



International Journal of Innovative Technologies in Social Science

e-ISSN: 2544-9435

Operating Publisher
SciFormat Publishing Inc.
ISNI: 0000 0005 1449 8214

2734 17 Avenue SW,
Calgary, Alberta, T3E0A7,
Canada
+15878858911
editorial-office@sciformat.ca

ARTICLE TITLE	CLINICAL SIGNIFICANCE OF HFE C282Y AND H63D HETEROZYGOSITY: A REVIEW OF CURRENT GUIDELINES AND EVIDENCE
----------------------	---

DOI	https://doi.org/10.31435/ijitss.4(52).2026.6734
------------	---

RECEIVED	12 August 2026
-----------------	----------------

ACCEPTED	09 October 2026
-----------------	-----------------

PUBLISHED	15 October 2026
------------------	-----------------

LICENSE



The article is licensed under a **Creative Commons Attribution 4.0 International License**.

© The author(s) 2026.

This article is published as open access under the Creative Commons Attribution 4.0 International License (CC BY 4.0), allowing the author to retain copyright. The CC BY 4.0 License permits the content to be copied, adapted, displayed, distributed, republished, or reused for any purpose, including adaptation and commercial use, as long as proper attribution is provided.

CLINICAL SIGNIFICANCE OF HFE C282Y AND H63D HETEROZYGOSITY: A REVIEW OF CURRENT GUIDELINES AND EVIDENCE

Alicja Szemberg (Corresponding Author, Email: alicja.szemberg@gmail.com)

Medical University of Warsaw, Warsaw, Poland

ORCID ID: 0009-0001-8805-1755

Martyna Stawicka

Medical University of Warsaw, Warsaw, Poland

ORCID ID: 0009-0004-4140-739X

Weronika Patrycja Polar

Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland

ORCID ID: 0009-0005-2818-1119

Ewa Weronika Pływak

Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland

ORCID ID: 0009-0005-1419-5480

Zuzanna d'Obyrn

Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland

ORCID ID: 0009-0005-1285-1886

Julia Sadowska

Cardinal Stefan Wyszyński University in Warsaw, Warsaw, Poland

ORCID ID: 0009-0002-6946-7277

Karolina Strzelecka

Medical University of Warsaw, Warsaw, Poland

ORCID ID: 0009-0007-9797-9815

Ewa Jagodzińska

Szpital Kliniczny Dzieciątka Jezus, Warsaw, Poland

ORCID ID: 0009-0008-4089-2708

ABSTRACT

With the increasing availability of genetic testing, individuals heterozygous for the C282Y or H63D variant of the HFE gene are being identified more frequently. Although clinical management is better established for patients homozygous for C282Y, recommendations for those with heterozygous HFE genotypes remain less clearly defined. This narrative review included 29 sources identified through searches of PubMed and Google Scholar, and screening of the reference lists of relevant publications. It aimed to synthesise the available evidence on the clinical risk associated with HFE heterozygosity and to assess the appropriateness of diagnostic and therapeutic interventions in this population. The evidence base included professional society guidelines and position statements, observational studies, case reports, and review articles addressing genotype-phenotype relationships in HFE-related haemochromatosis. The available data indicate that heterozygosity for a single C282Y or H63D variant rarely results in clinically significant iron overload and do not support routine dietary restrictions or prophylactic phlebotomy in individuals with iron indices within normal limits. Similar conclusions apply to C282Y/H63D compound heterozygosity, although this genotype is associated with a slightly higher clinical risk. The review also highlights the limited guidance on family testing when the proband is a heterozygous carrier, as well as the clinical consequences of overinterpreting H63D. Overall, these findings support a phenotype-guided approach to the diagnostic and therapeutic management of individuals heterozygous for HFE variants.

KEYWORDS

HFE, Haemochromatosis, C282Y, H63D, Heterozygosity, Iron Overload

CITATION

Szemberg, A., Stawicka, M., Polar, W. P., Plywak, E. W., d'Obyrn, Z., Sadowska, J., Strzelecka, K., & Jagodzińska, E. (2026). Clinical significance of HFE C282Y and H63D heterozygosity: A review of current guidelines and evidence. *International Journal of Innovative Technologies in Social Science*, 4(52). [https://doi.org/10.31435/ijitss.4\(52\).2026.6734](https://doi.org/10.31435/ijitss.4(52).2026.6734)

COPYRIGHT

© The author(s) 2026. This article is published as open access under the **Creative Commons Attribution 4.0 International License (CC BY 4.0)**, allowing the author to retain copyright. The CC BY 4.0 License permits the content to be copied, adapted, displayed, distributed, republished, or reused for any purpose, including adaptation and commercial use, as long as proper attribution is provided.

1. Introduction

Haemochromatosis is a genetic disorder which causes excessive iron accumulation due to impaired regulation of iron homeostasis. Insufficient hepcidin activity leads to inadequate suppression of ferroportin and consequently to increased intestinal absorption of iron, and its enhanced release into the circulation. Sustained iron overload may result in iron deposition in tissues and organ damage, primarily in the liver. In the European population, HFE-related haemochromatosis is the most common form of the disease and homozygosity for the p.Cys282Tyr (C282Y) variant is associated with the greatest risk of its development (European Association for the Study of the Liver [EASL], 2022; Nemeth & Ganz, 2023).

However, genetic predisposition does not necessarily imply clinical manifestation of the disease. In a large population-based study of individuals of northern European descent, iron overload-related disease was identified in 28.4% of men and 1.2% of women with C282Y homozygosity, demonstrating incomplete and sex-dependent clinical penetrance (Allen et al., 2008). The distinction between a predisposing genotype and an iron-overload phenotype remains an important consideration in the context of heterozygosity for HFE variants, which is common in the general population. Among 10,556 blood donors from South Wales, simple C282Y heterozygosity was present in 1 in 7.9 individuals and H63D heterozygosity in 1 in 4.2. Despite their high prevalence, the presence of either variant was not associated with a significantly greater risk of iron accumulation in the study cohort (Jackson et al., 2001).

The clinical significance of the p.His63Asp (H63D) variant is particularly uncertain. Although H63D frequently occurs in the population, its influence on the development of iron overload remains unclear, raising questions about its role in routine haemochromatosis testing. Shearman et al. (2026) note that the presence of H63D may be misinterpreted and lead to unwarranted clinical action. Similarly, the EASL guidelines emphasise the controversial clinical value of H63D genotyping and indicate that, in individuals with HFE genotypes other than C282Y homozygosity, the diagnosis of haemochromatosis should not be based solely on genetic findings.

In clinical practice, the detection of an HFE variant may therefore precede any biochemical abnormalities and may sometimes occur in individuals who will never develop significant iron overload. Thus, it is crucial to determine the actual consequences of heterozygous carrier status and the extent of management it requires. It remains uncertain whether the identification of such a genotype justifies regular monitoring of iron-related parameters or dietary modifications, or whether these decisions should depend primarily on biochemical evidence of iron overload and the presence of additional risk factors.

Current guidelines clearly define the management of confirmed haemochromatosis, but the approach to individuals heterozygous for an HFE variant is not distinctly established. These documents focus principally on the diagnosis and treatment of iron overload, while the appropriate scope of monitoring and potential preventive measures for heterozygotes without biochemical abnormalities remains unsettled.

The aim of this review is therefore to synthesise current evidence on the clinical significance of heterozygosity for HFE variants, particularly C282Y and H63D, and to assess the appropriate clinical approach when they are identified in the absence of iron overload.

2. Methodology

This study is a narrative review of the literature based on searches of PubMed and Google Scholar conducted between August and September 2026. The search strategy involved combinations of the following terms: "HFE", "haemochromatosis", "heterozygous"/"heterozygosity", "C282Y", "H63D", "iron overload", "phlebotomy" and "cascade testing". Only sources published in English were eligible for inclusion, with no restriction on publication date.

The analysis encompassed primary research (observational, cohort, and experimental studies), case reports and review articles, including systematic reviews, as well as clinical practice guidelines and position statements from professional organisations, such as EASL, ACG, EMQN and ESHG. These sources addressed the relationship between HFE genotype and phenotypic expression or the principles of clinical management in carriers of HFE variants. The reference lists of these guidelines and review articles were also screened, which enabled the identification of additional primary studies not otherwise retrieved.

The final analysis comprised 29 sources. The findings are presented thematically according to the principal aspects of the topic: mechanisms of HFE-related iron overload and clinical penetrance of particular HFE genotypes, factors modifying phenotypic expression, recommendations regarding biochemical testing, indications for interventions aimed at reducing iron stores, family screening after identification of carriers, and the clinical consequences of overinterpreting the H63D variant.

3. HFE Genotypes and Their Clinical Significance

The pathophysiology of type 1 hereditary haemochromatosis is based on the HFE-hepcidin-ferroportin axis. When transferrin saturation is low, HFE forms a complex with the transferrin receptor 1 (TfR1). As transferrin saturation increases, HFE is displaced from TfR1 and binds to the transferrin receptor 2 (TfR2) on hepatocytes. Together with signalling involving hemojuvelin (HJV), this iron-sensing pathway promotes transcription of hepcidin, the principal hormone regulating systemic iron homeostasis (Ginzburg, 2019). Heparin binds to ferroportin, which is the only known cellular iron exporter responsible for iron release from enterocytes and macrophages into the circulation, and induces its internalisation and degradation, thereby reducing iron entry into plasma (Nemeth & Ganz, 2023).

Pathogenic HFE variants impair normal iron sensing and may result in hepcidin production that is inappropriately low relative to body iron stores (Ginzburg, 2019). This results in persistent ferroportin activity which promotes excessive intestinal iron absorption and progressive iron accumulation in tissues, particularly in the liver, pancreas, and heart (Barton & Parker, 2024; Nemeth & Ganz, 2023).

The probability of clinically manifest disease varies markedly according to the particular allelic combination. Burke et al. (2000) estimated the risk associated with individual HFE genotypes in a pooled analysis of 14 case-control studies. The strongest association was observed for C282Y/C282Y homozygosity (OR = 4383; 95% CI: 1374 to > 10,000), with an attributable fraction (AF) of 0.73. C282Y/H63D compound heterozygosity was associated with a substantially lower, although still increased, risk (OR = 32; 95% CI: 18.5-55.4; AF = 0.06), as was H63D/H63D homozygosity (OR = 5.7; 95% CI: 3.2-10.1; AF = 0.01). For simple heterozygosity, the reported odds ratios were 4.1 (95% CI: 2.9-5.8; AF = 0.03) for C282Y and 1.9 (95% CI: 1.5-2.5; AF = 0.03) for H63D, among the lowest values across the genotypes analysed. The estimates for C282Y heterozygosity also showed significant heterogeneity between the component studies. Burke et al.

(2000) further examined genotype penetrance in the general population. For C282Y/C282Y homozygosity, penetrance was below 50% when lower prevalences of clinically manifest disease were assumed and fell to 10-20% when the prevalence was assumed to be 1:1000. Considerably lower values were obtained for the remaining genotypes: 0.3-1.4% for C282Y/H63D compound heterozygosity and only 0.04-0.2% for H63D/H63D homozygosity.

Clinical expression of the C282Y/C282Y genotype ranges from asymptomatic biochemical abnormalities to severe organ damage. Consequently, the estimated proportion of patients with overt disease depends heavily on which manifestations are included in the diagnostic criteria. Allen et al. (2008) used a broad definition requiring documented iron overload together with at least one related clinical feature. According to that, the disease was present in 28.4% of men and 1.2% of women with C282Y/C282Y. Grosse et al. (2018), by contrast, analysed four cohort studies of older men homozygous for C282Y and restricted the outcome to the most severe forms of liver disease: cirrhosis or hepatocellular carcinoma. In a pooled analysis, severe liver disease was present in 9.0% of older male C282Y homozygotes (95% CI: 2.6-15.3%). Taken together, these results indicate that most carriers of the C282Y/C282Y genotype do not develop its most serious complications.

C282Y/H63D compound heterozygosity has substantially lower clinical penetrance than C282Y/C282Y homozygosity, as supported by data from long-term clinical follow-up. In the largest North American cohort of this genotype reported to date, Hasan et al. (2022) analysed 247 individuals over a period of up to 10 years after initial genotyping. Iron overload-related disease was ultimately identified in 5.3%, which the authors interpreted as evidence of the generally low clinical penetrance of this genotype.

Simple heterozygosity for either C282Y or H63D appears to have only a limited clinical effect. Aranda et al. (2010) reported a statistically significant increase in transferrin saturation among carriers of either variant, but at levels not associated with organ damage. This is consistent with the findings of Jackson et al. (2001), whose study of 10,556 blood donors found no significantly higher risk of overall iron overload in simple heterozygotes than in individuals without these variants. Thus, simple heterozygosity may be associated with detectable biochemical differences without corresponding organ injury in most individuals.

Nevertheless, clinically significant iron overload has occasionally been reported in simple heterozygotes. Turbiville et al. (2019) described a woman heterozygous for C282Y who required regular phlebotomy because of markedly elevated iron levels, while Abeyagunawardena et al. (2024) reported a similar course in a man heterozygous for H63D, with iron deposition involving the liver and pancreas. Such cases may have an additional genetic explanation. In a study of six C282Y heterozygotes with unexplained iron overload (Aguilar-Martinez et al., 2011), extended sequencing revealed an additional, rare HFE variant on the second allele. Routine testing limited to C282Y and H63D may therefore label such individuals as simple heterozygotes while failing to identify an underlying compound heterozygous state.

However, the HFE genotype is not the only possible explanation for such discrepancies. Fabri et al. (2026) described a woman heterozygous for C282Y who developed cirrhosis in the setting of severe iron overload and coexisting autoimmune hepatitis. The authors considered liver disease itself as a possible contributor to disturbed iron metabolism. Regardless of whether an unexpected iron-overload phenotype reflects an unrecognised genetic variant or concomitant liver disease, abnormal iron indices in a heterozygous carrier require further evaluation rather than being dismissed on the basis of a generally low-risk genotype.

HFE genotyping is therefore informative but insufficient on its own for assessing clinical risk in an individual patient. This is illustrated by findings at both extremes of the spectrum. Although C282Y/C282Y homozygosity has the strongest established association with haemochromatosis, its estimated penetrance is generally below 50% and becomes even lower when more stringent clinical endpoints are applied. Simple heterozygosity, on the other hand, is generally regarded as a low-risk state, yet clinically relevant iron overload has been documented in isolated cases, sometimes in the presence of another potential explanation such as a rare HFE variant or concomitant liver disease. HFE genotype should therefore be interpreted as one component of clinical assessment rather than as an independent determinant of individual risk.

4. Factors Influencing the Clinical Expression of HFE Variants

The phenotypic expression of HFE variants is diverse and may depend on additional physiological, environmental and genetic factors which are particularly important when interpreting abnormal iron indices in heterozygous individuals. These include sex and the associated physiological loss of iron, comorbidities, and additional genetic variation that may account for a clinical picture disproportionate to that expected from the identified HFE genotype.

Sex is among the best characterised factors influencing the clinical manifestations of HFE genotypes. In the cohort of 247 individuals with C282Y/H63D genotype studied by Hasan et al. (2022), documented iron overload, defined as substantial hepatic iron deposition confirmed histologically or serum ferritin concentration $> 1000 \mu\text{g/L}$, was more frequent in men than in women (13.5% vs. 4.3%). Sex-related differences were also observed in the prospective population-based study by Gurrin et al. (2009). Among women with C282Y/H63D who were premenopausal at baseline, mean serum ferritin concentration increased from 42.5 to 109.3 $\mu\text{g/L}$ during follow-up ($p < 0.001$). A similar increase, from 35.0 to 64.4 $\mu\text{g/L}$ ($p < 0.001$), occurred in women without the C282Y and H63D variants. Despite these biochemical changes, the development of iron overload-related disease remained uncommon. Therefore, the difference between women and men may be partly connected to physiological iron loss before menopause, although this does not fully account for sex-related variability in clinical expression.

A similar pattern has been observed in women with simple C282Y or H63D heterozygosity. In a 12-year observational study by Zaloumis et al. (2015), ferritin levels increased after the menopause both in women who were heterozygous for one of these variants and in women without them, while mean iron indices remained within reference ranges. Furthermore, no cases of documented iron overload were identified among women carrying a single C282Y or H63D variant. These observations suggest that a postmenopausal rise in ferritin level may primarily reflect reduced physiological iron loss and does not establish HFE-related iron overload in heterozygous carriers.

Another group of factors includes metabolic disorders, liver disease and excessive alcohol consumption, all of which may affect serum ferritin concentration and hepatic status independently of HFE genotype. This is particularly important in the interpretation of hyperferritinaemia, as it is not a specific marker of increased iron stores. Walsh et al. (2006) studied 249 individuals with C282Y/H63D, 21 of whom had ferritin levels $> 1000 \mu\text{g/L}$. Information about comorbidities and other factors was available for 18 of these patients, and every one had at least one potential contributor to elevated ferritin, including hepatic steatosis, obesity, diabetes, or excessive alcohol intake. Liver biopsy was performed in a separate subgroup of 51 individuals. Fibrosis was found in 13 cases, with 12 of these also presenting with steatosis, excessive alcohol consumption or diabetes. Notably, the only patient with cirrhosis also had two of these factors: diabetes and excessive alcohol consumption. The authors also raised the possibility that iron accumulation may interact with other hepatotoxic factors, although the available data did not allow the nature of this relationship to be determined. Accordingly, both hyperferritinaemia and hepatic abnormalities in C282Y/H63D compound heterozygotes require consideration of alternative or additional causes. Metabolic diseases and excessive alcohol consumption may contribute to liver injury, but the extent to which they modify genotype-associated risk remains uncertain.

Evidence regarding simple heterozygotes further illustrates the need for thorough interpretation of marked hyperferritinaemia. Zaloumis et al. (2015) described three individuals heterozygous for C282Y or H63D with serum ferritin concentrations above $1000 \mu\text{g/L}$, yet their transferrin saturation remained below 55%. The authors suggested that the elevated ferritin levels could reflect coexisting factors rather than genotype-related iron overload. In addition, the frequency of the assessed disease markers in simple heterozygotes was similar to that observed in individuals without HFE variants. Hence, an isolated increase in ferritin concentration in a heterozygous carrier should be interpreted together with transferrin saturation, other iron indices, and alternative causes of hyperferritinaemia.

Additional genetic variation may also explain unexpectedly severe iron accumulation in an individual with simple heterozygosity. Aguilar-Martinez et al. (2011) performed extended molecular testing in six patients with iron overload who had initially been classified as simple C282Y heterozygotes. As a result, four rare HFE variants were identified, three of which had not previously been described. The authors further proposed that when confirmed iron overload cannot be explained by additional HFE variants, investigation of other genes involved in iron homeostasis, including HAMP, HJV, TFR2, and SLC40A1, may be warranted. These observations do not imply that simple C282Y heterozygosity itself carries a high risk of pathological iron accumulation. On the contrary, they show that in cases of severe iron overload atypical for this genotype,

an additional cause should be considered rather than attributing this condition to the presence of a single common variant.

The significance of heterozygous HFE genotypes should therefore be interpreted in the context of factors that can influence both iron indices and their clinical meaning. Sex and the reduction in physiological iron loss after menopause affect biochemical parameters, whereas metabolic disorders and liver disease have a dual role: they may complicate the interpretation of hyperferritinaemia while also contributing to hepatic injury in the presence of excess iron accumulation. Conversely, unexpectedly severe iron overload may point to additional genetic determinants. Considering these factors could help to distinguish abnormalities possibly related to HFE status from those requiring another explanation and provide a basis for deciding whether a heterozygous individual without established iron overload requires any specific follow-up.

5. Biochemical Monitoring After Identification of an HFE Variant

Recommendations for further management following the identification of HFE variants differ depending on the genotype and the presence of biochemical features of iron overload. The American College of Gastroenterology (ACG) recommends that patients with the H63D variant but without C282Y "should be counseled that they are not at increased risk of iron overload" (Kowdley et al., 2019, p. 1203), yet this is a conditional recommendation based on evidence of very low quality. The guideline does not specify a separate schedule of biochemical surveillance for this group.

Different guidance applies to C282Y/H63D compound heterozygosity. EASL (2022) states that in asymptomatic individuals with this genotype, who show no signs of iron overload, the risk of developing excessive iron accumulation is low. However, depending on the clinical assessment, monitoring of iron parameters may be recommended. The frequency of monitoring should be adjusted to the patient's age and individual risk profile. EASL (2022) further recommends that management of C282Y/H63D compound heterozygotes and H63D/H63D homozygotes should be guided by the phenotypic presentation and additional risk factors rather than the genotype alone. Although this is a strong recommendation, it is supported by level 5 evidence, corresponding to expert opinion or mechanism-based reasoning.

For simple C282Y heterozygosity, neither guideline provides a dedicated recommendation, and for H63D the ACG statement is limited to counselling. Neither document defines a specific follow-up protocol for simple heterozygotes. In practice, the extent of further evaluation in individuals with HFE genotypes other than C282Y/C282Y therefore depends more on individual clinical assessment than on a clearly defined evidence-based standard.

6. Indications for Iron-Reducing Interventions in Heterozygous Individuals Without Iron Overload

The identification of heterozygosity for an HFE variant may raise the question of whether the genetic test result alone is sufficient to justify measures intended to limit iron accumulation. Potential strategies include reducing dietary iron intake or performing prophylactic phlebotomy. However, the rationale for such interventions depends not only on their impact on iron homeostasis, but also on the actual risk associated with a particular genotype. This assessment is crucial in individuals who carry an HFE variant but have no biochemical evidence of iron overload.

Iron absorption in simple C282Y heterozygotes was directly examined by Hunt and Zeng (2004) and Roe et al. (2005). In the former study, 11 healthy individuals heterozygous for C282Y were compared with 12 participants without C282Y or H63D. After adjusting for ferritin levels, no significant differences were found between these groups in the absorption of either haem or non-haem iron, from both a standard and a fortified meal (Hunt & Zeng, 2004). Roe et al. (2005) obtained similar results in 15 men heterozygous for C282Y and 15 men without the variant. Non-haem iron absorption from a high-bioavailability diet was 7.6% and 6.8%, respectively, while absorption from iron-fortified cereal products was 5.3% and 4.9%. None of these comparisons reached statistical significance. The small sample sizes do not exclude subtle differences in absorption between the studied groups, but the findings do not indicate a marked increase in dietary iron absorption among simple C282Y heterozygotes.

The relationship between iron intake and iron stores in women with different HFE genotypes was the subject of a study by Greenwood et al. (2005). No significant difference in ferritin concentration was found between carriers of a single C282Y variant and those without it. Based on these results, the authors did not demonstrate the need for C282Y heterozygotes to restrict their meat intake beyond recommendations

applicable to a healthy diet. When combined with data from iron absorption studies, these observations do not support special dietary restrictions solely because a single C282Y variant is present.

Evidence on dietary modification is more extensive in patients with confirmed iron overload, although its relevance to heterozygous individuals with normal iron indices is limited. Moretti et al. (2013) systematically reviewed studies of dietary influences on iron balance in hereditary haemochromatosis. The available literature indicated that diet can modify iron bioavailability and gastrointestinal absorption. However, no prospective randomised trials evaluating the efficacy of dietary interventions were identified, and few data were available on their effects on disease course or quality of life. Therefore, the possibility of modifying iron absorption through diet does not determine its clinical value. Since the significance of such interventions remains uncertain even in patients with haemochromatosis, their routine use in heterozygous individuals without iron overload is difficult to justify.

This interpretation is also consistent with the EASL (2022) recommendations. Even patients with diagnosed haemochromatosis are generally advised to follow a healthy diet comparable to that recommended for the general population rather than a restrictive low-iron diet. Furthermore, the guidelines recommend avoiding iron supplementation and products fortified with iron. Reduced red-meat intake and avoidance of vitamin C supplementation are also addressed, although these are weak recommendations. No corresponding recommendation calls for reduced dietary iron intake solely because of simple C282Y or H63D heterozygosity. Taken together with the findings of Hunt and Zeng (2004), Roe et al. (2005), and Greenwood et al. (2005), current knowledge does not justify routine long-term dietary iron restriction in simple heterozygotes with normal iron indices.

The role of prophylactic iron removal by phlebotomy is even more uncertain. No studies have directly assessed whether initiating phlebotomy provides clinical benefit or prevents subsequent iron accumulation in individuals with simple C282Y or H63D heterozygosity, or C282Y/H63D compound heterozygosity, without evidence of iron overload. This takes on particular significance considering the low risk of documented iron overload described previously. The absence of such data does not prove that prophylactic phlebotomy is ineffective. Nevertheless, when the underlying probability of clinically relevant iron accumulation is low, the lack of demonstrated benefit substantially weakens the justification for its routine use.

C282Y/H63D compound heterozygosity warrants separate consideration because its clinical significance is greater than that of simple heterozygosity, although the overall risk of substantial iron overload remains low. EASL (2022) indicates that in this group phlebotomy may be considered when iron overload has been confirmed by magnetic resonance imaging or liver biopsy. However, this is a weak recommendation based on level 5 evidence.

The importance of a phenotype-based approach is emphasised by the fact that repeated phlebotomy is not entirely without burden or risk for the patient. In discussing phlebotomy for hyperferritinaemia, Beaton and Adams (2012) highlighted the possibility of iron-deficiency anaemia and of perpetuating an incorrect diagnosis when treatment is continued despite the absence of a valid indication. More recent data on the burden of repeated iron depletion come from Infanti et al. (2024), who enrolled 30 individuals with C282Y/C282Y, C282Y/H63D, or H63D/H63D genotypes and elevated serum ferritin levels. Among the 27 participants who completed the study, anaemia occurred in 13 (48%), and fatigue was reported after 25% of phlebotomy procedures. These findings raise further concerns about the use of phlebotomy in individuals without confirmed iron overload.

A positive genetic test alone is therefore insufficient to justify interventions aimed at reducing body iron stores. In heterozygous individuals without biochemical evidence of excessive iron accumulation, neither chronic dietary restriction nor prophylactic phlebotomy has demonstrated clinical benefit. Such measures should not be initiated solely on the basis of genetic predisposition.

7. Family Screening After Identification of an HFE Carrier

The ACG guideline (Kowdley et al., 2019) and the EASL recommendations (2022) discuss screening of first-degree relatives primarily in the context of patients with haemochromatosis. According to Barton and Parker (2024), siblings of a proband who carry a single C282Y variant are not considered at risk of HFE-related iron overload, even though their serum iron parameters may be abnormal, and no further testing of their own children or partners is proposed. Testing of a reproductive partner is discussed only when the proband has diagnosed disease and is intended to assess the probability of a disease-associated genotype in offspring. However, none of these sources describes a situation in which family testing would be initiated by the carrier of a single HFE variant. By contrast, the European Molecular Genetics Quality Network (EMQN) guidelines advise against systematic testing of adult first-degree relatives of C282Y heterozygotes and report that no consensus was reached on testing clinically asymptomatic adult relatives of C282Y/H63D compound heterozygotes (Porto et al., 2016).

Nevertheless, general principles of genetic counselling for autosomal recessive diseases do not restrict cascade testing to affected individuals (Vears et al., 2023). An identified carrier may also serve as the starting point for testing within a family, yet detection of a single pathogenic variant does not automatically establish the need for further evaluation of relatives. The recommendations of the European Society of Human Genetics indicate that cascade testing to identify carriers of autosomal recessive disorders may be considered when, among other criteria, the carrier frequency in the population is high (at least 1:100), so that the reproductive partner can also be offered testing, the condition is serious, and clinical or reproductive options are available (de Wert et al., 2026).

Applying these general criteria to HFE does not provide an unequivocal basis for extending testing to the family of a simple heterozygous carrier. C282Y and H63D variants are common: the C282Y allele frequency reaches 6.2%, while H63D is widely distributed and is more prevalent than C282Y (Kowdley et al., 2019). Their high prevalence is therefore one factor considered when evaluating the appropriateness of cascade testing. However, the criterion of disease severity is less straightforward because penetrance of HFE genotypes associated with haemochromatosis is incomplete and the probability of clinically significant disease varies markedly between genotypes. Population frequency alone is therefore insufficient to justify family testing after identification of simple heterozygosity.

This ambiguity is reflected in the guidelines on haemochromatosis. Apart from the EMQN recommendation concerning relatives of C282Y heterozygotes, they do not specify the management pathway when family testing would begin with an individual carrying a single HFE variant. In particular, current sources do not establish whether, or under what circumstances, identification of simple heterozygosity should prompt testing of a reproductive partner.

8. Clinical Consequences of Overinterpreting the H63D Variant

In clinical practice, the presence of H63D may be regarded as a confirmation of haemochromatosis or as an explanation for non-specific symptoms and laboratory abnormalities, even though the genetic finding alone does not support such conclusions. This overinterpretation is reflected in the term ‘H63D syndrome’, which appears in the literature. Shearman et al. (2026) emphasise that this terminology assigns undue clinical significance to H63D and that there is no basis for recognising the syndrome as a distinct clinical entity.

This phenomenon also has practical implications, evident in decisions regarding the patient’s further management. The EMQN guidelines note that expanding HFE testing to include H63D may generate additional investigations of limited diagnostic value and may cause anxiety related to the result (Porto et al., 2016). The relevance of this issue is supported by data from Speechley et al. (2012). In the group with simple H63D heterozygosity and normal ferritin levels and transferrin saturation, the use of medical services during the 12 months after disclosure of the result was 3% higher than in a comparison group without C282Y or H63D and with normal iron indices (RR = 1.03; 95% CI: 1.02-1.04; $p < 0.001$). The study could not determine whether this difference was directly related to the genetic result or whether the additional healthcare use was clinically justified. It therefore does not demonstrate a direct causal link between H63D detection and unnecessary medical consultations, but it does suggest that disclosure of an equivocal genetic finding may influence subsequent use of healthcare services.

These concerns are also reflected in the debate over routine H63D genotyping. EASL (2022) considers the value of H63D testing controversial. Testing for this variant may be performed in selected clinical situations, but its detection should not dictate treatment decisions. A more definitive stance was presented by Shearman

et al. (2026), who propose limiting routine HFE testing to the C282Y variant. In their view, H63D may have a role as part of extended genetic assessment in selected patients with unequivocally confirmed iron overload, but it should not be used for risk assessment in asymptomatic individuals or for cascade testing. The discrepancy between these positions illustrates that the debate surrounding H63D now extends beyond its clinical significance to whether the result should actually be obtained and reported when it does not influence subsequent management.

Conclusions

The clinical relevance of HFE heterozygosity is determined primarily by the patient's phenotype, not by the genetic finding itself. In individuals with simple C282Y or H63D heterozygosity and no biochemical evidence of iron accumulation, the probability of overt disease is low. However, this does not mean that iron overload never occurs in carriers. Abnormal iron indices therefore require further evaluation despite the generally favourable risk profile of these genotypes. C282Y/H63D compound heterozygosity requires closer attention, but management should likewise be based on abnormalities in iron metabolism and the presence of additional risk factors.

With regard to iron-reducing interventions, specific dietary restrictions or prophylactic phlebotomy are not justified in the absence of iron overload. Follow-up recommendations, however, vary according to genotype. No dedicated schedule for periodic monitoring of iron indices is specified for simple C282Y or H63D heterozygosity, whereas for C282Y/H63D compound heterozygosity EASL (2022) allows monitoring adjusted to age and individual risk profile. Moreover, current recommendations focus mainly on relatives of patients with diagnosed haemochromatosis. Among the reviewed documents, only the EMQN guidelines address relatives of simple C282Y heterozygotes and advise against their systematic testing, while the role of testing a reproductive partner after identification of a single variant remains undefined.

The H63D variant exemplifies the potential consequences of overinterpreting genetic findings. As testing becomes increasingly available, it is crucial not only to identify a predisposition but also to determine its clinical implications. Genotype information should contribute to patient management rather than result in carrier status being regarded as disease.

Further research is required to define more precisely the risk associated with HFE heterozygosity and the optimal scope of management in this population. Until then, decisions regarding monitoring, possible iron-reducing interventions, and family testing should remain individualised according to the patient's biochemical and clinical phenotype together with additional risk factors.

Conflict of Interest: No conflicts of interests to declare.

Author Contributions: All authors contributed to the article. All authors have read and approved the final version of the manuscript and agreed to its submission and publication.

Funding: This research received no external funding.

REFERENCES

1. Abeyagunawardena, I., Mayura, S., Samarasingha, P., Nawinne, A., & Karunatilake, H. (2024). Iron overload in histidine-to-aspartic acid substitution at 63 (H63D) gene heterozygous hereditary hemochromatosis with erythrocytosis: A case report. *Cureus*, 16(12), e76335. <https://doi.org/10.7759/cureus.76335>
2. Aguilar-Martinez, P., Grandchamp, B., Cunat, S., Cadet, E., Blanc, F., Nourrit, M., Lassoued, K., Schved, J.-F., & Rochette, J. (2011). Iron overload in HFE C282Y heterozygotes at first genetic testing: A strategy for identifying rare HFE variants. *Haematologica*, 96(4), 507–514. <https://doi.org/10.3324/haematol.2010.029751>
3. Allen, K. J., Gurrin, L. C., Constantine, C. C., Osborne, N. J., Delatycki, M. B., Nicoll, A. J., McLaren, C. E., Bahlo, M., Nisselle, A. E., Vulpe, C. D., Anderson, G. J., Southey, M. C., Giles, G. G., English, D. R., Hopper, J. L., Olynyk, J. K., Powell, L. W., & Gertig, D. M. (2008). Iron-overload-related disease in HFE hereditary hemochromatosis. *New England Journal of Medicine*, 358(3), 221–230. <https://doi.org/10.1056/NEJMoa073286>
4. Aranda, N., Viteri, F. E., Montserrat, C., & Arija, V. (2010). Effects of C282Y, H63D, and S65C HFE gene mutations, diet, and life-style factors on iron status in a general Mediterranean population from Tarragona, Spain. *Annals of Hematology*, 89(8), 767–773. <https://doi.org/10.1007/s00277-010-0901-9>

5. Barton, J. C., & Parker, C. J. (2024). HFE-related hemochromatosis. In M. P. Adam, S. Bick, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, & A. Amemiya (Eds.), *GeneReviews® [Internet]*. University of Washington, Seattle. Retrieved August 2026, from <https://www.ncbi.nlm.nih.gov/books/NBK1440/> (Original work published 2000)
6. Beaton, M. D., & Adams, P. C. (2012). Treatment of hyperferritinemia. *Annals of Hepatology*, 11(3), 294–300. [https://doi.org/10.1016/S1665-2681\(19\)30923-8](https://doi.org/10.1016/S1665-2681(19)30923-8)
7. Burke, W., Imperatore, G., McDonnell, S. M., Baron, R. C., & Khoury, M. J. (2000). Contribution of different HFE genotypes to iron overload disease: A pooled analysis. *Genetics in Medicine*, 2(5), 271–277. <https://doi.org/10.1097/00125817-200009000-00001>
8. de Wert, G., van El, C. G., Clarke, A., Cordier, C., Fellmann, F., Genuardi, M., Hentze, S., Kayserili, H., Macek, M., MacLeod, R., Melegh, B., Mendes, A., Rial-Sebbag, E., Stefánsdóttir, V., Tranebjærg, L., Ulph, F., & Forzano, F. (2026). Cascade counselling and testing. Recommendations of the European Society of Human Genetics. *European Journal of Human Genetics*, 34(2), 171–184. <https://doi.org/10.1038/s41431-025-01945-3>
9. European Association for the Study of the Liver. (2022). EASL clinical practice guidelines on haemochromatosis. *Journal of Hepatology*, 77(2), 479–502. <https://doi.org/10.1016/j.jhep.2022.03.033>
10. Fabri, R. F. S., Rodrigues, R. M. D. M., & Prabakar, D. (2026). Severe hepatic iron overload and cirrhosis in an HFE C282Y heterozygote with autoimmune hepatitis: A case of genotype-phenotype discordance. *Cureus*, 18(3), e105851. <https://doi.org/10.7759/cureus.105851>
11. Ginzburg, Y. Z. (2019). Hfeidin-ferroportin axis in health and disease. *Vitamins and Hormones*, 110, 17–45. <https://doi.org/10.1016/bs.vh.2019.01.002>
12. Greenwood, D. C., Cade, J. E., Moreton, J. A., O'Hara, B., Burley, V. J., Randerson-Moor, J. A., Kukalich, K., Thompson, D., Worwood, M., & Bishop, D. T. (2005). HFE genotype modifies the influence of heme iron intake on iron status. *Epidemiology*, 16(6), 802–805. <https://doi.org/10.1097/01.ede.0000181306.85583.ea>
13. Grosse, S. D., Gurrin, L. C., Bertalli, N. A., & Allen, K. J. (2018). Clinical penetrance in hereditary hemochromatosis: Estimates of the cumulative incidence of severe liver disease among HFE C282Y homozygotes. *Genetics in Medicine*, 20(4), 383–389. <https://doi.org/10.1038/gim.2017.121>
14. Gurrin, L. C., Bertalli, N. A., Dalton, G. W., Osborne, N. J., Constantine, C. C., McLaren, C. E., English, D. R., Gertig, D. M., Delatycki, M. B., Nicoll, A. J., Southey, M. C., Hopper, J. L., Giles, G. G., Anderson, G. J., Olynyk, J. K., Powell, L. W., & Allen, K. J. (2009). HFE C282Y/H63D compound heterozygotes are at low risk of hemochromatosis-related morbidity. *Hepatology*, 50(1), 94–101. <https://doi.org/10.1002/hep.22972>
15. Hasan, S. M. M., Farrell, J., & Borgaonkar, M. (2022). C282Y/H63D compound heterozygosity is a low penetrance genotype for iron overload-related disease. *Journal of the Canadian Association of Gastroenterology*, 5(5), 240–247. <https://doi.org/10.1093/jcag/gwac025>
16. Hunt, J. R., & Zeng, H. (2004). Iron absorption by heterozygous carriers of the HFE C282Y mutation associated with hemochromatosis. *American Journal of Clinical Nutrition*, 80(4), 924–931. <https://doi.org/10.1093/ajcn/80.4.924>
17. Infanti, L., Leitner, G., Moe, M., Pehlic, V., Cattaneo, M., Benkert, P., Holbro, A., Passweg, J., Worel, N., & Buser, A. (2024). Blood donation for iron removal in individuals with HFE mutations: Study of efficacy and safety and short review on hemochromatosis and blood donation. *Frontiers in Medicine*, 11, 1362941. <https://doi.org/10.3389/fmed.2024.1362941>
18. Jackson, H. A., Carter, K., Darke, C., Guttridge, M. G., Ravine, D., Hutton, R. D., Napier, J. A., & Worwood, M. (2001). HFE mutations, iron deficiency and overload in 10,500 blood donors. *British Journal of Haematology*, 114(2), 474–484. <https://doi.org/10.1046/j.1365-2141.2001.02949.x>
19. Kowdley, K. V., Brown, K. E., Ahn, J., & Sundaram, V. (2019). ACG clinical guideline: Hereditary hemochromatosis. *American Journal of Gastroenterology*, 114(8), 1202–1218. <https://doi.org/10.14309/ajg.0000000000000315>
20. Moretti, D., van Doorn, G. M., Swinkels, D. W., & Melse-Boonstra, A. (2013). Relevance of dietary iron intake and bioavailability in the management of HFE hemochromatosis: A systematic review. *American Journal of Clinical Nutrition*, 98(2), 468–479. <https://doi.org/10.3945/ajcn.112.048264>

21. Nemeth, E., & Ganz, T. (2023). Heparidin and iron in health and disease. *Annual Review of Medicine*, 74, 261–277. <https://doi.org/10.1146/annurev-med-043021-032816>
22. Porto, G., Brissot, P., Swinkels, D. W., Zoller, H., Kamarainen, O., Patton, S., Alonso, I., Morris, M., & Keeney, S. (2016). EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis (HH). *European Journal of Human Genetics*, 24(4), 479–495. <https://doi.org/10.1038/ejhg.2015.128>
23. Roe, M. A., Heath, A. L. M., Oyston, S. L., Macrow, C., Hoogewerff, J. A., Foxall, R., Dainty, J. R., Majsak-Newman, G., Willis, G., & Fairweather-Tait, S. J. (2005). Iron absorption in male C282Y heterozygotes. *American Journal of Clinical Nutrition*, 81(4), 814–821. <https://doi.org/10.1093/ajcn/81.4.814>
24. Shearman, J., Olynyk, J. K., Distant, S., Porto, G., & Sanchez, M. (2026). HFE and hemochromatosis: Time to reconsider the diagnostic role of the p.His63Asp variant. *Blood Red Cells & Iron*, 2(1), 100034. <https://doi.org/10.1016/j.brci.2025.100034>
25. Speechley, M., Alter, D., Guo, H., Harrison, H., & Adams, P. C. (2012). Effect of ambiguous hemochromatosis gene test results on physician utilization. *Medical Care*, 50(5), 394–398. <https://doi.org/10.1097/MLR.0b013e318245a06e>
26. Turbiville, D., Du, X., Yo, J., Jana, B. R., & Dong, J. (2019). Iron overload in an HFE heterozygous carrier: A case report and literature review. *Laboratory Medicine*, 50(2), 212–217. <https://doi.org/10.1093/labmed/lmy065>
27. Vears, D. F., Boyle, J., Jacobs, C., McInerney-Leo, A., & Newson, A. J. (2023). Human Genetics Society of Australasia position statement: Genetic carrier testing for recessive conditions. *Twin Research and Human Genetics*, 26(2), 188–194. <https://doi.org/10.1017/thg.2023.15>
28. Walsh, A., Dixon, J. L., Ramm, G. A., Hewett, D. G., Lincoln, D. J., Anderson, G. J., Subramaniam, V. N., Dodemaide, J., Cavanaugh, J. A., Bassett, M. L., & Powell, L. W. (2006). The clinical relevance of compound heterozygosity for the C282Y and H63D substitutions in hemochromatosis. *Clinical Gastroenterology and Hepatology*, 4(11), 1403–1410. <https://doi.org/10.1016/j.cgh.2006.07.009>
29. Zaloumis, S. G., Allen, K. J., Bertalli, N. A., Turkovic, L., Delatycki, M. B., Nicoll, A. J., McLaren, C. E., English, D. R., Hopper, J. L., Giles, G. G., Anderson, G. J., Olynyk, J. K., Powell, L. W., & Gurrin, L. C. (2015). The natural history of HFE simple heterozygosity for C282Y and H63D: A prospective twelve year study. *Journal of Gastroenterology and Hepatology*, 30(4), 719–725. <https://doi.org/10.1111/jgh.12804>