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HFE and Hemochromatosis: Time to Reconsider the Diagnostic Role of the p.His63Asp Variant

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Abstract:
Hemochromatosis, primarily caused by homozygosity for the HFE p.Cys282Tyr variant, is the most frequent genetic iron overload disorder in populations of Northern European ancestry. Since the discovery of the HFE gene, genetic testing for HFE-related hemochromatosis has frequently included both the p.Cys282Tyr and the more common p.His63Asp variant. However, growing evidence indicates that the p.His63Asp variant lacks clear pathogenic significance in most clinical contexts related to iron overload, and its routine reporting may lead to diagnostic confusion and inappropriate management. This manuscript calls for a reassessment of current genetic testing practices in hemochromatosis, recommending that testing for HFE-related hemochromatosis be restricted to the p.Cys282Tyr variant. This position is grounded in current scientific evidence and aims to improve diagnostic accuracy, reduce patient harm, and promote more consistent clinical interpretation.

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HFE and Hemochromatosis: Time to Reconsider the Diagnostic Role of the p.His63Asp Variant

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Abstract

Hemochromatosis, primarily caused by homozygosity for the *HFE* p.Cys282Tyr variant, is the most frequent genetic iron overload disorder in populations of Northern European ancestry. Since the discovery of the *HFE* gene, genetic testing for HFE-related hemochromatosis has frequently included both the p.Cys282Tyr and the more common p.His63Asp variant. However, growing evidence indicates that the p.His63Asp variant lacks clear pathogenic significance in most clinical contexts related to iron overload, and its routine reporting may lead to diagnostic confusion and inappropriate management. This manuscript calls for a clarification of current genetic testing practices in hemochromatosis, recommending that testing for *HFE*-related hemochromatosis be restricted to the p.Cys282Tyr variant. This position is grounded in current scientific evidence and aims to improve diagnostic accuracy, reduce patient harm, and promote more consistent clinical interpretation.

Introduction

Hemochromatosis is a common inherited metabolic disorder characterized by excessive iron accumulation. Although rare forms of hemochromatosis do exist, HFE-related hemochromatosis is now recognized as the most common single-gene disorder in populations of Northern European descent (1).

Given ongoing uncertainty and debate surrounding the clinical significance of the p.His63Asp variant, a critical clarification of its role in diagnostic and management strategies is warranted. We have conducted a comprehensive review of the current evidence regarding the clinical interpretation of the p.His63Asp variant in hemochromatosis. Based on this analysis, we have formulated a series of focused consensus statements intended to guide its use in diverse clinical settings. These recommendations aim to provide greater clarity for patients, their families, and healthcare providers. In clinical scenarios that fall outside the scope of these recommendations, referral to a specialist with expertise in iron overload disorders is strongly advised to reduce the risk of misdiagnosis or inappropriate attribution of hemochromatosis.

The history of hemochromatosis spans over a century of evolving concepts, diagnostic criteria, and shifting paradigms in etiology. The condition was first described by A. Trousseau in the 1860s, who observed a triad of diabetes, skin hyperpigmentation, and liver disease, though he did not identify iron overload as the underlying cause (2). In 1889, F. D. von Recklinghausen coined the term ‘Hemochromatosis’ and linked the syndrome to iron accumulation, establishing the foundation for viewing it as a systemic iron storage disorder (3). The most comprehensive early description came from J. H. Sheldon in 1935, who published a monograph detailing the clinical and pathological features and emphasized tissue iron deposition, especially in the liver, as the defining diagnostic criterion (4). At this time, diagnosis relied on clinical features (such as bronze diabetes, cirrhosis, and cardiomyopathy) and histopathologic confirmation of iron overload via liver biopsy. It was not until 1975, when M. Simon and colleagues demonstrated a strong association between idiopathic hemochromatosis and the HLA-A3 and HLA-B7 or B14 alleles, providing the first genetic linkage evidence (5,6). Subsequent advances in the 1980s and 1990s shifted diagnostic criteria toward biochemical markers, as elevated serum iron, high transferrin saturation, and increased serum ferritin levels became hallmark indicators of the disease (7,8). A major breakthrough occurred in 1996 when J. Feder and colleagues identified mutations in the ‘HFE’ gene as the genetic basis for most adult cases of hemochromatosis, cementing its identity as a Mendelian disorder and establishing genetic testing as a diagnostic cornerstone (9).

In recent years, the understanding of hemochromatosis has deepened further. It is now recognized as an endocrine liver disease caused by inappropriately low levels of hepcidin, the master regulator of systemic iron homeostasis (10-12). This reclassification highlights the

primary defect in iron sensing and regulation rather than merely iron storage. In clinical practice, invasive liver biopsy, once essential for diagnosis and fibrosis staging, has largely been replaced by non-invasive magnetic resonance imaging (MRI)-based iron quantification and transient elastography, allowing for accurate assessment of hepatic iron burden and fibrosis without procedural risks (13). Over time, diagnostic criteria have evolved from symptom-based observation and autopsy findings to biochemical, genetic, and now imaging-based and molecular-functional definitions (14). This evolution has not been without controversy, as illustrated by the so-called 'H63D syndrome', a concept lacking peer-reviewed clinical evidence, where overinterpretation of this *HFE* variant led to inflated disease attribution and confusion in both clinical and research settings.

The discovery and recognition of *HFE*

The discovery of mutations in the '*HLA-H*' gene, identified in 1996 through positional cloning (9), which was later renamed *HFE* (for 'high Fe') (15, 16) (OMIM *613609), marked a major breakthrough in understanding the genetic basis of hemochromatosis (OMIM # 235200). In their seminal study, Feder *et al.* identified two variants in the *HLA-H* gene, the p.Cys282Tyr variant (C282Y, rs1800562, NM_000410.4:c.845G>A) and the p.His63Asp variant (H63D, rs1799945, NM_000410.4:c.187C>G) (9). Of the 178 well-characterized North American hemochromatosis patients, 148 were homozygous for the p.Cys282Tyr variant. Among the nine heterozygous for p.Cys282Tyr, eight also carried the p.His63Asp variant, making them compound heterozygotes. One additional patient was homozygous for p.His63Asp (9). These two variants were found to occur on separate haplotypes, within a genomic linkage region spanning several kilobases, suggesting independent evolutionary origins. Due to its high allele frequency in control populations and similar prevalence (21%) among patients lacking p.Cys282Tyr, the pathogenic significance of p.His63Asp was uncertain; nevertheless, the observation that 86% of the 178 patients were either homozygous for p.Cys282Tyr or compound heterozygous for p.Cys282Tyr/p.His63Asp was initially interpreted as supporting the involvement of p.His63Asp in the pathogenesis of hemochromatosis. Altogether, this work highlighted a key distinction between disease-associated alleles (individual variants) and disease-associated genotypes (specific combinations) that confer risk. Homozygosity for p.Cys282Tyr mutation emerged as the predominant genotype in hemochromatosis, present in up to 80–90% of clinically diagnosed cases in European populations.

An early letter by Beutler (17), published soon after the discovery of *HFE*, proposed that p.His63Asp could contribute to hemochromatosis in compound heterozygotes, albeit with very low penetrance. While appropriately cautious, this early framing nonetheless supported consideration of p.His63Asp as clinically relevant and may have influenced how its significance was subsequently interpreted in diagnostic contexts.

Subsequent population studies of *HFE* variants confirmed that p.Cys282Tyr is the ancestral hemochromatosis mutation. Its high prevalence among Northern Europeans mirrors the geographic distribution of hemochromatosis and supports the hypothesis that the mutation originated in an early Celtic population, later spreading through Celtic migrations facilitated by Viking maritime expertise (18*)*. In contrast, the p.His63Asp variant shows a broader and less disease-correlated distribution, consistent with it being a more ancient allele. In certain European populations, such as Basques, Catalans, and the Dutch, the p.His63Asp allele frequency exceeds 25% (19), while p.Cys282Tyr occurs at less than half that rate. Notably, in the United Kingdom, p.His63Asp is approximately twice as frequent as p.Cys282Tyr (20).

Statement 1

The original scientific study reporting the strong association between hemochromatosis and the *HFE* genotype containing the p.Cys282Tyr variant in homozygosity, also reported additional genotypes containing the most common genetic variant, p.His63Asp (Feder *et al.* 1996).

The cellular consequences of the *HFE* variants

The p.Cys282Tyr variant was found to disrupt a key disulfide bond and prevent formation of the essential tertiary structure of the HFE protein, abrogating its association with β2-microglobulin, thus explaining its absence at the cell surface in subjects homozygous for this variant (9,21). The lack of cell-surface expression of HFE ultimately results in reduced transcription and secretion of hepcidin from the hepatocyte. Low circulating levels of hepcidin lead to unchecked release of iron from duodenal enterocytes and reticuloendothelial cells through the transmembrane iron transporter ferroportin (22).

Contrary to the consequence of the p.Cys282Tyr variant, the functional consequences of the p.His63Asp variant remain obscure. The variant was predicted to lead to an amino-acid transition with the putative peptide-binding domain of the protein. This does not lead itself to an immediately plausible mechanism resulting in the low circulating hepcidin hemochromatosis phenotype. The p.His63Asp variant does not abrogate *HFE* association with β2-microglobulin, but early studies suggested that it might indirectly alter the affinity of *HFE* to transferrin receptor (23). Work on mouse models of *HFE*-associated hemochromatosis suggested mild iron overload with p.His67Asp (the mouse equivalent to p.His63Asp in humans) homozygosity and compound heterozygosity with p.Cys294Tyr (the mouse equivalent to p.Cys282Tyr in humans) in comparison to the mouse equivalent of p.Cys282Tyr homozygosity but the relevance of these studies to human disease remain unclear (24).

Notably, in humans, p.Cys282Tyr/p.His63Asp compound heterozygotes, who frequently present with additional cofactors such as alcohol use, metabolic syndrome, or steatotic liver disease that

can influence iron metabolism, exhibit mildly reduced hepcidin production, although the overall clinical impact remains modest compared with p.Cys282Tyr homozygotes (25).

Statement 2

The exact molecular and functional consequences of the p.His63Asp variant remain unknown. Current evidence suggests that the effect on systemic iron metabolism of p.His63Asp containing genotypes is minimal, if any.

The limitations of p.His63Asp variant in contributing to a diagnosis of hemochromatosis

Early analyses of *HFE* mutations in European hemochromatosis patients revealed an even higher prevalence of p.Cys282Tyr homozygosity than the 83% reported in Feder's original study. In France (26), the United Kingdom (27), Norway (28), Spain (29) and Australia (30), the percentage of well-characterized individual patients who were p.Cys282Tyr homozygous ranging from 87.1% to 100%. Therefore, in light of the strong association between p.Cys282Ty and hemochromatosis, diagnostic laboratories rapidly adopted *HFE* mutation analysis to support early diagnosis of the condition. Despite the weak association of p.His63Asp containing genotypes with hemochromatosis, *HFE* genotyping, including both the p.Cys282Tyr and the p.His63Asp variants, was implemented in routine genetic testing for suspected hemochromatosis.

In a subsequent report (31), the relative risk for hemochromatosis in subjects with the p.Cys282Tyr/p.His63Asp compound heterozygous genotype was only 0.5%, compared with the more highly penetrant p.C282Y homozygous genotype. Therefore, it was concluded that despite analyses indicating a potential association of certain p.His63Asp containing genotypes and disease, there was a need to more precisely define the absolute risk linked to each *HFE* genotype, considering factors such as age, sex and environmental factors (31). Numerous subsequent case-control studies reported that only a relatively small proportion of hemochromatosis patients carried genotypes involving the p.His63Asp variant. A large meta-analysis including 202 studies with 66,263 cases and 226,515 controls found no statistically significant association between genotypes containing the p.His63Asp variant and liver disease or any other clinical outcomes typically associated with hemochromatosis, including heart disease, arthropathy and diabetes (32).

True iron overload associated with hemochromatosis requires documentation of elevation of the transferrin saturation combined with elevation of the serum ferritin level. Although elevated transferrin saturation is widely considered the most sensitive marker of the hemochromatosis phenotype, it is subjected to significant biological variability, influenced by variables such as circadian timing and fasting state at the time of sample collection. Serum ferritin is a nonspecific marker, as studies indicate that fewer than 10% of elevated ferritin levels are attributed to

hemochromatosis, with most cases instead associated with inflammation, chronic alcohol consumption or hepatic steatosis (33).

According to EASL guidelines (14), p.His63Asp genotyping is not routinely recommended but may be considered in specific clinical contexts, as its clinical relevance remains controversial.

An individual with a low-risk hemochromatosis genotype (including p.Cys282Tyr/p.His63Asp compound heterozygosity and p.His63Asp homozygosity) and a raised ferritin should not be considered to have hemochromatosis without further evidence of increased tissue iron overload – ideally confirmed prospectively by positive MRI, or in the absence of MRI, by measurements confirming elevation of body iron stores. The potential phenotypic expression in compound heterozygotes may be influenced by additional factors, including alcohol use, metabolic syndrome, and steatotic liver disease, which can modify the severity and onset of iron-related abnormalities (34,35).

Statement 3

The p.His63Asp variant when present in compound heterozygosity with p.Cys282Tyr can be considered to confer a risk for mild iron overload, commonly in association with other risk factors (including alcohol use, metabolic syndrome and steatotic liver disease). It is not considered to be a disease-causing genotype on its own.

Testing for p.His63Asp has limited utility in the evaluation of patients with significant iron overload

Since the identification of the *HFE* gene it has been widely recognized that homozygosity for p.Cys282Tyr mutation is the most common risk factor for iron overload in humans. Rarer forms of genetic iron overload are recognized, and their phenotype may vary significantly from *HFE*-associated hemochromatosis. Such patients require specialist assessment and management. This should include quantification of liver iron concentration by MRI, assessment of cardiac and endocrine functions (13, 36). Genetic analysis in such instances should include not only testing for p.His63Asp but should include sequencing the entire *HFE* gene and other genes involved in iron metabolism (*HJV*, *TFR2*, *HAMP*, *SLC40A1)* (37). Clearly, this is beyond the capabilities of most first line routine clinical testing services, highlighting the necessity for specialist consultation in a more in-depth second-line investigation.

Statement 4

Regardless of their p.His63Asp status, patients with ‘unexplained’ significant tissue iron overload — demonstrated either by a direct measure of liver iron concentration (prospective determination) or by quantitative phlebotomy (retrospective determination) — and without *HFE*

p.Cys282Tyr homozygosity, should be tested for other pathogenic variants in *HFE* and non-*HFE* genes.

Assessment of p.His63Asp has no role in screening and case-finding

Cascade screening has been recommended in international guidelines and a practiced feature of hemochromatosis care since its hereditary nature was established. The identification of the *HFE* gene, particularly the p.Cys282Tyr variant, greatly expanded the potential for early genetic testing in siblings and offspring of individuals with p.Cys282Tyr homozygosity. Genetic-based testing in families where the affected proband has a genotype different from p.Cys282Tyr homozygosity (including p.Cys282Tyr/p.His63Asp compound heterozygosity and p.His63Asp homozygosity) can be considered “uninformative” (i.e. the predictive value of testing is too low to be of value). Therefore, assessment of p.His63Asp for family cascade or population screening is not commonly practiced and not recommended (38).

The largest population study of *HFE* variants analyzed p.Cys282Tyr and p.His63Asp in over 450,000 UK residents followed up for 7 years (20). Whereas p.Cys282Tyr homozygosity was strongly associated with hemochromatosis, liver disease, liver cancer, joint and neurological conditions, the risk in p.Cys282Tyr/p.His63Asp compound heterozygotes was modest, and no increased morbidity was observed in p.His63Asp homozygotes (20, 39). After 13 years, the cumulative hemochromatosis diagnosis in nearly 5000 male compound heterozygotes was only 5.4% by age 80 years, compared to 56.4% in p.Cys282Tyr homozygotes (40). Similar studies in Australia (41, 42) and Denmark (43, 44) confirmed no increased risk of liver disease, diabetes, or iron overload in individuals with p.His63Asp-containing genotypes.

In countries where hemochromatosis and the p.Cys282Tyr variant have a high prevalence, there has been a longstanding debate regarding the potential value of population screening (45). To date, no country has yet adopted population screening and although there is an increasing recognition that testing for p.Cys282Tyr might be one model to consider, there is no evidence that testing for p.His63Asp would contribute to such a program. There is no evidence to suggest that prospective testing for p.His63Asp in individuals without evidence of iron overload is beneficial.

Statement 5

The inclusion of the p.His63Asp variant for assessing hemochromatosis risk in asymptomatic individuals (including cascade or family testing and population screenings) offers no clinical benefit and is not recommended.

Research testing and direct to consumers genetic tests

Given the prevalence of hemochromatosis and of genotypes bearing the p.Cys282Tyr variant in certain populations, there is an increasing demand for genetic variant analysis from clinicians in both secondary and primary care settings. Genetic risk must be communicated to affected individuals. Any such communication needs to be clear, evidence-based, and clinically balanced. Moreover, a growing number of individuals are subjected to genetic testing outside the framework and oversight of their established healthcare providers. Many people currently receive highly technical and personalized genetic data directly from research bodies and commercial organizations. In many countries this practice is being promoted by industry leaders, offering to cover more and more genes as well as partnering with patients' associations to expand access. Results inferring a disease risk can lead to anxiety and distress for patients, often resulting in additional consultations and medical tests.

The American College of Medical Genetics and Genomics (ACMG) define only p.Cys282Tyr homozygosity as an "actionable" incidental genetic finding, i.e. a finding of sufficient significance to warrant communication to the patient/participant/customer along with a clinical assessment and management (46). Given the high prevalence of p.His63Asp across the world, the unlimited testing of this variant creates a huge potential for misdiagnosis and even unnecessary treatment. This should be consistently resisted (47) and any laboratory performing and reporting on genetic information (including research and commercial partners) must acknowledge their clinical responsibility.

Conclusion and Recommendations

The p.His63Asp variant in *HFE* remains a genetic variant of interest. However, there is insufficient evidence to justify the continued routine reporting of this variant in all referrals. This variant has never been informative in the context of family analysis and therefore it should not be reported in cascade screening. Testing for p.His63Asp may be considered as part of second-line genetic analysis in the assessment of a patient with unequivocal evidence of iron overload (38), alongside analysis of other *HFE* regions and non-*HFE* hemochromatosis genes (*HJV, TFR2, HAMP, SLC40A1*), preferably in specialized centers with expertise in interpreting pathogenic variants in hemochromatosis.

Without more selective and clinically informed reporting, colleagues will continue to be overwhelmed with patients presenting modest elevations of serum ferritin and what are essentially low- or no-risk genotypes (i.e. as p.Cys282Tyr/p.His63Asp compound heterozygotes or p.His63Asp homozygotes). We have no evidence that these genotypes alone confer serious risk or that the individual would ever benefit from iron reduction/venesection treatment (20, 40).

We have a responsibility to make an early yet accurate diagnosis of hemochromatosis before recommending treatment. A false diagnosis such as could occur in any individual with isolated hyperferritinemia and a low risk *HFE* genotype (but without compelling evidence of iron

overload) is unacceptable. It may lead to unnecessary venesection, as well as potential stigma and psychological harm. Given the wide range of non-specific symptoms associated with hemochromatosis and iron overload, patients may mistakenly attribute new symptoms to their (incorrect) diagnosis. This misattribution can lead to delays in reporting symptoms, undergoing proper investigation and receiving timely diagnosis of other potentially serious health conditions. In the context of emerging targeted molecular therapies for hemochromatosis, and to rigorously assess the long-term efficacy of such therapies in broader populations, it is essential to focus on the patient group at greatest risk of serious disease. An early “proof-of-concept” study evaluating the hepcidin mimetic rusfertide (PTG-300, Protagonist Inc.) showed promising tolerance and potential benefit (48). However, of the 16 participants, only five were confirmed p.Cys282Tyr homozygotes, while others carried other genotypes containing the p.His63Asp variant. Future trials should therefore concentrate on individuals homozygous for p.Cys282Tyr, who represent the group most likely to benefit from targeted intervention.

Overall, in patients with confirmed tissue iron overload, testing for the HFE p.His63Asp variant may have limited clinical utility, especially when broader genetic and environmental factors are considered. Routine reporting of this variant, particularly in individuals without clear evidence of iron overload, offers no diagnostic benefit and should be discouraged to prevent misinterpretation and unnecessary clinical actions.

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Author contributions

M.S. initiated the discussion on this topic. All authors contributed to manuscript writing and revision.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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