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Investigation of HFE Mutations Associated with Hemochromatosis the Case of Ordu Province

Hemokromatozis ile İlişkili HFE Mutasyonlarının Araştırılması: Ordu İli Örneği

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ABSTRACT

Objective: Hereditary hemochromatosis (HH) is a common autosomal recessive disorder characterised by increased intestinal iron absorption and progressive systemic iron overload. Although the C282Y variant is the predominant cause of HH in Northern European populations, the distribution of *HFE* variants varies geographically, and data from different regions of Türkiye remain limited. This study aimed to evaluate the molecular spectrum of *HFE* gene variants and their biochemical correlates in patients investigated for HH in Ordu province, located in the Black Sea region of Türkiye.

Methods: A retrospective analysis was conducted of 100 patients who underwent *HFE* gene sequencing between January 2024 and October 2025. Demographic characteristics, hemoglobin, serum ferritin, serum iron, transferrin saturation (TS), and *HFE* genotypes were obtained from medical records. PCR-based amplification followed by next-generation sequencing on the Illumina MiSeq platform was performed. Variants were classified using international databases, including ClinVar, Franklin, VarSome, HGMD Public®, gnomAD, and dbSNP. Comparisons between variant carriers and non-carriers were analysed using the Mann–Whitney U and chi-square tests.

Results: Among the 1000 patients (53 males, 47 females; mean age 47.2 ± 17.1 years), 64 (64.0%) exhibited a wild-type *HFE* genotype, while 36 (36.0%) carried at least one variant. The c.187C>G (H63D) variant was the most common, with 30 heterozygotes and 3 homozygotes, followed by one case each of C282Y homozygosity, c.76 + 2 dup heterozygosity, and H63D + c.76+2 dup compound heterozygosity. Ferritin, serum iron, and hemoglobin levels did not differ significantly between variant

ÖZ

Amaç: Herediter hemokromatozis (HH), artmış intestinal demir emilimi ve ilerleyici sistemik demir yüklenmesi ile karakterize yaygın bir otozomal resesif hastalıktır. C282Y varyantı Kuzey Avrupa popülasyonlarında HH'nin baskın nedeni olmakla birlikte, HFE varyantlarının dağılımı coğrafi olarak değişkenlik göstermektedir ve Türkiye'nin farklı bölgelerine ait veriler halen sınırlıdır. Bu çalışma, Türkiye'nin Karadeniz Bölgesi'nde yer alan Ordu ilinde HH açısından araştırılan hastalarda *HFE* geni varyantlarının moleküler spektrumunu ve bunların biyokimyasal korelatlarını değerlendirmeyi amaçlamıştır.

Yöntemler: Ocak 2024 ile Ekim 2025 tarihleri arasında *HFE* gen dizilemesi yapılan 100 hastanın retrospektif analizi gerçekleştirildi. Demografik özellikler, hemoglobin, serum ferritin, serum demiri, transferrin satürasyonu (TS) ve *HFE* genotipleri tıbbi kayıtlardan elde edildi. PCR temelli amplifikasyonun ardından Illumina MiSeq platformunda yeni nesil dizileme uygulandı. Varyantlar; ClinVar, Franklin, VarSome, HGMD Public®, gnomAD ve dbSNP dâhil uluslararası veri tabanları kullanılarak sınıflandırıldı. Varyant taşıyıcıları ile taşıyıcı olmayanlar arasındaki karşılaştırmalar Mann-Whitney U ve ki-kare testleri ile analiz edildi.

Bulgular: Yüz hastanın (53 erkek, 47 kadın; ortalama yaş 47,2 ± 17,1 yıl) 64'ünde (%64,0) vahşi tip *HFE* genotipi saptanırken, 36'sında (%36,0) en az bir varyant bulundu. En sık görülen varyant, 30 heterozigot ve 3 homozigot olgu ile c.187C>G (H63D) idi. Bunu birer olgu şeklinde C282Y homozigotluğu, c.76+2dup heterozigotluğu ve H63D + c.76+2dup bileşik heterozigotluğu izledi. Ferritin, serum demiri ve hemoglobin düzeyleri açısından varyant taşıyıcıları ile taşıyıcı olmayanlar arasında

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ABSTRACT

carriers and non-carriers (p = 0.869, 0.204, and 0.674, respectively). However, elevated TS (> 45%) was observed significantly more often in variant carriers than in wild-type individuals (41.7% vs. 20.3%, p = 0.040), indicating an approximately 2.8-fold higher likelihood of high TS among carriers. The proportion of participants with ferritin > 400 µg/L did not differ significantly between groups. The highest ferritin level (6104 µg/L) was observed in a heterozygous H63D female patient with severe anemia, while the highest TS (99%) was detected in a patient without any detectable variants.

Conclusion: In this Black Sea cohort, H63D was the predominant *HFE* variant, whereas C282Y was rare, consistent with regional genetic patterns in Türkiye. Although *HFE* variants were not associated with significant differences in ferritin, their strong association with elevated TS suggests a measurable impact on iron handling. These findings highlight the importance of TS in the diagnostic evaluation of HH in regions where C282Y-related HH is uncommon. Larger multicenter studies incorporating imaging-based iron quantification are needed to better define genotype–phenotype relationships in the Turkish population.

Keywords: Hereditary hemochromatosis, *HFE* gene, iron overload, transferrin saturation

ÖZ

anlamlı fark saptanmadı (sırasıyla p = 0,869, 0,204 ve 0.674). Bununla birlikte, yüksek TS düzeyi (> %45) varyant taşıyıcılarında vahşi tip bireylere göre anlamlı olarak daha sık gözlendi (%41,7'ye karşı %20,3, p = 0,040); bu durum, taşıyıcılarda yüksek TS görülme olasılığının yaklaşık 2.8 kat daha fazla olduğunu göstermektedir. Ferritin düzeyinin > 400 µg/L olma oranı açısından gruplar arasında anlamlı fark bulunmadı. En yüksek ferritin düzeyi (6104 µg/L), ağır anemisi olan H63D heterozigot kadın bir hastada saptanırken; en yüksek TS değeri (%99), saptanabilir herhangi bir varyantı olmayan bir hastada gözlendi.

Sonuç: Bu Karadeniz kohortunda H63D başlıca HFE varyantı olarak saptanırken, C282Y'nin nadir olduğu görüldü; bu bulgu Türkiye'deki bölgesel genetik örüntülerle uyumludur. HFE varyantları ferritin düzeylerinde anlamlı farklılıklarla ilişkili olmasa da, yüksek TS ile gösterdikleri güçlü ilişki demir metabolizması üzerinde ölçülebilir bir etkilerine işaret etmektedir. Bu bulgular, C282Y ile ilişkili HH'nin nadir olduğu bölgelerde HH'nin tanısal değerlendirmesinde TS'nin önemini vurgulamaktadır. Türk popülasyonunda genotip-fenotip ilişkilerini daha iyi tanımlayabilmek için görüntüleme temelli demir kantifikasyonunu içeren daha geniş, çok merkezli çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Herediter hemokromatozis, *HFE* geni, demir birikimi, transferrin satürasyonu

INTRODUCTION

Hereditary hemochromatosis (HH) is one of the most prevalent autosomal recessive metabolic disorders worldwide. It is characterised by excessive intestinal iron absorption and progressive parenchymal iron deposition in multiple organs, primarily the liver, pancreas, heart, joints, and pituitary gland (1). The resultant iron overload leads to tissue injury and, if untreated, may cause cirrhosis, diabetes mellitus, cardiomyopathy, hypogonadism, arthropathy, and even hepatocellular carcinoma (HCC) (2). Although environmental and secondary causes of iron overload exist, HH represents the major genetic form, with its pathogenesis predominantly linked to pathogenic variants in the *HFE* gene (2).

The *HFE* gene, located on chromosome 6p21.3 within the major histocompatibility complex region, spans approximately 12 kb and contains six exons encoding a 343-amino-acid transmembrane glycoprotein (3). The HFE protein has been demonstrated to interact with the transferrin receptor 1 (TfR1), thereby playing a pivotal role in modulating hepcidin expression. This, in turn, is the central regulator of systemic iron homeostasis (4). Under normal physiological conditions, the HFE–TfR1 complex regulates iron uptake by hepatocytes and enterocytes through feedback signalling that limits dietary iron absorption. Disruption of this pathway through *HFE* variants leads to insufficient hepcidin synthesis, unchecked intestinal iron absorption, and progressive systemic iron overload (5).

The most prevalent pathogenic *HFE* variants are *c.845G>A(p. Cys282Tyr, C282Y) and c.187C>G(p.His63Asp, H63D),* which account for the vast majority of cases, especially in populations of Northern European descent (6). The C282Y variant has been demonstrated to disrupt a critical disulfide bond that is necessary for the proper folding and cell-surface expression of the *HFE* protein. This results in impaired TfR1 binding and dysregulated iron sensing (7). Homozygosity for the *C282Y* allele has been demonstrated to be responsible for over 80–90% of clinically diagnosed HH cases in Western populations, whereas compound heterozygosity for C282Y/H63D has been shown to be associated with a milder, often incomplete phenotype (8). The *H63D* variant, while more common globally, is generally considered a low-penetrance variant that contributes to iron overload only in the presence of other genetic or environmental risk factors such as alcohol intake, viral hepatitis, or metabolic syndrome (9).

In addition to these classical variants, a number of rare or population-specific variants in *HFE* and non-*HFE* genes, including *TFR2, HAMP, HJV, and SLC40A1,* have been reported to cause distinct subtypes of HH with variable clinical penetrance (10). Studies from Southern Europe, the Mediterranean basin, and the Middle East have revealed a more diverse variant spectrum, underscoring the importance of regional genetic screening and local genotype–phenotype correlation analyses (10).

The prevalence of HH varies considerably across populations and ethnic groups. The C282Y allele frequency has been estimated at approximately 6–10% in Northern European populations, 2–4% in Southern European populations, and less than 1% in Asian and African populations, reflecting its well-established Northwestern European origin and founder effect. In contrast, studies conducted in Türkiye have consistently demonstrated that the H63D variant is the predominant *HFE* variant, whereas C282Y remains relatively rare. This distribution pattern likely reflects the complex population genetics, historical migration routes, and admixture events that have shaped the genetic landscape of Anatolia. Nevertheless, comprehensive data regarding the molecular spectrum and regional variability of *HFE* variants within different Turkish subpopulations remain limited. These limitations underscores the importance of region-specific genetic studies aimed at clarifying both carrier frequencies and the clinical relevance of *HFE* variants in the Turkish population. Clinically, HH displays substantial variability in onset age, biochemical profile, and organ involvement, even among

individuals harbouring identical genotypes (11). This heterogeneity reflects the interplay among genetic background, dietary factors, alcohol use, and comorbid conditions. Early recognition through genetic screening is therefore essential, as therapeutic phlebotomy when initiated before irreversible organ damage has occurred can effectively normalize iron levels and prevent complications (2,11). In recent years, next-generation sequencing and expanded molecular panels have greatly improved the detection of both common and rare *HFE* variants, facilitating earlier diagnosis and more precise classification of hereditary iron overload syndromes (10).

In view of these observations, a systematic evaluation of *HFE* gene variants within distinct geographic regions has the potential to enhance understanding of local variant profiles and their clinical correlates. The present study aims to determine the variant detection rate of the *HFE* gene am*ong* patients referred for suspected HH in Ordu Province and its surrounding region, to analyse their demographic and clinical characteristics, and to contribute data to the scientific literature.

MATERIALS AND METHODS

A retrospective analysis was conducted of the medical records of patients who attended the Ordu University outpatient clinic between January 1, 2024, and October 1, 2025, with a preliminary diagnosis of hemochromatosis. All patients for whom *HFE* gene sequencing was requested were included in the study. This cohort represents a referral-based screening population rather than clinically confirmed HH cases. All consecutive patients who were referred with a preliminary clinical suspicion of HH and for whom *HFE* gene analysis was requested were included, regardless of whether they ultimately fulfilled established diagnostic criteria for HH based on biochemical or clinical parameters. Therefore, the study reflects the real-world diagnostic spectrum of patients evaluated for suspected HH in our region. However, the referral-based inclusion strategy may introduce selection bias and should be considered a methodological limitation when interpreting genotype–phenotype associations. The primary objective was to evaluate the frequency and distribution of *HFE* variants and to assess their association with iron-related biochemical parameters in a real-world referral cohort. The clinical findings and the variants identified in the patients were described. In the present study, patients' ages, sex, hemoglobin levels, ferritin levels, serum iron levels, transferrin saturation (TS), and *HFE* gene *anal*ysis results were obtained by retrospective review of their medical records.

A total of 100 patients who were referred to the Department of Medical Genetics at Ordu University Hospital with a preliminary diagnosis of hemochromatosis were evaluated. Peripheral blood samples were collected in EDTA tubes for DNA extraction.

The genomic DNA was isolated using the DiaRex® Whole Blood Genomic DNA Extraction Kit (Cat. No. BLD-5295, Diagen, Ankara). This process involved lysis, proteinase K digestion, ethanol precipitation, and column-based purification to obtain high-quality DNA. The measurement of nucleic acid concentrations was conducted utilising a Colibri Microvolume Spectrometer (Titertek-Berthold, Germany). The exons of the HFE *gene* were amplified via PCR and subjected to sequencing on the Illumina MiSeq platform using the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA). The variants were visualised using the Integrative Genomics Viewer (IGV) and classified according to the Franklin, VarSome, HGMD Public®, ClinVar, gnomAD, ExAC, KGP, and dbSNP databases.

The present study was conducted with the approval of the Non-Interventional Clinical Research Ethics Committee of Ordu University (decision number: 2025/227, date: 09.07.2025). This retrospective study was conducted using data obtained from medical records in accordance with institutional ethical standards.

RESULTS

A total of 100 patients (53 males and 47 females) who underwent *HFE* gene analysis were included in the study. The mean age of the patients was 47.2 ± 17.1 years (range: 2–81 years). Regarding biochemical parameters, serum ferritin levels were available for 97 patients, with a mean ferritin concentration of 447.9 ± 726.7 µg/L (min: 9.27; max: 6104). Serum iron levels were available in 93 patients, with an average of 105.2 ± 49.6 µg/dL (min: 27.8; max: 268.3). The measurement of hemoglobin values was conducted in all patients, yielding a mean of 14.3 ± 2.2 g/dL (min: 8.4; max: 19.1). TS data were available for 65 patients (65% of the cohort), and all TS-based statistical analyses were performed within this subset, with a mean TS of 41.7 ± 24.4% (min: 5.67%; max: 99%). The baseline demographic and biochemical characteristics of the study population are summarized in Table 1.

Genotype analysis revealed that 64 of the 100 patients (64.0%) had no HFE variants (wild-type). Among the 36 patients carrying at least one variant, diverse genotypic patterns were observed:

- 30 patients (30.0%) were identified as heterozygous for c.187C>G (p.His63Asp, H63D).
- patients (3.0%) were identified as homozygous for H63D.
- 1 patient (1.0%) was identified as homozygous for c.845G>A (p.Cys282Tyr, C282Y).
- 1 patient (1.0%) was identified as heterozygous for c.76+2 dup.
- 1 patient (1.0%) was found to carry both c.187C>G and c.76+2 dup as a compound heterozygote.

At the allele level, a total of 41 mutant alleles were identified: 37 c.187C>G (H63D), 2 c.845G>A (C282Y), and 2 c.76+2 dup.

When ferritin levels were assessed the upper reference limit of 400 µg/L, 34 patients (34.0%) had ferritin >400 µg/L. Using a TS threshold of 45%, elevated TS values were observed in 28 patients (28.0%).

Patients were then categorised into two main groups based on the presence of any *HFE* variant: those without variants (n = 64) and those carrying at least one *HFE* variant (n = 36). The comparative biochemical findings of variant carriers and non-carriers are shown in Table 2. The mean age of the participants did not differ significantly between the groups (47.3 ± 16.4 vs. 46.9 ± 18.7 years). Ferritin, serum iron, and hemoglobin levels were compared between variant carriers and non-carriers using the Mann–Whitney U test. This comparison revealed no statistically significant differences.

- ferritin ($p = 0.869$),
- serum iron ($p = 0.204$),
- hemoglobin ($p = 0.674$).

TS levels were found to be comparable between the groups, with mean values of 39.6 ± 23.6% recorded in the wild-type group and 44.7 ± 25.6% observed among variant carriers ($p = 0.453$).

Table 1. Baseline demographic and biochemical characteristics of the patients.

	n	Mean ± SD	Median (IQR)	Min–Max
Age	100	47.2 ± 17.1	49 (32–62)	2–81
Sex (male/female)	100	53 / 47	–	–
Hemoglobin (g/dL)	100	14.3 ± 2.2	14.4 (12.9–15.7)	8.4–19.1
Ferritin (µg/L)	97	447.9 ± 726.7	205 (95–405)	9.27–6104
Serum iron (µg/dL)	93	105.2 ± 49.6	97 (73–130)	27.8–268.3
Transferrin saturation (%)	65	41.7 ± 24.4	37 (22–54)	5.67–99

Min: Minimum, Max: Maximum, SD: Standard deviation, IQR: Interquartile range.

Table 2. Comparison of biochemical parameters between patients with and without HFE variants.

Parameter	No variant (n = 64)	≥1 variant (n = 36)	p–value
Ferritin (µg/L)	462 ± 841	420 ± 498	0.869
Serum iron (µg/dL)	110 ± 49	96 ± 48	0.204
Hemoglobin (g/dL)	14.3 ± 2.3	14.2 ± 1.9	0.674
Transferrin saturation (%)**	39.6 ± 23.6	44.7 ± 25.6	0.453
TS > 45% (n%)**	13 / 64 (20.3%)	15 / 36 (41.7%)	0.040*
Ferritin > 400 µg/L (n%)	23 / 64 (35.9%)	11 / 36 (30.6%)	0.745

*Statistically significant.
**Transferrin saturation analyses were performed only in patients with available TS data (n = 65); percentages are calculated within this subgroup.
TS: Transferrin saturation.

However, the frequency of elevated TS (TS > 45%) was notably higher in patients carrying *HFE* variants. Among the 65 patients with available TS data, 15 of 36 variant carriers (41.7%) and 13 of 64 non-carriers (20.3%) exhibited TS > 45%. Chi-square analysis demonstrated a statistically significant association between the presence of *HFE* variants and elevated TS ($\chi^2 = 4.21$, $p = 0.040$). On the basis of this finding, the likelihood of having TS > 45% was approximately 2.8 times higher in variant carriers compared non-carriers (odds ratio ≈ 2.80; 95% confidence interval: 1.14–6.89).

In contrast, no significant difference was observed in the proportion with ferritin > 400 µg/L between variant carriers (11/36; 30.6%) and non-carriers (23/64; 35.9%) ($p = 0.745$).

A detailed genotypic subgroup review showed that the highest ferritin value (6104 µg/L) occurred in a 78-year-old female patient who was heterozygous for H63D and had severe anemia (Hb: 8.4 g/dL, TS: 79.91%). The highest TS value, 99%, was observed in a male patient without any detectable *HFE* variant; ferritin was not available for this patient.

In summary, the most frequently observed *HFE* variant in this cohort was c.187C>G (H63D), predominantly in the heterozygous form. While no significant differences were observed between variant carriers and non-carriers in ferritin, serum iron, or hemoglobin levels, a significant association was detected between carriage of *HFE* variants and elevated TS. These findings suggest that *HFE* variants may influence iron loading primarily through TS, whereas ferritin levels in this cohort appear to be strongly influenced by additional modifying factors.

DISCUSSION

The present study provides a comprehensive evaluation of *HFE* gene variants and iron-related biochemical parameters in a clinically heterogeneous cohort from the Black Sea region of Türkiye. The present findings corroborate the predominance of the c.187C>G (H63D) variant and the rarity of the classical c.845G>A (C282Y) variant in accordance with population-based studies conducted in Mediterranean, Middle Eastern, and Central Asian populations (12,13). The low prevalence of C282Y in Türkiye has been repeatedly highlighted, suggesting that classical Northern European–type HH (HH type 1) is uncommon in this region, whereas H63D represents the major allele influencing iron parameters (14,15).

The absence of significant differences in ferritin and serum iron levels between variant carriers and non-carriers in this study aligns with the widely accepted notion that H63D has low penetrance and limited phenotypic expression in isolation (16). Numerous studies have demonstrated that even *H63D* homozygotes frequently do not develop clinically significant iron overload unless additional genetic or environmental modifiers are present, including metabolic syndrome, chronic liver disease, alcohol use or concurrent inflammatory disorders (17,18).

In contrast, the significantly higher TS values observed among variant carriers in our cohort provide biologically meaningful insight. TS has been shown to rise earlier than ferritin in the natural history of HH, reflecting increased ferroportin-mediated iron efflux and decreased hepcidin activity. These phenomena may be subtly influenced by H63D variants (19,20). It has been demonstrated through experimental studies that mutant *HFE* proteins exhibit altered interactions with TfR1 and transferrin receptor 2 (TfR2)

which suggests the potential to modify hepcidin regulation even in the absence of severe phenotypic expression (21,22). Therefore, the elevated TS among *HFE* variant carriers in our cohort may represent an early biochemical footprint of altered iron homeostasis.

Although mean TS values did not differ significantly between variant carriers and non-carriers, the categorical analysis using the clinically established threshold of TS > 45% revealed a statistically significant association with *HFE* variant carriage. This discrepancy may be explained by the clinical relevance of threshold-based interpretation in HH, where TS values exceeding 45% are widely accepted as early biochemical indicators of altered iron metabolism. Comparisons of continuous means may obscure clinically meaningful distributional shifts around diagnostic cut-off points, whereas dichotomized analyses better reflect real-world diagnostic decision-making. Nevertheless, the calculated odds ratio should be interpreted with caution given the retrospective design, the relatively limited sample size, and the availability of TS measurements in only a subset of patients.

However, ferritin levels did not differ significantly across genotypes. In view of ferritin's well-established role as an acute-phase reactant influenced by hepatic inflammation, obesity, and systemic metabolic conditions, this finding is not surprising (23,24). In this cohort, several individuals with markedly elevated ferritin levels exhibited concomitant anemia or other comorbidities. These findings support the hypothesis that ferritin lacks specificity in distinguishing genetic iron overload from secondary hyperferritinemia.

Beyond the primary biochemical findings, the genotype distribution in our cohort reflects a characteristic pattern observed in Southern European and Middle Eastern countries. Genetic epidemiology studies suggest that the C282Y allele originated in Northwestern Europe and underwent a founder expansion. However, H63D exhibits a much older and geographically widespread evolutionary history (12,13,15,25). The predominance of H63D in our population may therefore be attributed to ancient migration patterns and long-term allelic equilibrium, rather than to disease-driven selection.

The single case of C282Y homozygosity in the present dataset indicates the rarity of classical *HFE*-associated hemochromatosis genotypes in Türkiye. While C282Y homozygotes demonstrate the highest clinical penetrance and strongest association with progressive iron overload, even among C282Y individuals the penetrance varies widely across ethnic groups and is strongly influenced by sex, dietary iron intake, alcohol consumption, viral hepatitis, metabolic syndrome and hormonal status (12,13,26). Therefore, the low prevalence of C282Y in our cohort is consistent with the limited number of clinically confirmed HH type 1 cases reported by hepatology centers across Türkiye.

In addition to the classical variants, one patient in our cohort was identified as carrying the c.76+2dup splice-site variant. This alteration affects the highly conserved +2 position of the donor splice site, a region that is critical for proper mRNA splicing. Variants occurring at canonical splice-site positions are generally considered likely to disrupt normal transcript processing and may result in exon skipping or aberrant protein products. However, functional validation data for c.76+2dup are currently limited, and its precise impact on HFE protein expression and hepcidin regulation remains unclear. Therefore, although the variant may have potential pathogenic relevance based on its predicted molecular consequence, cautious interpretation is warranted in the absence of experimental confirmation or segregation analysis.

A notable observation in the present study was the discordance between ferritin and TS across genotypes. This finding is biologically plausible: while TS reflects circulating iron that is immediately available for cellular uptake, ferritin reflects stored iron and is heavily influenced by inflammatory pathways. Systemic inflammation has been observed to stimulate ferritin synthesis via IL-6-mediated pathways and to suppress ferroportin, resulting in the paradoxical coexistence of high ferritin and low TS—commonly observed in anemia of chronic disease and non-alcoholic fatty liver disease related hyperferritinemia (23,24). In contrast, early HH is classically characterised by disproportionately elevated TS with normal or mildly elevated ferritin (20). The present findings support this model: variant carriers exhibited higher TS despite having ferritin levels comparable to those of wild-type individuals. Such discrepancies emphasise the clinical importance of TS as an early diagnostic parameter, particularly in regions where H63D predominates and C282Y-based HH is rare.

The mild phenotype of H63D carriers observed in the present dataset is consistent with molecular functional studies demonstrating that the H63D variant results in minimal disruption of the *HFE–TfR1–TfR2–hepcidin* axis compared with C282Y, H63D mutant proteins have been observed to retain a partial ability to form functional complexes, resulting in a subtle reduction rather than complete absence of hepcidin signaling (21,22). Consequently, many H63D carriers may present with mild biochemical alterations without clinically significant iron deposition. However, environmental interactions, such as obesity, alcohol consumption, or metabolic syndrome have been demonstrated to amplify these effects and unmask subclinical dysregulation (17,18).

This study has several limitations. First, its referral-based design meant that all participants were included based on a preliminary clinical suspicion of HH rather than on confirmed diagnostic criteria, resulting in a heterogeneous diagnostic spectrum. This may limit the generalizability of genotype–phenotype associations and partly explain the relatively high proportion of wild-type individuals and the modest biochemical differences observed. Second, TS measurements were available for only 65% of the cohort and TS-based analyses were therefore restricted to this subgroup, potentially reducing statistical power and influencing the interpretation of the results. Finally, the relatively small sample size (n = 100) and the restriction of genetic analysis to the *HFE* gene—without sequencing other hemochromatosis-related genes—limit broader conclusions, and non-*HFE* genetic causes of iron overload cannot be excluded.

CONCLUSION

This study confirms that the *HFE* variant profile in Türkiye is characterised by a high prevalence of the H63D variant and a very low frequency of C282Y. While H63D does not significantly influence ferritin levels, it is associated with a measurable increase in TS, suggesting a mild effect on iron transport rather than substantial iron accumulation. These findings highlight the diagnostic value of TS in populations where classical C282Y-related hemochromatosis is rare.

Further multicenter and prospective studies using comprehensive clinical and imaging-based assessments are required to clarify genotype–phenotype relationships and to optimise diagnostic strategies for iron metabolism disorders in the Turkish population.

Ethics

Ethics Committee Approval: The present study was conducted with the approval of the Non-Interventional Clinical Research Ethics Committee of Ordu University (decision number: 2025/227, date: 09.07.2025).

Informed Consent: This retrospective study was conducted using data obtained from medical records in accordance with institutional ethical standards.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ç.D., A.O.G., M.Ö., Concept: Ç.D., A.O.G., M.Ö., Design: Ç.D., A.O.G., M.Ö., Data Collection or Processing: Ç.D., A.O.G., M.Ö., Analysis or Interpretation: Ç.D., A.O.G., M.Ö., Literature Search: Ç.D., A.O.G., M.Ö., Writing: Ç.D., A.O.G., M.Ö.

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