The association between HFE p.C282Y gene polymorphism with cardiac biomarker in coronary artery diseases

Noor Kitab Al-Hasnawi [a]*, Halla.G.Mahmood [b] , Ahmed Hussein Al-Mayali [c].

[a] Department of Clinical Biochemistry, College of Medicine, University of Karbala, Iraq.

[b] Department of Clinical Biochemistry, College of Medicine, University of baghdad, Iraq.

[c] Department of Internal Medicine College of Medicine, University of Kerbala, Iraq.

**Corresponding author at: Department of Clinical biochemistry/College of Medicine/University of Kerbala, Iraq. Tel: 009647709045001*

Email: Nour.Kitab1109f@comed.uobaghdad.edu.iq

ABSTRACT

***Background*:** The most prevalent form of coronary artery disease (CAD) encompasses stable and unstable angina as well as myocardial infarction. This chronic ailment is characterized by the narrowing of arteries due to the accumulation of lipid-filled protrusions inside the arterial walls. The HFE p.C282Y gene polymorphism, specifically located in exon 4 with the nucleotide change c.845G→A (rs1800562), has been identified as a genetic factor related with coronary artery disorders.

Aim: The objective of this study is to evaluate the correlation between the HFE p.C282Y gene polymorphism (specifically located in exon 4; c.845G→A; rs1800562) and the occurrence of coronary artery disorders among the overall population.

Methods: A case-control research was conducted including a cohort of 100 patients diagnosed with coronary artery disorders and a control group of 100 persons who were deemed healthy. The Genotyping of (Polymerase chain reaction –restriction fragment length) polymorphism (PCR-RFLP) method was utilized to perform genotyping for the SNP gene polymorphism in the HFE gene.

***Results*:** The study investigated the frequency of genotypes and alleles of the HFE p.C282Y gene polymorphism in individuals with (CAD) and a control group. The analysis included multinomial logistic regression to assess the frequencies under (co-dominant, dominant, and recessive genetic) models. The genotype frequencies of rs1800562 exhibited adherence to the principles of (Hardy-Weinberg equilibrium) in both the CAD and Control groups. The statistical power of the present study to detect a significant difference at a significance level of 0.05 was found to be 91.2%.

Conclusion The HFE p.C282Y (exon 4; c.845G→A; rs1800562) SNP of HFE gene is associated with coronary artery disorders.

Keywords: for coronary artery diseases (CAD), HFE gen, SNP single nucleotide polymorphism p.C282Y.

INTRODUCTION

Coronary artery diseases (CAD) represent the primary cause of mortality on a global scale (1), accounting for approximately one-third or more of all fatalities in those aged 35 years and above (2). According to a study conducted in 2015, the prevalence of coronary artery disorders was seen in a population of 110 million individuals, resulting in a significant number of 8.9 million fatalities (3). Coronary artery diseases encompass a persistent medical condition characterized by the gradual formation of fatty deposits inside the arterial walls, resulting in the narrowing of the arteries. This condition is associated with various cardiovascular events, such as (angina, myocardial infarction, and sudden cardiac death) (4). Estrogen hormones exert a cardioprotective effect in females, resulting in a reduced susceptibility and occurrence of coronary artery disorders in comparison to males of similar age (5).

The HFE-protein is categorized as a transmembrane protein of type I, characterized by its close association with β2-microglobulin. Mutations that take place at either position 63 or position 282 of the HFE protein have the potential to interfere with its binding to β2-microglobulin, thereby compromising the interaction between HFE and the transferrin receptor. As a result, this disturbance results in an increased rate of iron absorption (6).

The HFE protein engages in protein-protein interactions at the cellular membrane to ascertain the iron levels within the organism (7).

When the HFE protein is linked to transferrin receptor 1, the receptor's ability to bind to transferrin protein is inhibited (8). The HFE protein governs the synthesis of hepcidin, a protein. The production of hepcidin occurs in the liver, and it plays a crucial role in regulating the absorption of dietary iron as well as the release of iron from storage locations throughout the body

(9). There exists a multitude of genes that are connected with the chance of developing coronary artery diseases, among which the HFE gene is included (10).

The HFE gene comprises a total of seven exons that collectively span a genomic region of 12 kilobases. The complete transcript encompasses a total of six exons. There are other variables, such as HFE, that function as upstream regulators of hepcidin transcription. The levels of HAMP expression were shown to be considerably reduced in untreated individuals with hemochromatosis, namely those with p.C282Y homozygosity and iron overload, in comparison to the control group (11).

The data presented above indicates that the regulation of hepcidin expression in relation to an excess of iron is greatly impacted by the HFE gene, and that the liver plays a crucial role in the pathogenesis of HFE-associated hemochromatosis (12). Furthermore, the findings of this study indicate that ferroprotein may have a role in the elimination of surplus iron from the liver (13). Therefore, individuals with p.C282Y homozygosity exhibit reduced hepcidin sensitivity to iron and have a shortfall of hepcidin, either relative or absolute (14).

The levels of mean (serum iron, transferrin saturation, and serum ferritin) are observed to be elevated in adults who possess the p.C282Y homozygosity genotype, in comparison to individuals with other prevalent HFE genotypes (15). The association between body iron reserves and the risk of (CHD) has been a subject of considerable scholarly discourse. The identification of HFE p.C282Y mutations, which are frequently observed in individuals with hereditary hemochromatosis, has been found to be associated with a notably heightened susceptibility to coronary heart disease (CHD). A recent proposal suggests that a clinical study could serve as the definitive method to evaluate the protective effects of iron against coronary heart disease (CHD) in individuals who are heterozygous for the C282Y gene variant .(16)

Serum ferritin is widely regarded as the most reliable biochemical indicator for evaluating iron reserves, making it a preferred test for inclusion in epidemiological investigations.

MATERIALS AND METHODS

STUDY SUBJECTS

The present study utilized a case-control research approach, employing a specific sample size of 200 participants, consisting of 100 individuals diagnosed with coronary artery disease (CAD). The study aimed to investigate the correlation between HFE p.C282Y (exon 4; c.845G→A; rs1800562) and coronary artery disease (CAD) by conducting a case-control analysis involving 100 control subjects. CAD was defined as the presence of a ≥70% organic stenosis in at least one segment of a major coronary artery or its primary branches, which was confirmed using coronary angiography. The study sample consisted of 100 individuals diagnosed with coronary artery disease, with 60 being male and 40 being female. These participants were selected from the cardiology center in Kerbala governorate, and the data collection period spanned from December 2022 to March 2023.The inclusion criteria encompassed two main factors: (1) patients who had a medical diagnosis of coronary artery disease (CAD) from physicians, and (2) participants who were above the age of 40 years. The exclusion criteria encompassed two specific conditions: (1) patients with liver illness and (2) patients with renal impairment. (3) Individuals diagnosed with diabetes mellitus or exhibiting abnormal results on a glucose tolerance test. The control group consisted of 100 individuals who were deemed to be in good health, with an equal distribution of 50 males and 50 women. These individuals were picked randomly from the overall population. The study's inclusion criteria encompassed the following factors: (1) Absence of prior medical history related to coronary artery disease (CAD); (2) Lack of familial history associated with CAD; (3) Matching of participants based on age, sex, and geographical distribution; (4) Body mass index (BMI) falling below 30 kg/m2 but beyond 18.5 kg/m2. Each case was required to complete a comprehensive questionnaire, which gathered data on many factors like (age, gender, family history, drug history, medical history), and other pertinent information. Additionally, measurements of (weight, height, and BMI) were taken for all participants. Informed consent has been obtained from all participants. The study protocol has received approval from the Ethical Committee of Baghdad Medical College.

GENOTYPIC DATA

Peripheral blood samples were obtained from both the coronary artery disease (CAD) group and the control group. These samples were collected in tubes containing EDTA as an anticoagulant. Subsequently, DNA extraction was performed on the whole-blood samples using the Rediaper genomic DNA extraction Kit, manufactured by Promega in the United States.Subsequently, the quantification and assessment of DNA concentration and purity were conducted using UV absorption at wavelengths of 260 and 280 nm, employing the Bio Drop instrument from the United Kingdom. The genotyping of the SNP HFE p.C282Y gene was conducted using the (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) (PCR-RFLP) method. This method involved the use of a thermocycler (Biometra, Germany) for the amplification and analysis of the HFE gene. The primer sequence corresponding to the HFE p.C282Y variant (located in exon 4; c.845G→A; rs1800562) gene was utilized.

C282Y(forward) 5’-TCCTCTTTCCTGTCAAGTGC-3'

C282Y(reverse) 5’-GATGACTCCAATGACTAGGG-3

The amplification reaction refers to a molecular biology technique that is used to increase the amount of a certain DNA sequence in a sample. In the single PCR reaction, a mixture consisting of two primers was utilized for the amplification of C282Y. The PCR mixture was created by combining 1µl of each (forward and reverse primer), each at a concentration of 10 pmol/µl, with 5 µl of extracted DNA. The resulting mixture was then brought to a total volume of 20 µl. Subsequently, the mixture was introduced into the lyophilization process to dehydrate the PCR premix formula, as indicated in Table 1.

Table (1): PCR program for amplification of 489 bp fragment flanking the C282Y mutation

Stage	Cycle	Step	Temp.	Time
1.*Denaturation*	1	1	95	5:00
2. *Annealing*	30	1	94	0:30
		2	61	0:30
		3	72	0:30
3. *Extension*	1	1	72	5:00
		2	4.0	HOLD

STATISTICAL ANALYSIS

The mean and standard deviation (M ± SD) are being discussed. The phenotypic data between the control and CAD groups were compared using the student's t-test and ANOVA test. This analysis was conducted using the SPSS Windows software developed by SPSS Inc. in Chicago, IL. The Hardy-Weinberg equilibrium was assessed by doing a chi-square test on genotype frequencies. This analysis was performed using the web-Assotest program available online. The calculation of genetic power was performed utilizing the web-based software OSSE. The study employed multivariate logistic regression analysis using the SPSS program to investigate the frequencies of genotypes and alleles in both the CAD and control groups. The analysis was conducted with and without considering age, sex, and BMI as covariates.

RESULTS

The study cohort included of 100 individuals, with 60 identified as male and 40 identified as female. The mean age of the patients was 58.59 years, with a standard deviation of 5.46. Additionally, the mean body mass index (BMI) was 27, with a standard deviation of 4. The control group had 50 male and 50 female individuals, with a mean age of 51.16 years and a standard deviation of 4.7 years. The individuals exhibited an average body mass index (BMI) of 23.76, accompanied with a standard deviation of 3.61. The examination of the HFE p.C282Y yielded outcomes that indicated the existence of distinct band widths that corresponded to various genotypes. In the case of the wild type (AA) genotype, the presence of two bands measuring 404 and 250 base pairs was detected. In the case of the heterozygous genotype (AG), three bands were detected, measuring 404, 250, and 242 base pairs.

Finally, for the homozygous genotype (GG), two bands measuring 404 and 242 base pairs were observed. The frequencies of genotypes and alleles for the HFE p.C282Y gene variant are presented in Table 2. The frequencies of genotypes observed in both the CAD and Control groups were found to be in accordance with the principles of (Hardy-Weinberg equilibrium). The statistical power of this study to detect a significant difference at a significance level of 0.05 was determined to be 91.2%. The findings of this study indicate a substantial association between the HFE gene polymorphism rs662799, namely the homozygous AA and heterozygous GA genotypes, and individuals diagnosed with coronary artery disease (CAD). Additionally, it was observed that the frequency of the A allele was higher in CAD patients.

The lipid profile, consisting of (total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and very low-density lipoprotein cholesterol (VLDL-C)), did not show statistical significance ($P > 0.05$) in individuals with coronary artery disease (CAD). There were no statistically significant differences observed in the lipid profile between individuals with GA and GG genotypes in the C282Y mutation group and the control group. Furthermore, there were no significant differences observed in the lipid profile, body mass index, and glycated hemoglobin levels between the patient groups with the GA and GG genotypes. It can be inferred that there exists a correlation between the iron status variables and the C282Y mutation status in the groups with ischemic heart disorders, as indicated in Table 3.

The study employed an analysis of variance (ANOVA) test to examine the clinical features, including (cholesterol levels, triglyceride levels, very low-density lipoprotein (VLDL) levels, high-density lipoprotein (HDL) levels, low-density lipoprotein cholesterol (LDL-C) levels, iron levels, ferritin levels, total iron-binding capacity (T.I.B.C.) levels, and high-sensitivity troponin I (hs-Trop I) levels), of participants with (CAD) in relation to HFE gene polymorphism. The study examined two genotypes, GA and GG, in relation to differences in ischemic heart disease among patients. The analysis revealed a statistically significant difference ($P < 0.05$) between the GA and GG genotypes with respect to the parameters of Iron, Ferritin, and T.I.B.C. The comparison of hs-Troponin I levels between individuals with two different genotypes, GA and GG, revealed no statistically significant difference in relation to the C282Y mutation refer to Table 3.

Table (2): Results of the Assessment of Genotype C282Y gen polymorphism in CAD patients and control groups.

Genotypes	Control n=100	CAD n=100	Unadjusted OR (95% CI)	Pvalue	Adjusted OR (95%CI)	P value
GG(Reference)	82	22				
GA	10	52	1.84 (0.71-3.66)	0.0001	1.76 (0.67-3.52)	0.0001
AA	8	26	1.51 (0.63-2.59)	0.0001	1.54 (0.56-2.61)	0.0004
Frequency of G allele	0.13	0.52		0.0001		

Table (3): Biochemical characteristics of CAD patients in relevance to the genotypes of C282Y gene polymorphism analyzed under co-dominant model.

Parameter	GA	AA	GG	P-value
BMI	28.47±5.14	28.16±4.18	28.35±6.15	0.3
F.B. S	99.44±8.78	95.77±9.89	96.88±11.59	0.3
HbA1C	4.67±1.78	4.76±1.91	4.23±1.85	0.5
HDL-C	40.93±9.93	40.92±8.02	39.53±7.77	0.2
LDL-C	95.89±10.99	94.45±11.79	97.45±13.22	0.2
TC	180.67±11.33	185.40±13.51	186.78±16.42	0.2
TG	149.49±13.22	158.39±12.92	154.73±10.92	0.3
VLDL-C	29.18±2.62	31.63±2.86	30.80±2.08	0.3
Iron	235.8±98.31	259.14±56.42	88.97±26.12	0.001
T.I.B.C	179.76±12.34	177.23±101.33	249.12±78.11	0.001
Ferritin	155.47±47.15	138.16±89.14	69.32±24.5	0.001
hs-Trop I	314.28±59.12	320.26±61.33	299.57±89.14	0.2

Table (4): Biochemical characteristics of CAD patients in relevance to the genotypes of C282Y gene polymorphism analyzed under dominant model

Parameter	GA+AA	GG	*P*-value
BMI	29.18±5.22	28.35±6.15	0.5
F.B. S	94.47±10.89	96.88±11.59	0.7
HbA1C	4.56±1.78	4.23±1.85	0.7
HDL-C	41.54±8.97	39.53±7.77	0.2
LDL-C	95.491±12.82	97.45±13.22	0.06
TC	190.92±14.53	186.78±16.42	0.2
TG	155.51±11.61	154.73±10.92	0.3
VLDL-C	31.78±2.86	30.80±2.08	0.3
Iron	260.12±59.71	88.97±26.12	0.001
T.I.B.C	189.11±11.33	249.12±78.11	0.001
Ferritin	145.26±59.14	69.32±24.5	0.001
hs-Trop I	324.16±72.22	299.57±89.14	0.3

DISCUSSION

The findings from the analysis of genotype distribution of the HFE C282Y single nucleotide (SNP) across several inheritance models revealed a statistically significant elevation of the A allele frequency in individuals with (CAD) compared to the control group. A notable observation was made regarding the considerable diversity in serum iron levels among patients belonging to the three genotypic groups (GG, GA, AA). This disparity in genotype occurrence (GG, GA, AA) highlighted the aforementioned observation. The minor A allele frequency in individuals with coronary artery disease (CAD) was found to be considerably greater compared to the control group. The present study provides clear evidence that the A allele of the C282Y single nucleotide polymorphism (SNP) located in exon 4, specifically the c.845G→A variant (rs1800562), within the HFE gene is significantly linked with the presence of coronary artery disease (CAD). This finding suggests that the A allele may serve as a potential risk factor for the development of CAD.

According to Sumi et al., hereditary haemochromatosis is a prevalent condition among individuals of Caucasian descent, exhibiting an autosomal recessive mode of inheritance. The prevalence of the C282Y mutation associated with this condition is estimated to be around 10 times higher than that of cystic fibrosis (17). In a comparable manner, a comprehensive population study conducted in the United States yielded estimations for the prevalence of C282Y homozygosity among several ethnic groups, including Native Americans, Hispanics, African Americans, Pacific Islanders, and Asians. The findings indicated that the frequency of C282Y homozygosity in these groups was at least one quarter of that observed in the non-Hispanic white population (18–20).

The results of this study align with prior investigations undertaken on the identical subject matter (21–23), as well as with studies conducted on individuals of Chinese descent (24) and populations from Taiwan (25). However, an alternative study (26) presents contradictory results.

In order to elucidate the potential underlying factors contributing to the association between the C282Y (SNP) of the HFE gene and the pathogenesis of coronary artery disease (CAD), it is necessary to conduct a comprehensive analysis. Several potential mechanisms propose that HFE may play a catalytic role in iron overload, rather than a structural one. In this context, it is suggested that HFE could enhance the expression of the HFE gene, which encodes the human hemochromatosis protein (HFE). This protein is primarily found on the surface of liver and intestinal cells and is responsible for regulating the production of hepcidin protein, which is widely regarded as the key regulator of iron levels (27–29). Iron overload may not just occur in individuals who are homozygous bearers of the risk alleles. Individuals with diverse heterozygosity and heterozygosity for the C282Y mutations exhibit an indirect increase in levels of (serum iron, serum ferritin, and transferrin saturation). The observed slight changes indicate a higher probability for heterozygotes to have a gradual buildup of iron, perhaps resulting in pathological conditions in later stages of life.(32–30)

For a number of years, there has been a proposed link between the presence of iron deposition and the development of coronary artery disease. In a study conducted by Lee TS et al., it was observed in an animal model of atherosclerosis that the advancement of atherosclerosis is diminished by an iron deficient diet.(33,34)

In a study conducted by Sahu et al. (year), it was found that The key etiological cause for hereditary haemochromatosis in the Western population has been identified as the C282Y mutation inside the HFE gene. The objective of this study is to investigate the prevalence of the C282Y mutation in the HFE gene within a cohort of 100 individuals diagnosed with coronary artery disease (CAD) from the northern region of India. The coronary architecture of these patients was assessed through the utilization of coronary angiography. According to the article, a cohort study found that 19% of persons diagnosed with coronary artery disease (CAD) displayed a positive presence of the C282Y mutation. Based on a scholarly article, it has been shown that individuals who possess heterozygosity for the C282Y mutation exhibit a moderate increase in their blood iron concentration, transferrin saturation, and serum ferritin concentration (35).

Table 3 presents the analysis of the C282Y single nucleotide (SNP) of the HFE gene promoter region in relation to coronary artery disease (CAD). The analysis was conducted using co-dominant and dominant models, as shown in both table 3 and table 4. The analysis of phenotypic data in coronary artery disease (CAD) stratified by genotypes (AA, GA, and GG) under the co-dominant model of the C282Y polymorphism revealed an intriguing observation. It was found that carriers of the GA and AA genotypes exhibited elevated plasma levels of iron, ferritin, and total iron-binding capacity (T.I.B.C.). This finding is highly significant in relation to iron, ferritin, and T.I.B.C. levels when considering both the co-dominant and dominant models of the C282Y polymorphism analysis of phenotypic data in CAD. Furthermore, the results obtained from analyzing the genotypes GG and GA+AA in CAD patients without (T2DM) are similar to those observed in the co-dominant model. Notably, the association between (iron, ferritin, and T.I.B.C. levels) remain highly significant in this context.

The current investigation reveals a noteworthy elevation in the concentrations of Iron, Ferritin, and T.I.B.C. (P=0.0001) among patients possessing the AA+GA genotypes, as compared to those with the GG genotype. This observation suggests a potential influence of the C282Y variant on the regulation of hepcidin, a protein of interest.

The findings underscore the correlation between genotypic groups of the C282Y polymorphism and levels of blood markers (Iron, Ferritin, T.I.B.C.), which are considered significant risk factors for coronary artery disease. Moreover, the association between mutations in the C282Y gene (specifically in exon 4; c.845G→A; rs1800562) and the likelihood of developing coronary heart disease (CHD) has sparked significant scholarly discourse (36). The identification of HFE C282Y mutations, which are frequently observed in individuals with hereditary hemochromatosis, has been found to substantially elevate the susceptibility to coronary heart disease (CHD) (37,38). A recent proposal suggests that a clinical trial could serve as an optimal method for evaluating the protective benefits of iron depletion against coronary artery disease (CAD) among individuals who are heterozygous for the C282Y gene variant (39)

This edition of Clinical Chemistry presents findings indicating that there is no significant association between angiographically verified coronary atherosclerosis and either a biochemical marker (serum ferritin) or a genetic marker (C282Y carrier status) of body iron reserves. The study reported a low analytical genotyping error rate of 0.73% in a total of 7,663 findings obtained from 257 distinct laboratories (40).

This work aligns with previous research (41–45) by demonstrating that the frequency of the A allele in the CAD without T2DM group is significantly greater than that observed in the control group. However, it was not possible to establish that this allele is an autonomous risk factor for CAD. Additional research is required to ascertain whether this particular genetic variation may be used as a predictive indicator for clinical coronary artery disease (CAD) occurrences in individuals who do not have type 2 diabetes. Therefore, in contrast to the results observed in individuals with diabetes and coronary artery disease (CAD), it is possible that the C282Y polymorphism (exon 4; c.845G→A; rs1800562) may not play a significant role as a risk factor in the progression of CAD.

Conclusions

The gene polymorphism HFE C282Y (exon 4; c.845G→A; rs1800562) was found to be linked to coronary artery disease (CAD). Individuals who possess the homozygous genotype (GG) and heterozygous (GA) genotype of C282Y exhibit a significant correlation and heightened susceptibility to the development of coronary artery disease (CAD).

REFERENCES

1. Jahmunah V, Ng EYK, San TR, Acharya UR. Automated detection of coronary artery disease, myocardial infarction and congestive heart failure using GaborCNN model with ECG signals. Comput Biol Med. 2021;134:104457.

2. Ismail ASM, Aouf MA, Diab RH, Baghdady YK. Relation of the left ventricular geometric patten to coronary artery disease in hypertensive patients using a 320-detector-row CT scanner. The Egyptian Heart Journal. 2023;75(1):1–11.

3. Szarpak L, Mierzejewska M, Jurek J, Kochanowska A, Gasecka A, Truszewski Z, et al. Effect of Coronary Artery Disease on COVID-19—Prognosis and Risk Assessment: A Systematic Review and Meta-Analysis. Biology (Basel). 2022;11(2):221.

4. Crane LR, Katz MJ. Cardiac Patient Care: Coronary Artery Disease (CAD). 2022;

5. Trtica Majnarić L, Guljaš S, Bosnić Z, Šerić V, Wittlinger T. Neutrophil-to-lymphocyte ratio as a cardiovascular risk marker may be less efficient in women than in men. Biomolecules. 2021;11(4):528.

6. Baschant U, Altamura S, Steele-Perkins P, Muckenthaler MU, Spasić MV, Hofbauer LC, et al. Iron effects versus metabolic alterations in hereditary hemochromatosis driven bone loss. Trends in Endocrinology & Metabolism. 2022;

7. Bonilla DA, Moreno Y, Petro JL, Forero DA, Vargas-Molina S, Odriozola-Martínez A, et al. A Bioinformatics-assisted review on iron metabolism and immune system to identify potential biomarkers of exercise stress-induced immunosuppression. Biomedicines. 2022;10(3):724.

8. Sokolov A V, Isakova-Sivak IN, Mezhenskaya DA, Kostevich VA, Gorbunov NP, Elizarova AY, et al. Molecular mimicry of the receptor-binding domain of the SARS-CoV-2 spike protein: from the interaction of spike-specific antibodies with transferrin and lactoferrin to the antiviral effects of human recombinant lactoferrin. Biometals. 2023;36(3):437–62.

9. Nemeth E, Ganz T. Hepcidin and iron in health and disease. Annu Rev Med. 2023;74:261–77.

10. Vaitiekus D. Impact of Clinical and Genetic Risk Factors on Anthracycline-Based Chemotherapy Subclinical Cardiotoxicity: doctoral dissertation: medical and health sciences, medicine (M 001). 2021;

11. Henninger B, Alustiza J, Garbowski M, Gandon Y. Practical guide to quantification of hepatic iron with MRI. Eur Radiol. 2020;30:383–93.

12. Altamura S, Müdder K, Schlotterer A, Fleming T, Heidenreich E, Qiu R, et al. Iron aggravates hepatic insulin resistance in the absence of inflammation in a novel db/db mouse model with iron overload. Mol Metab. 2021;51:101235.

13. Xiao X, Moschetta GA, Xu Y, Fisher AL, Alfaro-Magallanes VM, Dev S, et al. Regulation of iron homeostasis by hepatocyte TfR1 requires HFE and contributes to hepcidin suppression in β-thalassemia. Blood, The Journal of the American Society of Hematology. 2023;141(4):422–32.

14. Li Y, Ouyang Q, Chen Z, Chen W, Zhang B, Zhang S, et al. Intracellular labile iron is a key regulator of hepcidin expression and iron metabolism. Hepatol Int. 2023;17(3):636–47.

15. Barton JC, Barton JC, Acton RT. Platelet counts in HFE p. C282Y/p. C282Y and wt/wt post-screening clinical evaluation participants. Platelets. 2023;34(1):2217933.

16. Barton JC, Edwards CQ, Acton RT. HFE hemochromatosis in African Americans: Prevalence estimates of iron overload and iron overload-related disease. Am J Med Sci. 2023;365(1):31–6.

17. Sumi MP, Guru SA, Sahu S, Khan B, Mp G, Saxena A. "Role of HFE gene in coronary artery disease"–A study from India. Clin Epidemiol Glob Health. 2020;8(2):469–72.

18. Skrlec I, Steiner R, Wagner J. Hereditary hemochromatosis gene mutations in patients with myocardial infarction. Molecular and experimental biology in medicine. 2019;2(1):24–8.

19. Lian J, Xu L, Huang Y, Le Y, Jiang D, Yang X, et al. Meta-analyses of HFE variants in coronary heart disease. Gene. 2013;527(1):167–73.

20. Hetet G, Elbaz A, Gariepy J, Nicaud V, Arveiler D, Morrison C, et al. Association studies between haemochromatosis gene mutations and the risk of cardiovascular diseases. Eur J Clin Invest. 2001;31(5):382–8.

21. MALTESE P, Poplavskaia E, Malyutkina I, Sirocco F, Bonizzato A, Capodicasa N, et al. Genetic tests for low-and middle-income countries: a literature review. Genetics and Molecular Research. 2017;16(1).

22. Schneidereith TA, Anderson G. Adult Health and Illness and Medical–Surgical Nursing. Lashley's Essentials of Clinical Genetics in Nursing Practice. 2015;

23. Xiao Q, Lauschke VM. The prevalence, genetic complexity and population-specific founder effects of human autosomal recessive disorders. NPJ Genom Med. 2021;6(1):41.

24. Battiloro E, Ombres D, Pascale E, D'Ambrosio E, Verna R, Arca M. Haemochromatosis gene mutations and risk of coronary artery disease. European Journal of Human Genetics. 2000;8(5):389–92.

25. Xiao Q, Lauschke VM. The prevalence, genetic complexity and population-specific founder effects of human autosomal recessive disorders. NPJ Genom Med. 2021;6(1):41.

26. Corradini E, Buzzetti E, Pietrangelo A. Genetic iron overload disorders. Mol Aspects Med. 2020;75:100896.

27. Nemeth E, Ganz T. Hepcidin and iron in health and disease. Annu Rev Med. 2023;74:261–77.

28. Anderson GJ, Bardou-Jacquet E. Revisiting hemochromatosis: genetic vs. phenotypic manifestations. Ann Transl Med. 2021;9(8).

29. Rana S, Prabhakar N. Iron disorders and hepcidin. Clinica Chimica Acta. 2021;523:454–68.

30. Mustafa BS. Defining Pathological Iron Status in Children with Thalassemia. Iraqi Journal of Pharmacy. 2022;19(2):108–16.

31. Nijaguna N, Vishnu Murthy GS, Sowmya K. Correlation with iron overload and thyroid dysfunction in children with thalassemia on regular transfusion therapy. Int J Biol Med Res. 2015;6(1):4756–61.

32. Gaur A. Thalassemia: Cardiac iron and chelators. Indian Pediatr. 2012;49:268–9.

33. Macdougall IC, Bircher AJ, Eckardt KU, Obrador GT, Pollock CA, Stenvinkel P, et al. Iron management in chronic kidney disease: conclusions from a "Kidney Disease: Improving Global Outcomes"(KDIGO) Controversies Conference. Kidney Int. 2016;89(1):28–39.

34. Guo Q, Qian C, Qian ZM. Iron metabolism and atherosclerosis. Trends in Endocrinology & Metabolism. 2023;

35. Sumi MP, Guru SA, Sahu S, Khan B, Mp G, Saxena A. "Role of HFE gene in coronary artery disease"–A study from India. Clin Epidemiol Glob Health. 2020;8(2):469–72.

36. Reyes C, Pons NA, Reñones CR, Gallisà JB, Val VA, Tebé C, et al. Association between serum ferritin and acute coronary heart disease: a population-based cohort study. Atherosclerosis. 2020;293:69–74.

37. Gutierrez-Bedmar M, Olmedo P, Gil F, Ruiz-Canela M, Martínez-González MA, Salas-Salvadó J, et al. Low serum iron levels and risk of cardiovascular disease in high risk elderly population: Nested case–control study in the PREvención con DIeta MEDiterránea (PREDIMED) trial. Clinical Nutrition. 2021;40(2):496–504.

38. Weidmann H, Bannasch JH, Waldeyer C, Shrivastava A, Appelbaum S, Ojeda-Echevarria FM, et al. Iron metabolism contributes to prognosis in coronary artery disease: prognostic value of the soluble transferrin receptor within the AtheroGene study. J Am Heart Assoc. 2020;9(9):e015480.

39. Al-Shaar L, Satija A, Wang DD, Rimm EB, Smith-Warner SA, Stampfer MJ, et al. Red meat intake and risk of coronary heart disease among US men: prospective cohort study. bmj. 2020;371.

40. Hou YCC, Yu HC, Martin R, Cirulli ET, Schenker-Ahmed NM, Hicks M, et al. Precision medicine integrating whole-genome sequencing, comprehensive metabolomics, and advanced imaging. Proceedings of the National Academy of Sciences. 2020;117(6):3053–62.

41. Sumi MP, Guru SA, Sahu S, Khan B, Mp G, Saxena A. "Role of HFE gene in coronary artery disease"–A study from India. Clin Epidemiol Glob Health. 2020;8(2):469–72.

42. Skrlec I, Steiner R, Wagner J. Hereditary hemochromatosis gene mutations in patients with myocardial infarction. Molecular and experimental biology in medicine. 2019;2(1):24–8.

43. Shizukuda Y, Rosing DR. Hereditary hemochromatosis with homozygous C282Y HFE mutation: possible clinical model to assess effects of elevated reactive oxygen species on the development of cardiovascular disease. Mol Cell Biochem. 2023;1–11.

44. Shizukuda Y, Tripodi DJ, Rosing DR. Iron overload or oxidative stress? Insight into a mechanism of early cardiac manifestations of asymptomatic hereditary hemochromatosis subjects with C282Y homozygosity. J Cardiovasc Transl Res. 2016;9:400–1.

45. Engberink MF, Povel CM, Durga J, Swinkels DW, de Kort WLAM, Schouten EG, et al. Hemochromatosis (HFE) genotype and atherosclerosis: increased susceptibility to iron-induced vascular damage in C282Y carriers? Atherosclerosis. 2010;211(2):520–5.