. Pietrangelo A. Hereditary hemochromatosis. Biochim Biophys Acta - Mol Cell Res. 2006;1763:700-710.
13. Neghina AM, Anghel A. Hemochromatosis Genotypes and Risk of Iron Overload—A Meta-Analysis. Ann Epidemiol. 2011;21:1-14.
14. Bacon BR, Adams PC, Kowdley K V., Powell LW, Tavill AS, American Association for the Study of Liver Diseases. Diagnosis and management of hemochromatosis: 2011 Practice Guideline by the American Association for the Study of Liver Diseases. Hepatology. 2011;54:328-343.
15. Brissot P. Diagnosis and current treatments for primary iron overload. Am J Hematol. 2007;82:1140-1141.
16. Tuomainen TP, Kontula K, Nyyssönen K, Lakka TA, Heliö T, Salonen JT. Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation : a prospective cohort study in men in eastern Finland. Circulation. 1999;100:1274-1279.
17. Adams PC. Hemochromatosis. Clin Liver Dis. 2004;8:735-53.
18. Ma J, Stampfer MJ. Body Iron Stores and the Risk of Coronary Heart Disease. Clin Chem. 2002;48:601-603.
19. de Valk B, Marx JJ. Iron, atherosclerosis, and ischemic heart disease. Arch Intern Med. 1999;159:1542-1548.
20. Adams PC, Barton JC. How I treat hemochromatosis. Blood. 2010;116:317-325.
21. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. Circulation. 2012;126:2020-35.
22. Pardo Silva MC, Njajou OT, Alizadeh BZ, Hofman A, Witteman JCM, van Duijn CM, Janssens AC. HFE gene mutations increase the risk of coronary heart disease in women. Eur J Epidemiol. 2010;25:643-649.
23. Haidari M, Javadi E, Sanati A, Hajilooi M, Ghanbili J. Association of increased ferritin with premature coronary stenosis in men. Clin Chem. 2001;47:1666-1672.
24. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation. 1992;86:803-811.
25. Sempos CT. Do body iron stores increase the risk of developing coronary heart disease? Am J Clin Nutr. 2002;76:501-3.
26. Gaenzer H, Marschang P, Sturm W, Neumayr G, Vogel W, Patsch J, Weiss G. Association between increased iron stores and impaired endothelial function in patients with hereditary hemochromatosis. J Am Coll Cardiol. 2002;40:2189-2194.
27. Carpenter J-P, Grasso AE, Porter JB, Shah F, Dooley J, Pennell DJ. On myocardial siderosis and left ventricular dysfunction in hemochromatosis. J Cardiovasc Magn Reson. 2013;15:24.
28. Steinberg KK, Cogswell ME, Chang JC, Caudill SP, McQuillan GM, Bowman BA, Grummer-Strawn LM, Sampson EJ, Khoury MJ, Gallagher ML. Prevalence of C282Y and H63D mutations in the hemochromatosis (HFE) gene in the United States. JAMA. 2001;285:2216-2222.
29. Tavill AS, Adams PC. A diagnostic approach to hemochromatosis. Can J Gastroenterol. 2006;20:535-540.
30. Candore G, Balistreri CR, Lio D, Mantovani V, Colonna-Romano G, Chiappelli M, Tampieri C, Licastro F, Branzi A, Averna M, Caruso M, Hoffmann E, Caruso C. Association between HFE mutations and acute myocardial infarction: a study in patients from Northern and Southern Italy. Blood Cells Mol Dis. 2003;31:57-62.
31. Whitfield JB, Cullen LM, Jazwinska EC, Powell LW, Heath AC, Zhu G, Duffy DL, Martin NG. Effects of HFE C282Y and H63D polymorphisms and polygenic background on iron stores in a large community sample of twins. Am J Hum Genet. 2000;66:1246-1258.