



HAL
open science

Hemochromatosis classification: update and recommendations by the BIOIRON Society

Domenico Girelli, Fabiana Busti, Pierre Brissot, Ioav Z Cabantchik, Martina U Muckenthaler, Graca Porto

► **To cite this version:**

Domenico Girelli, Fabiana Busti, Pierre Brissot, Ioav Z Cabantchik, Martina U Muckenthaler, et al.. Hemochromatosis classification: update and recommendations by the BIOIRON Society. *Blood*, 2022, 139 (20), pp.3018-3029. 10.1182/blood.2021011338 . hal-03418911

HAL Id: hal-03418911

<https://hal.science/hal-03418911v1>

Submitted on 14 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License



American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

Hemochromatosis classification: update and recommendations by the BIOIRON Society

Tracking no: BLD-2021-011338R2

Domenico Girelli (University of Verona, Italy) Fabiana Busti (University of Verona, Italy) Pierre Brissot (INSERM, Univ-Rennes 1, INRA, Institut Numecan, France) Ioav Cabantchik (Hebrew University, Jerusalem, Israel) Martina Muckenthaler (University of Heidelberg, Germany) Graca Porto (i3S/IBMC, Portugal)

Abstract:

Hemochromatosis (HC) is a genetically heterogeneous disorder in which uncontrolled intestinal iron absorption may lead to progressive iron overload responsible for disabling and life-threatening complications such as arthritis, diabetes, heart failure, hepatic cirrhosis, and hepatocellular carcinoma.

The recent advances in the knowledge of pathophysiology and molecular basis of iron metabolism have highlighted that HC is caused by mutations in at least five genes, resulting in insufficient hepcidin production or, rarely, resistance to hepcidin action. This has led to an HC classification based on different molecular subtypes, mainly reflecting successive gene discovery. This scheme was difficult to adopt in clinical practice and therefore needs revision. Here we present recommendations for unambiguous HC classification developed by a working group of the International Society for the Study of Iron in Biology and Medicine (BIOIRON Society) including both clinicians and basic scientists during a meeting in Heidelberg, Germany. We propose to deemphasize the use of the molecular subtype criteria in favor of a classification addressing both clinical issues and molecular complexity. Ferroportin Disease (former type 4a) has been excluded because of its distinct phenotype. The novel classification aims to be of practical help whenever a detailed molecular characterization of HC is not readily available.

Conflict of interest: COI declared - see note

COI notes: TG and EN are co-founder of Intrinsic LifeScience, and consultant for Protagonist, Vifor Pharma, Ionis Pharmaceuticals, and Disc Medicine. JR is consultant for Bond Biosciences and Gilead. The other authors declare no competing financial interests.

Preprint server: No;

Author contributions and disclosures: DG, PBr, GP, MM and IC led the panel and conceived the manuscript. DG and FB co-wrote the manuscript. PA, EB-J, PBi, BB, CC, RE, RF, TG, OL, GM, EN, APie, APip, DP, JR, MS, PS, DS, HZ critically revised and edited the manuscript

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement:

Clinical trial registration information (if any):

1 **Special Report**

2

3 **Article Title:** Hemochromatosis classification: update and recommendations by the
4 BIOIRON Society

5 **Short Title:** New Hemochromatosis classification by BIOIRON Society

6

7 **Authors:** Domenico Girelli^{1*}, Fabiana Busti^{1*}, Pierre Brissot^{2*}, Ioav Cabantchik³, Martina U.
8 Muckenthaler⁴, Graça Porto⁵, on behalf of the Nomenclature Committee of the International
9 Society for the Study of Iron in Biology and Medicine (BIOIRON Society)**

10

11 ¹Department of Medicine, Section of Internal Medicine, EuroBloodNet Center, University of Verona and
12 Azienda Ospedaliera Universitaria Integrata Verona, Italy

13 ²INSERM, Univ-Rennes, INRA, UMR 1241, Institut NuMeCan, Rennes, France

14 ³Della Pergola Chair, Alexander Silberman Institute of Life Sciences, Hebrew University, Jerusalem, Israel

15 ⁴Department of Pediatric Oncology, Hematology, and Immunology and Molecular Medicine Partnership Unit,
16 University of Heidelberg, Heidelberg, Germany; Molecular Medicine Partnership Unit, European Molecular
17 Biology Laboratory, Heidelberg, Germany; Translational Lung Research Center, German Center for Lung
18 Research, Heidelberg, Germany; German Centre for Cardiovascular Research, Partner Site Heidelberg,
19 Mannheim, Germany

20 ¹⁷Institute for Molecular and Cell Biology (IBMC), i3S (Instituto de Investigação e Inovação em Saúde),
21 Universidade do Porto, Porto, Portugal; Clinical Hematology, Santo António Hospital - Porto University

22

23

24 *equal contributors

25 **Full list of Authors and affiliations is reported at the end of the manuscript

26

27

28

29 **Corresponding author:** Domenico Girelli, Department of Medicine, Section of Internal

30 Medicine, Policlinico Giambattista Rossi, 37134 Verona, Italy. E-mail:

31 domenico.girelli@univr.it. Tel. +39 045 812 4262. Fax +39 045 802 7496.

32

33

34

35

36

37 **Text word count:** 4,031

38 **Abstract word count:** 200

39 **Number of figures:** 3

40 **Number of tables:** 4

41 **Number of references: 110**

42

43

44

45

46

47

48 **Abstract**

49 Hemochromatosis (HC) is a genetically heterogeneous disorder in which uncontrolled
50 intestinal iron absorption may lead to progressive iron overload responsible for disabling
51 and life-threatening complications such as arthritis, diabetes, heart failure, hepatic
52 cirrhosis, and hepatocellular carcinoma.

53 The recent advances in the knowledge of pathophysiology and molecular basis of iron
54 metabolism have highlighted that HC is caused by mutations in at least five genes,
55 resulting in insufficient hepcidin production or, rarely, resistance to hepcidin action. This
56 has led to an HC classification based on different molecular subtypes, mainly reflecting
57 successive gene discovery. This scheme was difficult to adopt in clinical practice and
58 therefore needs revision. Here we present recommendations for unambiguous HC
59 classification developed by a working group of the International Society for the Study of
60 Iron in Biology and Medicine (BIOIRON Society) including both clinicians and basic
61 scientists during a meeting in Heidelberg, Germany. We propose to deemphasize the use
62 of the molecular subtype criteria in favor of a classification addressing both clinical issues
63 and molecular complexity. Ferroportin Disease (former type 4a) has been excluded
64 because of its distinct phenotype. The novel classification aims to be of practical help
65 whenever a detailed molecular characterization of HC is not readily available.

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82 **Historical Perspective**

83 It is commonly accepted that the term "hemochromatosis" was coined by the German
84 pathologist von Recklinghausen in 1889. Of note, this was during the *Versammlung*
85 *Deutscher Naturforscher* (meeting of German scientists) held in Heidelberg, like the
86 BIOIRON Society (formerly IBIS) meeting in 2019, which led to the current report.
87 Following the description of patients with "bronze diabetes and cirrhosis" by French
88 physicians led by Armand Trousseau in the mid-1800s, von Recklinghausen hypothesized
89 that something circulating in the blood ("Hemo-") was responsible for skin and organ
90 damage and pigmentation ("-chromatosis") (HC). Recognizing excess iron as the etiology
91 of organ toxicity took several decades, and was attributable to Joseph Sheldon in 1935,
92 who was also the first to suggest the genetic origin of the metabolic defect (for a
93 comprehensive historical review, see¹). Overall, these pioneers' works clearly defined a
94 clinical-pathological entity caused by progressive iron accumulation and characterized by
95 multi-organ damage (mainly in liver, pancreas, joints, heart, and endocrine glands), without
96 signs of anemia (to the contrary, some patients show mildly increased Hb levels)²⁻⁵. In the
97 1950s, ferrokinetic studies revealed abnormally increased intestinal iron absorption as the
98 key pathophysiological feature of HC,⁶ and repeated/frequent phlebotomies were
99 established as the mainstay of treatment⁷. In 1977, the seminal work by Marcel Simon and
100 colleagues reported the tight linkage between the major histocompatibility complex (MHC)
101 and the putative hemochromatosis gene on chromosome 6p, definitively demonstrating
102 the genetic origin of the disease⁸. This paved the way to the discovery, in 1996, of the
103 "hemochromatosis gene" *HFE* (alias "high Fe", official full name "homeostatic iron
104 regulator")⁹, which provided additional information about HC. Initially, it appeared that up to
105 95% of HC cases could be attributed to homozygosity for a single nucleotide change (845
106 G→A) causing the substitution of cysteine by tyrosine at amino acid 282 (*p.Cys282Tyr* or
107 C282Y variant)¹⁰⁻¹³. A second *HFE* polymorphism, *p.His63Asp*, was detected, whose
108 minor role became clearer later¹⁴. The meaning of the *p.Cys282Tyr/p.His63Asp* compound
109 heterozygosity is discussed in detail below. The high frequency of *p.Cys282Tyr*
110 homozygosity in the original studies resulted from the inclusion of patients mostly of
111 Northern European ancestry, a region where the variant had originated around 4,000
112 BC.^{15,16} Indeed, the *p.Cys282Tyr* variant, frequent in certain geographical regions,¹⁷ is rare
113 or even absent in large areas of the world, including Asian and African countries, as well
114 as in native Americans.^{18,19} Subsequent studies in Southern Europe, in the Mediterranean

115 area, and in Brazil found that at least one-third of subjects with a defined HC phenotype
116 were negative for *p.Cys282Tyr* at *HFE* genetic testing.^{20,21}

117 Over time, it became evident that the genetic basis of HC was more heterogeneous
118 than initially assumed, and several variants in other iron-controlling genes (collectively
119 referred to as “non-*HFE* genes”) were progressively associated with the disorder. These
120 include variants on genes coding for a second receptor for transferrin (*TFR2*),²²⁻²⁴
121 ferroportin (*SLC40A1*),²⁵ hepcidin (*HAMP*),^{26,27} and hemojuvelin (*HJV*).^{28,29} In particular,
122 the discovery of variants in the *HAMP* and *HJV* genes made it possible to define a severe
123 early-onset (*juvenile*) form of HC, with early cardiac and endocrine impairments, as a
124 molecularly distinct entity.

125 Hepcidin is the master regulator of iron homeostasis³⁰⁻³³ and its identification has
126 represented a considerable advance in the comprehension of the pathophysiological
127 mechanisms underlying HC. For detailed reviews on hepcidin discovery, functions and
128 regulation, readers are referred elsewhere³⁴⁻³⁷. Briefly, hepcidin is a small peptide
129 hormone produced by the liver, that negatively controls circulating iron levels. Through
130 interaction with ferroportin³⁸⁻⁴⁰ (its receptor and the only cellular iron exporter so far
131 identified in humans), hepcidin inhibits the absorption of dietary iron in the duodenum and
132 its release by spleen macrophages involved in recycling iron from senescent erythrocytes.
133 Molecular defects causing hepcidin deficiency result in uncontrolled intestinal iron
134 absorption, with progressive iron accumulation in tissues, ultimately leading to HC.¹ In
135 most cases gene defects cause insufficient production of hepcidin, while rarely ferroportin
136 resistance to hepcidin is observed (see below). As illustrated in **Figure 1**, hepcidin
137 regulation by iron is quite complex and involves numerous proteins^{41,42} whose alterations
138 can compromise hormone synthesis or function. For this reason, identifying the molecular
139 causes of HC is far from simple and requires a deep knowledge of its pathogenetic basis,
140 which is still not completely clarified. In our view, the term “hemochromatosis” should be
141 reserved for this unique clinical entity caused by genetic lesions that primarily affect the
142 hepcidin-ferroportin system and not used to describe clinically distinct iron overload
143 conditions with other causes (see discussion below).

144

145 **The current clinical scenario**

146 Unlike in the past, fully expressed and potentially lethal HC (with liver cirrhosis,
147 diabetes, endocrine dysfunction, and heart failure) is seen rarely in current clinical
148 practice.⁴³ This can be ascribed to increased awareness of the disease, and, mostly, to the

149 routine assessment of iron biomarkers, particularly serum ferritin. Unfortunately, this is
150 counterbalanced by an increased diagnostic challenge for non-experts in the iron field,
151 primarily due to the lack of specificity of ferritin.

152 Ferritin is an essentially intracellular protein that serves to store iron safely. It is also
153 present at very low concentration ($\mu\text{g/L}$) in serum, likely through secretion by
154 macrophages⁴⁴. Normal values usually range from 30 to 200 or 300 $\mu\text{g/L}$ in females and
155 males, respectively. The function of secreted extracellular ferritin remains largely
156 unknown.^{45,46} Several common conditions lead to increased serum ferritin levels, including
157 virtually all inflammatory disorders, hepatic cytolysis (e.g., during acute or chronic liver
158 disease), or the metabolic syndrome.^{47,48} This translates into a huge number of
159 consultations, overuse of the “first-level” genetic test looking for the presence of the
160 common variants in the *HFE* gene, and even misdiagnosis due to incorrect interpretation
161 of the results.⁴⁹

162 The glycoprotein transferrin is the extracellular carrier of iron that is detectable at high
163 concentration in blood (g/L; the third most abundant protein after albumin). Transferrin
164 saturation (TSAT) is calculated as the ratio between serum iron and transferrin (multiplied
165 by the correction factor 1.42) or, less reliably, between serum iron and total iron binding
166 capacity, and expressed as a percentage. TSAT is much less requested in clinical practice
167 but is much more informative about a possible diagnosis of HC. Normal TSAT varies
168 between 20 and 45%. It has been estimated that hyperferritinemia with normal TSAT is
169 associated with increased iron stores in less than 10% of cases.⁵⁰ Importantly, TSAT
170 elevation is the hallmark of HC. In HC patients, high TSAT reflects the increased pool of
171 circulating iron due to insufficient hepcidin production and typically precedes the rise of
172 serum ferritin by several years.⁵¹ TSAT tends to remain elevated even in subjects
173 effectively iron-depleted by phlebotomies. Occasional reports of normal TSAT in
174 *p.Cys282Tyr* homozygotes with hyperferritinemia should always prompt the search for
175 additional cofactors that raise ferritin, such as metabolic syndrome or alcohol intake.^{52,53}

176 Liver biopsy was once regarded as the gold standard for the diagnosis of HC, because
177 it can reveal iron deposition in hepatocytes with the typical decreasing gradient from the
178 periportal zone (most exposed to iron coming from the gut) to the central-lobular zone in
179 the hepatic acinus. However, in current practice the demonstration of *p.Cys282Tyr*
180 homozygosity along with elevated serum ferritin and TSAT is considered sufficient to make
181 the diagnosis of HC. Liver biopsy remains useful for prognostic purposes in HC patients
182 with serum ferritin levels repeatedly $>1,000 \mu\text{g/L}$, allowing the early identification of

183 advanced fibrosis or even subclinical cirrhosis. These conditions require close surveillance
184 for hepatocellular carcinoma even after iron depletion.⁵¹ Nowadays, liver biopsy is seldom
185 performed due to its invasiveness, costs, and the increasing availability of non-invasive
186 tools. Indeed, magnetic resonance imaging (MRI) techniques have largely replaced it for
187 the determination of liver iron concentration (LIC). This is obtained indirectly by using
188 various MRI protocols, for which there is still no consensus on the best one. The choice of
189 the protocol mainly depends on local expertise, as well as on the available equipment and
190 software (for detailed reviews, see ⁵⁴⁻⁵⁶). Moreover, hepatic transient elastography
191 (Fibroscan) is a reliable non-invasive method for detecting liver fibrosis in HC patients,
192 limiting the need for liver biopsy to those with indeterminate results.⁵⁷

193 Currently, HC is typically suspected in subjects with no or minimal symptoms, increased
194 serum ferritin levels without alternative explanation, high TSAT, and evidence of increased
195 liver iron stores by MRI. Making the diagnosis at this preclinical early stage⁵¹ has the
196 undoubted advantage of preventing organ damage by a relatively simple and cost-
197 effective treatment (phlebotomy), as well as of allowing normal life expectancy.^{58,59}

198 The HFE genetic test, available in most laboratories, identifies the commonest inherited
199 defect in Caucasians predisposing to *HFE*-related HC, i.e. *p.Cys282Tyr* homozygosity.
200 Therefore, in Caucasians, an HFE genetic test is indicated when high TSAT is confirmed
201 irrespective of parenchymal IO demonstration. *p.Cys282Tyr* homozygosity has a variable
202 and difficult to predict clinical penetrance,⁶⁰⁻⁶⁴ with several inherited and acquired modifiers
203 potentially contributing to the final phenotype. For this reason, *HFE*-related HC should not
204 be viewed as a simple monogenic disorder, but rather as the complex result of the
205 interplay of environmental, lifestyle, and still unidentified genetic cofactors.¹

206 Regarding the compound *p.Cys282Tyr* and *p.His63Asp* heterozygosity, compelling
207 evidence exists that this genotype *per se* is characterized by minimal or no clinical
208 penetrance ^{65,66}. Thus, it cannot be considered diagnostic for HC (for a detailed discussion
209 see¹⁴), but at most as a susceptibility factor that can be associated with mild-to-moderate
210 IO only in case of digenic inheritance⁶⁷ (see below) or when other predominant causes of
211 liver disease are present, namely non-alcoholic fatty liver disease (NAFLD), alcohol, or
212 HCV. Of note, the latter two are known to cause acquired hepcidin suppression^{41,68,69}.

213 According to existing guidelines, whenever a subject with *p.Cys282Tyr/p.His63Asp*
214 compound heterozygosity has evidence of IO, a secondary cause of liver disease should
215 be sought and treated^{70,71}, with phlebotomies possibly considered as an adjunctive
216 treatment. On the other hand, the negative effects of an automatic HC (mis)diagnosis in

217 *p.Cys282Tyr/p.His63Asp* compound heterozygotes are commonly seen at referral centers.
218 They include patients' and family members' unnecessary anxiety, incomplete prior
219 investigations (e.g., serum ferritin but neither TSAT nor MRI), overlooking of other causes,
220 and/or unnecessary treatment by phlebotomies.

221 Clinical elements that should raise a definite suspicion of HC are reported in **Table 1**. In
222 Caucasians with a negative first-level *HFE* test (i.e., *p.Cys282Tyr* homozygosity is not
223 detected) and in non-Caucasians, a second-level genetic test should be considered in
224 order to identify rarer variants in the *HFE* or in other genes known to be linked to hepcidin
225 control. In general, these types of HC are less influenced by cofactors, and characterized
226 by a more severe and homogeneous clinical picture appearing at a younger age.⁷² Their
227 molecular diagnosis is often complex, since variants in HC genes other than *HFE* are
228 typically private, i.e. restricted to members of only one or a few families. To this end,
229 modern approaches based on Next-Generation-Sequencing (NGS) have greatly expanded
230 the diagnostic possibilities in rarer HC, while at the same time opening enormous
231 challenges of interpretation of the results. NGS is generally available only at referral
232 centers and requires specific expertise to avoid misdiagnosis, with long wait time for
233 results (see below). Nonetheless, treatment of patients with a defined HC phenotype
234 should not be delayed pending the result of the genetic test. Recently, NGS methods have
235 also made it possible to estimate the global prevalence of *HFE* and non-*HFE* HC in
236 different populations,¹⁹ as summarized in **Table 2**.

237 **Figure 2** illustrates a possible algorithm for diagnosis of HC, starting from clinical,
238 biochemical and imaging studies to molecular confirmation.

239

240 **The nomenclature of genetic disorders**

241 HC nomenclature suffers from a common problem of classifying genetic diseases. In
242 contrast to genes, diseases lack a standardized way to review official names and symbols
243 by formal committees (<https://ghr.nlm.nih.gov/primer/mutationsanddisorders/naming>).
244 Disease nomenclature is often derived from the name(s) of the physician(s) who first
245 described the condition, one major sign or symptom, or the biochemical/genetic underlying
246 defect. However, the growing comprehension of the pathophysiological or molecular
247 mechanisms that regulate diseases, as well as the identification of new phenotypes, may
248 require revision of the initial name by experts, in order to improve its usefulness in clinical
249 practice.

250 Proper nomenclature, in fact, is an essential prerequisite for clear and effective
251 communication about a particular condition. Ideally, it should unequivocally evoke
252 disorders sharing the same pathogenesis and treatment, eventually helping clinicians to
253 provide an accurate diagnosis and management.

254

255 **What hemochromatosis is (and what it is not)**

256 Nomenclature and case definition of HC have long been recognized as a potential source
257 of confusion⁷³, especially when dealing with the report of genetic tests¹⁴. Among experts,
258 there was a common feeling that the HC classification needs to be revised in view of the
259 increasing awareness and knowledge of IO disorders. To this end, the BIOIRON Society
260 promoted a two-step process. The starting point was the preparation of a survey that was
261 sent to working group participants, including both expert clinicians and basic scientists
262 actively involved in the iron metabolism field, and consisting of nearly all who discovered
263 the hemochromatosis genes and hepcidin. The survey questions and the summary of
264 responses are available in the Supplementary Materials (S1). This was followed by a
265 critical collegial discussion during a specific session of the most recent Biennial Meeting of
266 the BIOIRON Society in Heidelberg. The recommendations reported here are the result of
267 such discussion, where the panelists eventually agreed on the novel classification. As an
268 integral part of the process, the panelists agreed on a robust definition of HC, based on
269 clinical presentation and widely available tools, as a prerequisite for genetic testing. The
270 main clinical, biochemical and imaging studies for the suspicion of HC are reported in
271 **Table 1**. Rigorously speaking, the term “hemochromatosis” should be reserved for a
272 unique genetic clinical-pathological condition characterized by increased TSAT, IO in the
273 liver (but not in the spleen), prevalent involvement of peri-portal hepatocytes with iron-
274 spared Kupffer cells, and signs and/or symptoms associated with IO. The panelists also
275 emphasized that the term “hemochromatosis” itself implies an IO of genetic origin, which is
276 why they would recommend avoiding the unnecessary use of qualifiers such as
277 “hereditary”, “genetic” or “primary”. Indeed, genetic defects in the hepcidin/ferroportin
278 regulatory axis (caused by variants in hepcidin regulators, the hepcidin gene itself, or in
279 ferroportin) are responsible for inadequate production or activity of hepcidin, or lack of
280 hepcidin responsiveness of ferroportin. Finally, the panelists agreed that the definition of
281 HC should also include the absence of hematological signs of a primary/predominant red
282 blood cell disorder, such as anemia or reticulocytosis (see **Table 1** for some exceptions to
283 this rule). This is needed to distinguish HC from other iron overload conditions, often

284 referred to as “iron-loading anemias”,^{74,75} which are similarly characterized by increased
285 TSAT and are nearly always genetically determined. In these conditions, the hepcidin
286 suppression is caused by factors released by erythropoietin-stimulated erythroblasts (e.g.
287 erythroferrone or ERFE),⁷⁶ as a consequence of ineffective erythropoiesis or compensated
288 chronic hemolysis, and not by variants in genes affecting the hepcidin-ferroportin axis. The
289 prototype of this group is non-transfusion dependent thalassemia (NTDT).⁷⁷ IO also occurs
290 in transfusion dependent inherited anemias, but in this case, it is mainly due to
291 transfusions *per se*, and hepcidin levels tend to be increased,⁷⁸ especially immediately
292 after the transfusion because of suppression of erythropoiesis.⁷⁹ **Figure 3** illustrates the
293 main mechanisms underlying the development of IO in hemochromatosis and iron loading
294 anemias. As mentioned above, the majority of iron loading anemias are inherited (for
295 recent comprehensive reviews, see^{36,80-82}), including forms caused by variants in the
296 hemoglobin genes, in genes coding for red blood cell enzymes or membrane structures,
297 as well as congenital sideroblastic⁸¹ or dyserythropoietic⁸² anemias. Sometimes, variants
298 in genes directly regulating iron transport and utilization (such as *DMT1*, transferrin,
299 ceruloplasmin, and others) may be implicated as well.⁸⁰ Finally, IO due to hepcidin
300 inhibition can also occur independently of transfusions in some forms of myelodysplastic
301 syndromes⁸³, especially those characterized by ringed sideroblasts and increased
302 ineffective erythropoiesis associated with acquired somatic mutations in *SF3B1*⁸⁴. In any
303 case, all these conditions should never be regarded as hemochromatosis because of the
304 distinct pathogenesis and treatment (e.g., phlebotomies are often not feasible).

305

306 **The former classifications: strengths and shortcomings**

307 The HC classifications reported by authoritative textbooks and recent reviews and
308 guidelines are based on a schema (**Table 3**), in which numbers and letters reflect the
309 chronology of first descriptions of genotype-phenotype correlations.^{51,71,85,86} Four types are
310 included, with type 2 and type 4 further subdivided into subtypes A and B. They have the
311 advantage of being very informative from a molecular point of view, and officially endorsed
312 by OMIM (the Online Mendelian Inheritance of Man database), but also present several
313 caveats and inconsistencies. The main limitations of the current classifications are listed
314 below.

315 **a. *Poor applicability in clinical practice (Limitations due to costs and lack of***
316 ***widespread expertise).***

317 Apart from the genetic test looking for the common *HFE* variants, the identification of
318 the molecular defect causing rarer forms of HC is currently offered by few laboratories,
319 heterogeneously distributed and scattered worldwide. This requires that patients should
320 travel, or DNA should be sent to referral centers, with inevitable discomforts, delays and
321 costs. Moreover, although the second-level genetic test (mainly based on NGS approach)
322 has recently improved, with gradually decreasing costs, it demands advanced experience
323 for a rigorous interpretation, which can take several weeks.^{14,87-89} Moreover, some cases
324 of HC still remain molecularly undiagnosed even after NGS, suggesting the possibility of
325 unknown gene(s) yet to be discovered.⁸⁹⁻⁹¹ For this reason, the cooperation between
326 geneticists, bioinformaticians, and clinicians is necessary to resolve the most difficult
327 cases. EuroBloodNet, the network connecting experts on rare hematological diseases, is
328 making great efforts in this direction (for details, see www.eurobloodnet.eu).

329 **b. Numerical subtypes do not capture the complex molecular pathogenesis of**
330 **HC.**

331 Recent applications of NGS have highlighted that some patients with a provisional
332 diagnosis of *non-HFE* HC cannot be ascribed to any of the numerical subtypes listed in the
333 previous classification (**Table 3**). Essentially there are two reasons:

334 1) Some show a “digenic” inheritance, deriving from the combination of pathogenic
335 variants in two different genes involved in iron metabolism (e.g., single *p.Cys282Tyr* +
336 heterozygous variants in *HJV*, *HAMP* or *TFR2*).^{90,92-95} Although there are still only few
337 cases reported, digenic inheritance must also be considered in cases whose *HFE*
338 genotype *per se* does not fully explain the clinical picture, for example in patients with
339 *p.Cys282Tyr* homozygosity and very early/severe IO.

340 2) Others do not display variants in any of the five classical hemochromatosis genes
341 (i.e., *HFE*, *HAMP*, *HJV*, *TFR2*, and *SLC40A1*). Recently, some small case-series⁹⁶⁻⁹⁸ have
342 reported moderate late-onset IO in patients carrying variants in the *BMP6* gene, encoding
343 one of the major activators of hepcidin expression in response to iron⁹⁹. The role of such
344 variants is still controversial, as they have been detected mostly in patients with a
345 substantial burden of acquired cofactors.¹⁰⁰ Nonetheless, they broaden the spectrum of
346 genetic defects potentially responsible for HC.

347 **c. Former Type 4A HC**

348 Former Type 4A HC actually represents an IO syndrome characterized by distinctive
349 clinical, biochemical, and pathological features, which do not fit the definition of HC.^{101,102}
350 They include normal to low TSAT, iron retention in spleen and hepatic macrophages, and,

351 sometimes, poor tolerance to standard phlebotomies. The underlying molecular defect is
352 the presence of loss-of-function (LOF) variants in the *SLC40A1* gene, that reduce
353 expression or iron export capability of ferroportin at the cell surface. Therefore, iron is
354 trapped inside iron-recycling macrophages (primarily in the spleen), resulting in a reduction
355 of circulating iron, and a tendency to iron-restricted erythropoiesis. The corresponding
356 clinical features are normal-to-low TSAT and, sometimes, the development of mild anemia
357 after phlebotomies. Both these elements are clearly at variance with the case definition of
358 HC according to **Table 1**. Another peculiarity is represented by autosomal dominant
359 inheritance. Taking into consideration all these aspects, many authors have suggested
360 adopting a specific terminology for this condition, such as Ferroportin disease (FD).^{14,102} In
361 spite of very high ferritin levels which may be evident even in young-adult subjects, FD
362 phenotype is generally milder than in *HFE*-related HC, possibly because of the lower
363 toxicity of iron accumulation in macrophages as compared to hepatocytes.^{103, 102,103}

364 On the other hand, very rare gain-of-function (GOF) variants in the *SLC40A1* gene lead
365 to ferroportin resistance to hepcidin, and cause IO conditions phenotypically and
366 biochemically indistinguishable from hepcidin-deficient HC (former Type 4B HC).¹⁰⁴
367 Variants that interfere with hepcidin binding to ferroportin and also impair ferroportin
368 stability or ability to export iron are also possible, potentially leading to a mixed or
369 intermediate phenotype variably influenced by age or environmental factors.

370 **d. Former Type 2 molecular subtypes are not always Juvenile forms and vice**
371 **versa**

372 As mentioned before, the term *juvenile* hemochromatosis classically designates an
373 early-onset (within the second or third decades of life), fully-expressed HC phenotype
374 showing similar penetrance in both genders and a tendency to present with cardiac and
375 endocrine dysfunctions.^{27,105} This phenotype is generally due to variants in *HJV* and
376 *HAMP* genes causing much more severe iron hyperabsorption than the *HFE* mutations.
377 However, recent studies have highlighted some age-overlap at diagnosis between the
378 various molecular subtypes of HC.⁷² Therefore, the term *juvenile* HC can be ambiguous if
379 invariably attributed to variants in the *HAMP* and *HJV* genes, because in some of these
380 patients the disease is diagnosed in adulthood. Similarly, the term can be misleading in
381 HC patients with defects in genes other than *HJV* or *HAMP*, but with early-onset severe
382 phenotypes.

383

384 **New classification of HC proposed by the working group**

385 As a result of the two-step process described previously, the panelists propose a new
386 classification of HC (shown in **Table 4**), addressing both clinical issues (thereby
387 addressing the needs of general clinicians and subspecialists) and molecular precision.
388 The emphasis on clinical features obviates the current challenges represented by second-
389 level genetic testing for detecting rare variants in the *HFE* and non-*HFE* genes, which
390 could lead to delayed diagnosis and treatment. When criteria listed on **Table 1** are fulfilled,
391 the diagnosis of *HFE*-related HC can be made in the presence of *p.Cys282Tyr*
392 homozygosity. If an appropriately investigated patient has an unequivocal HC phenotype
393 without cofactors but is not a *p.Cys282Tyr* homozygote (and this includes compound
394 *p.Cys282Tyr* and *p.His63Asp* heterozygosity or *p.His63Asp* homozygosity), a provisional
395 diagnosis of "molecularly undefined" HC can be made, and phlebotomies started. In this
396 case, quantification of the total amount of iron removed by phlebotomies will serve as an
397 additional marker of IO. The panelists agree that, whenever possible, an accurate
398 molecular characterization remains important in these patients, especially for cascade
399 screening of asymptomatic siblings, or other first-degree relatives. To this end, patients
400 should be referred (or DNA should be sent) to a specialized center. Indeed, second-level
401 genetic tests have limitations that include costs, time-delay, and poor availability in certain
402 regions, and require a high level of expertise for interpretation. Based on NGS results,
403 some cases could be reclassified into *HFE*-related, digenic or non-*HFE* HC (as shown in
404 **Figure 2**).

405 Based on all the above considerations, we suggest adopting a new, more workable
406 classification of HC (shown in **Table 4**) capable of capturing the growing genetic
407 complexity of HC highlighted by NGS. In fact, the type-numerical classification does not
408 allow assignation of any subtypes to patients with complex genotypes deriving from
409 variants in two genes ("digenic" HC), nor to those who remain undefined after sequencing
410 of known HC genes.

411 Finally, the panelists agreed to abandon the current terminology of Type 4A and 4B HC,
412 related to LOF and GOF variants in the ferroportin gene, respectively. While Type B is in
413 all respects a form of HC (and it should be referred to as ferroportin-related HC), Type 4A
414 has the unique characteristics we described previously. Thus, it should be definitively
415 renamed as "Ferroportin Disease" and included in inherited rare disorders of iron
416 metabolism other than HC. It is important to recall that ferroportin mutations are
417 characterized by an autosomal dominant inheritance pattern, with important implications
418 for genetic testing of family members.

419 In summary, the novel classification proposed here is based on a pathophysiological
420 cornerstone (hepcidin deficiency) and a distinct clinical/biochemical phenotype. It
421 recognizes the difficulties of a complete molecular characterization and has the potential of
422 being easily shareable between practicing physicians and referral centers. Avoiding any
423 ambiguity is essential for clear and effective communication, that will facilitate proper
424 diagnosis and treatment of HC.

425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452

453 The Authors (Members of the Nomenclature Committee of the International Society for the
454 Study of Iron in Biology and Medicine - BIOIRON Society) and affiliations (alphabetical
455 order) are as follows:

456 **Paul C Adams¹, Edouard Bardou-Jacquet², Patricia Bignell³, Pierre Brissot⁴, Fabiana**
457 **Busti⁵, Barbara Butzeck⁶, Ioav Cabantchik⁷, Clara Camaschella⁸, Robert Evans⁹,**
458 **Robert Fleming¹⁰, Tomas Ganz¹¹, Domenico Girelli⁵, Olivier Loréal¹², Giacomo**
459 **Marchi⁵, Martina U. Muckenthaler¹³, Elizabeta Nemeth¹⁴, Antonello Pietrangelo¹⁵,**
460 **Alberto Piperno¹⁶, Graça Porto¹⁷, Dianne Prince⁹, John D. Ryan¹⁸, Mayka Sanchez¹⁹,**
461 **Paulo Santos²⁰, Dorine W. Swinkels^{21,22}, Heinz Zoller²².**

462
463 ¹Department of Medicine, University of Western Ontario, London, Ontario, Canada.

464 ²Univ Rennes, CHU Rennes, National Reference Center for Hemochromatosis and iron metabolism
465 disorder, F-35000, Rennes, France.

466 ³Oxford University Hospitals NHS Foundation Trust, The Churchill Hospital, Oxford, UK

467 ⁴INSERM, Univ-Rennes, INRA, UMR 1241, Institut NuMeCan, Rennes, France

468 ⁵Department of Medicine, Section of Internal Medicine, EuroBloodNet Center, University of Verona and
469 Azienda Ospedaliera Universitaria Integrata Verona, Italy

470 ⁶European Federation of Associations of Patients with Haemochromatosis EFAPH, Croissy-sur-Seine,
471 France.

472 ⁷Della Pergola Chair, Alexander Silberman Institute of Life Sciences, Hebrew University, Jerusalem, Israel

473 ⁸Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy

474 ⁹Haemochromatosis International*, Bratton Fleming, Devon, UK

475 ¹⁰Saint Louis University, St. Louis, Missouri, USA

476 ¹¹David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA

477 ¹²INSERM, University of Rennes, INRAE, UMR 1241, AEM2 Platform, Nutrition Metabolisms and Cancer
478 (NuMeCan) Institute, Rennes, France.

479 ¹³Department of Pediatric Oncology, Hematology, and Immunology and Molecular Medicine Partnership Unit,
480 University of Heidelberg, Heidelberg, Germany; Molecular Medicine Partnership Unit, European Molecular
481 Biology Laboratory, Heidelberg, Germany; Translational Lung Research Center, German Center for Lung
482 Research, Heidelberg, Germany; German Centre for Cardiovascular Research, Partner Site Heidelberg,
483 Mannheim, Germany

484 ¹⁴Center for Iron Disorders, David Geffen School of Medicine, University of California, Los Angeles, CA, USA

485 ¹⁵Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia; Internal Medicine
486 and Centre for Hemochromatosis and Heredometabolic Liver Diseases, ERN -EuroBloodNet Center,
487 Azienda Ospedaliero-Universitaria di Modena, Policlinico, Modena, Italy

488 ¹⁶University of Milano-Bicocca - Department of Medicine and Surgery, Monza, Italy; Medical Genetics -
489 ASST-Monza, S. Gerardo Hospital, Monza, Italy; Centre for Rare Diseases - Disorders of Iron Metabolism -
490 ASST-Monza, EuroBloodNet Referral Centre, S. Gerardo Hospital, Monza, Italy

491 ¹⁷Institute for Molecular and Cell Biology (IBMC), i3S (Instituto de Investigação e Inovação em Saúde),
492 Universidade do Porto, Porto, Portugal; Clinical Hematology, Santo António Hospital - Porto University
493 Hospital Center (CHUP), Porto, Portugal; Molecular Pathology and Immunology, Abel Salazar Institute for
494 Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal

495 ¹⁸Hepatology Unit, Beaumont Hospital/Royal College of Surgeons in Ireland, Dublin, Ireland

496 ¹⁹Department of Basic Sciences, Faculty of Medicine and Health Sciences, Universitat Internacional de
497 Catalunya (UIC); Sant Cugat del Valles, Barcelona, Spain

498 ²⁰Department of Pharmacology, Universidade Federal de Sao Paulo, São Paulo, Brazil

499 ²¹Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud University Medical
500 Center, Nijmegen, Netherlands.

501 ²²Department of Blood Cell Research and Unit of Transfusion Medicine, Sanquin Research and Blood Bank,
502 Amsterdam, The Netherlands.

503 ²³Medical University of Innsbruck, Department of Medicine I, Christian Doppler Laboratory on Iron and
504 Phosphate Biology, Innsbruck, Austria.

505
506 *Hemochromatosis International: Global Alliance linking associations of patients with Hemochromatosis
507 worldwide (<http://haemochromatosis-international.org/>)
508
509

510 **Authorship and conflict-of-interest statements**

511 PBr, DG, GP, MM and IC led the panel and conceived the manuscript. DG and FB co-
512 wrote the manuscript. PA, EB-J, PBi, BB, CC, RE, RF, TG, OL, GM, EN, APie, APip, DP,
513 JR, MS, PS, DS, HZ critically revised and edited the manuscript. TG and EN are co-
514 founder of Intrinsic LifeScience, and consultant for Protagonist, Vifor Pharma, Ionis
515 Pharmaceuticals, and Disc Medicine. JR is consultant for Bond Biosciences and Gilead.
516 The other authors declare no competing financial interests.
517

518 **References**

- 519 1. Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment.
520 *Gastroenterology*. 2010;139(2):393-408, 408 e391-392.
- 521 2. Barton JC, Bertoli LF, Rothenberg BE. Peripheral blood erythrocyte parameters in
522 hemochromatosis: evidence for increased erythrocyte hemoglobin content. *J Lab Clin Med*.
523 2000;135(1):96-104.
- 524 3. Beutler E, Felitti V, Gelbart T, Waalen J. Haematological effects of the C282Y HFE mutation in
525 homozygous and heterozygous states among subjects of northern and southern European ancestry. *Br*
526 *J Haematol*. 2003;120(5):887-893.
- 527 4. Datz C, Haas T, Rinner H, Sandhofer F, Patsch W, Paulweber B. Heterozygosity for the C282Y
528 mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation,
529 and hemoglobin in young women: a protective role against iron deficiency? *Clin Chem*.
530 1998;44(12):2429-2432.
- 531 5. McLaren CE, Barton JC, Gordeuk VR, et al. Determinants and characteristics of mean
532 corpuscular volume and hemoglobin concentration in white HFE C282Y homozygotes in the
533 hemochromatosis and iron overload screening study. *Am J Hematol*. 2007;82(10):898-905.
- 534 6. Alper T, Savage DV, Bothwell TH. Radioiron studies in a case of hemochromatosis. *J Lab Clin*
535 *Med*. 1951;37(5):665-675.
- 536 7. Crosby WH. A history of phlebotomy therapy for hemochromatosis. *Am J Med Sci*.
537 1991;301(1):28-31.
- 538 8. Simon M, Bourel M, Genetet B, Fauchet R. Idiopathic hemochromatosis. Demonstration of
539 recessive transmission and early detection by family HLA typing. *N Engl J Med*. 1977;297(19):1017-
540 1021.
- 541 9. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with
542 hereditary haemochromatosis. *Nat Genet*. 1996;13(4):399-408.
- 543 10. Jazwinska EC. Current status in identifying the haemochromatosis gene. *J Gastroenterol*
544 *Hepatol*. 1996;11(11):1041-1043.
- 545 11. Jazwinska EC, Cullen LM, Busfield F, et al. Haemochromatosis and HLA-H. *Nat Genet*.
546 1996;14(3):249-251.
- 547 12. Jazwinska EC, Powell LW. Hemochromatosis and "HLA-H": definite! *Hepatology*.
548 1997;25(2):495-496.
- 549 13. Cullen LM, Gao X, Easteal S, Jazwinska EC. The hemochromatosis 845 G-->A and 187 C-->G
550 mutations: prevalence in non-Caucasian populations. *Am J Hum Genet*. 1998;62(6):1403-1407.
- 551 14. Porto G, Brissot P, Swinkels DW, et al. EMQN best practice guidelines for the molecular genetic
552 diagnosis of hereditary hemochromatosis (HH). *Eur J Hum Genet*. 2016;24(4):479-495.
- 553 15. Distant S, Robson KJ, Graham-Campbell J, Arnaiz-Villena A, Brissot P, Worwood M. The
554 origin and spread of the HFE-C282Y haemochromatosis mutation. *Hum Genet*. 2004;115(4):269-279.
- 555 16. Cassidy LM, Martiniano R, Murphy EM, et al. Neolithic and Bronze Age migration to Ireland and
556 establishment of the insular Atlantic genome. *Proc Natl Acad Sci U S A*. 2016;113(2):368-373.
- 557 17. McCullough JM, Heath KM, Smith AM. Hemochromatosis: Niche Construction and the Genetic
558 Domino Effect in the European Neolithic. *Hum Biol*. 2015;87(1):39-58.
- 559 18. Lok CY, Merryweather-Clarke AT, Viprakasit V, et al. Iron overload in the Asian community.
560 *Blood*. 2009;114(1):20-25.
- 561 19. Wallace DF, Subramaniam VN. The global prevalence of HFE and non-HFE hemochromatosis
562 estimated from analysis of next-generation sequencing data. *Genet Med*. 2016;18(6):618-626.
- 563 20. Piperno A, Sampietro M, Pietrangelo A, et al. Heterogeneity of hemochromatosis in Italy.
564 *Gastroenterology*. 1998;114(5):996-1002.

- 565 21. Santos PC, Cancado RD, Pereira AC, et al. Hereditary hemochromatosis: mutations in genes
566 involved in iron homeostasis in Brazilian patients. *Blood Cells Mol Dis.* 2011;46(4):302-307.
- 567 22. Camaschella C, Roetto A, Cali A, et al. The gene TFR2 is mutated in a new type of
568 haemochromatosis mapping to 7q22. *Nat Genet.* 2000;25(1):14-15.
- 569 23. Roetto A, Daraio F, Alberti F, et al. Hemochromatosis due to mutations in transferrin receptor 2.
570 *Blood Cells Mol Dis.* 2002;29(3):465-470.
- 571 24. Girelli D, Bozzini C, Roetto A, et al. Clinical and pathologic findings in hemochromatosis type 3
572 due to a novel mutation in transferrin receptor 2 gene. *Gastroenterology.* 2002;122(5):1295-1302.
- 573 25. Montosi G, Donovan A, Totaro A, et al. Autosomal-dominant hemochromatosis is associated
574 with a mutation in the ferroportin (SLC11A3) gene. *J Clin Invest.* 2001;108(4):619-623.
- 575 26. Roetto A, Papanikolaou G, Politou M, et al. Mutant antimicrobial peptide hepcidin is associated
576 with severe juvenile hemochromatosis. *Nat Genet.* 2003;33(1):21-22.
- 577 27. Camaschella C, Roetto A, De Gobbi M. Juvenile hemochromatosis. *Semin Hematol.*
578 2002;39(4):242-248.
- 579 28. Papanikolaou G, Samuels ME, Ludwig EH, et al. Mutations in HFE2 cause iron overload in
580 chromosome 1q-linked juvenile hemochromatosis. *Nat Genet.* 2004;36(1):77-82.
- 581 29. Lanzara C, Roetto A, Daraio F, et al. Spectrum of hemojuvelin gene mutations in 1q-linked
582 juvenile hemochromatosis. *Blood.* 2004;103(11):4317-4321.
- 583 30. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized
584 in the liver. *J Biol Chem.* 2001;276(11):7806-7810.
- 585 31. Pigeon C, Ilyin G, Courselaud B, et al. A new mouse liver-specific gene, encoding a protein
586 homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *J Biol*
587 *Chem.* 2001;276(11):7811-7819.
- 588 32. Andrews NC. Forging a field: the golden age of iron biology. *Blood.* 2008;112(2):219-230.
- 589 33. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophys Acta.* 2012;1823(9):1434-
590 1443.
- 591 34. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian
592 iron metabolism. *Cell.* 2010;142(1):24-38.
- 593 35. Ganz T. Hepcidin and iron regulation, 10 years later. *Blood.* 2011;117(17):4425-4433.
- 594 36. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin
595 era. *Haematologica.* 2020.
- 596 37. Muckenthaler MU, Rivella S, Hentze MW, Galy B. A Red Carpet for Iron Metabolism. *Cell.*
597 2017;168(3):344-361.
- 598 38. Aschemeyer S, Qiao B, Stefanova D, et al. Structure-function analysis of ferroportin defines the
599 binding site and an alternative mechanism of action of hepcidin. *Blood.* 2018;131(8):899-910.
- 600 39. Pan Y, Ren Z, Gao S, et al. Structural basis of ion transport and inhibition in ferroportin. *Nat*
601 *Commun.* 2020;11(1):5686.
- 602 40. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin Regulates Cellular Iron Efflux by Binding to
603 Ferroportin and Inducing Its Internalization. *Science.* 2004;306(5704):2090-2093.
- 604 41. Girelli D, Nemeth E, Swinkels DW. Hepcidin in the diagnosis of iron disorders. *Blood.*
605 2016;127(23):2809-2813.
- 606 42. Wang CY, Babitt JL. Liver iron sensing and body iron homeostasis. *Blood.* 2019;133(1):18-29.
- 607 43. Deugnier Y, Morcet J, Laine F, et al. Reduced phenotypic expression in genetic
608 hemochromatosis with time: Role of exposure to non-genetic modifiers. *J Hepatol.* 2019;70(1):118-125.
- 609 44. Cohen LA, Gutierrez L, Weiss A, et al. Serum ferritin is derived primarily from macrophages
610 through a nonclassical secretory pathway. *Blood.* 2010;116(9):1574-1584.
- 611 45. Arosio P, Elia L, Poli M. Ferritin, cellular iron storage and regulation. *IUBMB Life.*
612 2017;69(6):414-422.
- 613 46. Arosio P, Carmona F, Gozzelino R, Maccarinelli F, Poli M. The importance of eukaryotic ferritins
614 in iron handling and cytoprotection. *Biochem J.* 2015;472(1):1-15.
- 615 47. Knovich MA, Storey JA, Coffman LG, Torti SV, Torti FM. Ferritin for the clinician. *Blood Rev.*
616 2009;23(3):95-104.
- 617 48. Sandnes M, Ulvik RJ, Vorland M, Reikvam H. Hyperferritinemia-A Clinical Overview. *J Clin*
618 *Med.* 2021;10(9).
- 619 49. Cullis JO, Fitzsimons EJ, Griffiths WJ, Tsochatzis E, Thomas DW, British Society for H.
620 Investigation and management of a raised serum ferritin. *Br J Haematol.* 2018;181(3):331-340.
- 621 50. Adams PC, Barton JC. A diagnostic approach to hyperferritinemia with a non-elevated
622 transferrin saturation. *J Hepatol.* 2011;55(2):453-458.

623 51. Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loreal O. Haemochromatosis.
624 *Nat Rev Dis Primers*. 2018;4:18016.

625 52. Adams PC, Reboussin DM, Press RD, et al. Biological variability of transferrin saturation and
626 unsaturated iron-binding capacity. *Am J Med*. 2007;120(11):999 e991-997.

627 53. Adams PCaBJC. Iron overload and cirrhosis in referred HFE p.C282Y homozygotes with normal
628 transferrin saturation and elevated serum ferritin. *Can Liver Journal*. 2020;3(2):188-193.

629 54. Golfeyz S, Lewis S, Weisberg IS. Hemochromatosis: pathophysiology, evaluation, and
630 management of hepatic iron overload with a focus on MRI. *Expert Rev Gastroenterol Hepatol*.
631 2018;12(8):767-778.

632 55. Henninger B, Alustiza J, Garbowski M, Gandon Y. Practical guide to quantification of hepatic
633 iron with MRI. *Eur Radiol*. 2020;30(1):383-393.

634 56. Franca M, Carvalho JG. MR imaging assessment and quantification of liver iron. *Abdom Radiol*
635 (NY). 2020;45(11):3400-3412.

636 57. Legros L, Bardou-Jacquet E, Latournerie M, et al. Non-invasive assessment of liver fibrosis in
637 C282Y homozygous HFE hemochromatosis. *Liver Int*. 2015;35(6):1731-1738.

638 58. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and
639 causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N Engl J Med*.
640 1985;313(20):1256-1262.

641 59. Prabhu A, Cargill T, Roberts N, Ryan JD. Systematic Review of the Clinical Outcomes of Iron
642 Reduction in Hereditary Hemochromatosis. *Hepatology*. 2020;72(4):1469-1482.

643 60. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G--> A (C282Y) HFE
644 hereditary haemochromatosis mutation in the USA. *Lancet*. 2002;359(9302):211-218.

645 61. Waalen J, Nordestgaard BG, Beutler E. The penetrance of hereditary hemochromatosis. *Best*
646 *Pract Res Clin Haematol*. 2005;18(2):203-220.

647 62. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and iron-overload screening in
648 a racially diverse population. *N Engl J Med*. 2005;352(17):1769-1778.

649 63. Pilling LC, Tamosauskaite J, Jones G, et al. Common conditions associated with hereditary
650 haemochromatosis genetic variants: cohort study in UK Biobank. *BMJ*. 2019;364:k5222.

651 64. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary
652 hemochromatosis. *N Engl J Med*. 2008;358(3):221-230.

653 65. Gurrin LC, Bertalli NA, Dalton GW, et al. HFE C282Y/H63D compound heterozygotes are at low
654 risk of hemochromatosis-related morbidity. *Hepatology*. 2009;50(1):94-101.

655 66. Walsh A, Dixon JL, Ramm GA, et al. The clinical relevance of compound heterozygosity for the
656 C282Y and H63D substitutions in hemochromatosis. *Clin Gastroenterol Hepatol*. 2006;4(11):1403-
657 1410.

658 67. Biasiotto G, Roetto A, Daraio F, et al. Identification of new mutations of hepcidin and
659 hemojuvelin in patients with HFE C282Y allele. *Blood Cells Mol Dis*. 2004;33(3):338-343.

660 68. Dostalíková-Cimburova M, Balusikova K, Kratka K, et al. Role of duodenal iron transporters and
661 hepcidin in patients with alcoholic liver disease. *J Cell Mol Med*. 2014;18(9):1840-1850.

662 69. Girelli D, Pasino M, Goodnough JB, et al. Reduced serum hepcidin levels in patients with
663 chronic hepatitis C. *J Hepatol*. 2009;51(5):845-852.

664 70. European Association For The Study Of The L. EASL clinical practice guidelines for HFE
665 hemochromatosis. *J Hepatol*. 2010;53(1):3-22.

666 71. Kowdley KV, Brown KE, Ahn J, Sundaram V. ACG Clinical Guideline: Hereditary
667 Hemochromatosis. *Am J Gastroenterol*. 2019;114(8):1202-1218.

668 72. Sandhu K, Flintoff K, Chatfield MD, et al. Phenotypic analysis of hemochromatosis subtypes
669 reveals variations in severity of iron overload and clinical disease. *Blood*. 2018;132(1):101-110.

670 73. Adams PC. Hemochromatosis case definition: out of focus? *Nat Clin Pract Gastroenterol*
671 *Hepatol*. 2006;3(4):178-179.

672 74. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading
673 anaemias. *Br J Haematol*. 2016;172(4):512-523.

674 75. Finch C. Regulators of iron balance in humans. *Blood*. 1994;84(6):1697-1702.

675 76. Coffey R, Ganz T. Erythroferrone: An Erythroid Regulator of Hepcidin and Iron Metabolism.
676 *Hemasphere*. 2018;2(2):e35.

677 77. Musallam KM, Cappellini MD, Wood JC, Taher AT. Iron overload in non-transfusion-dependent
678 thalassemia: a clinical perspective. *Blood Rev*. 2012;26 Suppl 1:S16-19.

679 78. Origa R, Galanello R, Ganz T, et al. Liver iron concentrations and urinary hepcidin in beta-
680 thalassemia. *Haematologica*. 2007;92(5):583-588.

681 79. Pasricha SR, Frazer DM, Bowden DK, Anderson GJ. Transfusion suppresses erythropoiesis
682 and increases hepcidin in adult patients with beta-thalassemia major: a longitudinal study. *Blood*.
683 2013;122(1):124-133.

684 80. Brissot P, Bernard DG, Brissot E, Loréal O, Troadec MB. Rare anemias due to genetic iron
685 metabolism defects. *Mutat Res*. 2018;777:52-63.

686 81. Ducamp S, Fleming MD. The molecular genetics of sideroblastic anemia. *Blood*.
687 2019;133(1):59-69.

688 82. Iolascon A, Andolfo I, Russo R. Congenital dyserythropoietic anemias. *Blood*.
689 2020;136(11):1274-1283.

690 83. Santini V, Girelli D, Sanna A, et al. Hepcidin levels and their determinants in different types of
691 myelodysplastic syndromes. *PLoS One*. 2011;6(8):e23109.

692 84. Bondu S, Alary AS, Lefevre C, et al. A variant erythroferrone disrupts iron homeostasis in
693 SF3B1-mutated myelodysplastic syndrome. *Sci Transl Med*. 2019;11(500).

694 85. Fleming RE, Ponka P. Iron overload in human disease. *N Engl J Med*. 2012;366(4):348-359.

695 86. Powell LW, Seckington RC, Deugnier Y. Haemochromatosis. *Lancet*. 2016;388(10045):706-
696 716.

697 87. Manolio TA, Fowler DM, Starita LM, et al. Bedside Back to Bench: Building Bridges between
698 Basic and Clinical Genomic Research. *Cell*. 2017;169(1):6-12.

699 88. Fernandez-Marmiesse A, Gouveia S, Couce ML. NGS Technologies as a Turning Point in Rare
700 Disease Research , Diagnosis and Treatment. *Curr Med Chem*. 2018;25(3):404-432.

701 89. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children.
702 *Nat Rev Genet*. 2018;19(5):325.

703 90. Badar S, Busti F, Ferrarini A, et al. Identification of novel mutations in hemochromatosis genes
704 by targeted next generation sequencing in Italian patients with unexplained iron overload. *Am J*
705 *Hematol*. 2016;91(4):420-425.

706 91. McDonald CJ, Ostini L, Wallace DF, Lyons A, Crawford DH, Subramaniam VN. Next-generation
707 sequencing: Application of a novel platform to analyze atypical iron disorders. *J Hepatol*.
708 2015;63(5):1288-1293.

709 92. Merryweather-Clarke AT, Cadet E, Bomford A, et al. Digenic inheritance of mutations in HAMP
710 and HFE results in different types of haemochromatosis. *Hum Mol Genet*. 2003;12(17):2241-2247.

711 93. Gan EK, Trinder D, Ayonrinde OT, Olynyk JK. Genetics of hereditary hemochromatosis: a
712 clinical perspective. *Expert Rev Endocrinol Metab*. 2009;4(3):225-239.

713 94. Rametta R, Dongiovanni P, Baselli GA, et al. Impact of natural neuromedin-B receptor variants
714 on iron metabolism. *Am J Hematol*. 2020;95(2):167-177.

715 95. Lanktree MB, Sadikovic B, Waye JS, et al. Clinical evaluation of a hemochromatosis next-
716 generation sequencing gene panel. *Eur J Haematol*. 2017;98(3):228-234.

717 96. Piubelli C, Castagna A, Marchi G, et al. Identification of new BMP6 pro-peptide mutations in
718 patients with iron overload. *Am J Hematol*. 2017;92(6):562-568.

719 97. Daher R, Kannengiesser C, Houamel D, et al. Heterozygous Mutations in BMP6 Pro-peptide
720 Lead to Inappropriate Hepcidin Synthesis and Moderate Iron Overload in Humans. *Gastroenterology*.
721 2016;150(3):672-683 e674.

722 98. Alvarenga AM, da Silva NK, Fonseca PFS, et al. Novel mutations in the bone morphogenetic
723 protein 6 gene in patients with iron overload and non-homozygous genotype for the HFE p.Cys282Tyr
724 mutation. *Blood Cells Mol Dis*. 2020;84:102444.

725 99. Xiao X, Alfaro-Magallanes VM, Babitt JL. Bone morphogenetic proteins in iron homeostasis.
726 *Bone*. 2020;138:115495.

727 100. McDonald CJ, Rishi G, Secondes ES, et al. Evaluation of a bone morphogenetic protein 6
728 variant as a cause of iron loading. *Hum Genomics*. 2018;12(1):23.

729 101. Pietrangelo A. The ferroportin disease. *Blood Cells Mol Dis*. 2004;32(1):131-138.

730 102. Pietrangelo A. Ferroportin disease: pathogenesis, diagnosis and treatment. *Haematologica*.
731 2017;102(12):1972-1984.

732 103. Vlasveld LT, Janssen R, Bardou-Jacquet E, et al. Twenty Years of Ferroportin Disease: A
733 Review or An Update of Published Clinical, Biochemical, Molecular, and Functional Features.
734 *Pharmaceuticals (Basel)*. 2019;12(3).

735 104. Bardou-Jacquet E, Ben Ali Z, Beaumont-Epinette MP, Loreal O, Jouanolle AM, Brissot P. Non-
736 HFE hemochromatosis: pathophysiological and diagnostic aspects. *Clin Res Hepatol Gastroenterol*.
737 2014;38(2):143-154.

738 105. De Gobbi M, Roetto A, Piperno A, et al. Natural history of juvenile haemochromatosis. *Br J*
739 *Haematol*. 2002;117(4):973-979.

- 740 106. Beutler E, Griffin MJ, Gelbart T, West C. A previously undescribed nonsense mutation of the
741 HFE gene. *Clin Genet.* 2002;61(1):40-42.
- 742 107. Mendes AI, Ferro A, Martins R, et al. Non-classical hereditary hemochromatosis in Portugal:
743 novel mutations identified in iron metabolism-related genes. *Ann Hematol.* 2009;88(3):229-234.
- 744 108. Zhang W, Wang X, Duan W, et al. HFE-Related Hemochromatosis in a Chinese Patient: The
745 First Reported Case. *Front Genet.* 2020;11:77.
- 746 109. Hamdi-Roze H, Beaumont-Epinette MP, Ben Ali Z, et al. Rare HFE variants are the most
747 frequent cause of hemochromatosis in non-c282y homozygous patients with hemochromatosis. *Am J*
748 *Hematol.* 2016;91(12):1202-1205.
- 749 110. Le Gac G, Congiu R, Gourlaouen I, Cau M, Ferec C, Melis MA. Homozygous deletion of HFE is
750 the common cause of hemochromatosis in Sardinia. *Haematologica.* 2010;95(4):685-687.

751

752

753

754

755

756

757

758

759

760

761

762

763

764

765

766

767

768

769

770

771

772

773

774

775

776 **Table 1. Main clinical, biochemical and imaging elements for the suspicion of Hemochromatosis**

777

Leading
+ TSAT >45% (mainstay)
+ S-Ferritin >200 µg/L (females) or >300 µg/L (males)
+ imaging evidence of liver IO (MRI* and/or biopsy**)
+ Iron deposits in hepatocytes (if biopsy is performed)
+ Absence of “predominant” acquired risk factors for hepcidin deficiency (e.g., alcohol abuse or end-stage liver disease) and iatrogenic iron overload (e.g. regular transfusions)
+ Absence of hematological signs of a primary red blood cell disorder, such as anemia*** (i.e. Hb>120 g/L in females, >130 g/L in males) and/or reticulocytosis
Not always present
± Signs and/or symptoms associated with IO:
- skin pigmentation, asthenia
- persistent increase of aminotransferases, hepatomegaly, cirrhosis, hepatocellular carcinoma
- joint pain, arthritis, chondrocalcinosis, reduced bone mineral density
- diabetes mellitus, hypopituitarism, hypoparathyroidism, hypogonadotropic hypogonadism
- cardiomyopathy, heart failure, cardiac arrhythmias

778 TSAT = transferrin saturation; IO = iron overload; MRI = Magnetic Resonance Imaging.

779 *Liver Iron Content (LIC) quantification by MRI can be obtained using different protocols, which vary
780 depending on local expertise and equipment. With these limitations, any LIC value higher than the upper
781 normal limit (generally set at 36-40 µmol/g dry weight) should lead to consideration of phlebotomies in HC
782 patients. Similarly, LIC >100-120 µmol/g and >240-300 µmol/g are generally considered as overt and severe
783 IO, respectively (see text and ref.^{55,56}).

784 **Liver biopsy should be considered in patients with ferritin >1,000 µg/L or signs of liver damage.

785 ***Exceptions may occur, e.g. in HC patients with diagnosis delayed after the appearance of liver cirrhosis, in
786 whom anemia may be observed because of hypersplenism or gastrointestinal bleeding, or subjects with
787 beta-thalassemia trait, whose coexistence is not rare in Mediterranean countries.

788

789

790

791

792

793

794

795

796

797 **Table 2. Combined pathogenic allele frequency for HC genes in the 1000 Genomes Project (1000G),**
 798 **Exome Sequencing Project (ESP), and Exome Aggregation Consortium (ExAc) datasets** (modified by
 799 Wallace et al.)¹⁹
 800

Gene	1000G	ESP6500	ExAc	Geographical distribution
<i>HFE</i> (<i>p.Cys282Tyr</i>)	0.013	0.048	0.0324	Highest prevalence in Northern Europe
<i>HFE</i> (non-<i>p.Cys282Tyr</i>)	0.001	0.0002	0.000307	
<i>HJV</i>		0.00074	0.000316	Highest prevalence in Southern Asia
<i>TFR2</i>	0.0004	0.0003	0.000102	Most frequent among non-Finnish European populations
<i>HAMP</i>	0.0002		0.0000165	Several populations
<i>SLC40A1</i>	0.0008	0.0009	0.00034	Several populations (highest prevalence among Africans)

801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824

825
826
827
828
829

Table 3. Former classification of HC

Classification	Gene involved and location	Inheritance	TSAT	Other clinical features
Type 1	<i>HFE</i> ; chr.6	AR	Increased	Adult-onset; more severe in males; highly variable clinical expression, with predominant liver damage and arthritis
Type 2A	<i>HJV</i> (hemojuvelin); chr.1	AR	Increased	Earlier onset (e.g.<30 years old); similar severity in both sexes; prevalent cardiac and endocrine involvement
Type 2B	<i>HAMP</i> (hepcidin); chr.19	AR	Increased	Earlier onset (e.g.<30 years old); similar severity in both sexes; prevalent cardiac and endocrine involvement
Type 3	<i>TFR2</i> (transferrin receptor 2); chr.7	AR	Increased	Very rare (look for parental consanguinity); clinically similar to Type 1, with an earlier onset
Type 4A	<i>SLC40A1</i> (ferroportin); chr.2	AD	Low-normal	Adult-onset; IO in the spleen; mild anemia; possible low tolerance to venesection
Type 4B	<i>SLC40A1</i> (ferroportin); chr.2	AD	Increased	Very rare; in general, clinically similar to Type 1, but more severe/early onset forms are reported

830
831
832
833
834
835
836
837
838
839
840
841

Novel classification	Molecular pattern	Note
<i>HFE</i> -related	<i>p.Cys282Tyr</i> homozygosity or compound heterozygosity of <i>p.Cys282Tyr</i> with other rare <i>HFE</i> pathogenic variants ¹⁰⁶⁻¹⁰⁹ or <i>HFE</i> deletion ¹¹⁰	Low penetrance; consider presence of host-related or environmental cofactors for IO In subjects with other <i>HFE</i> genotypes (e.g. <i>p.Cys282Tyr/His63Asp</i> compound heterozygosity or <i>p.His63Asp</i> homozygosity) consider second-line genetic testing for rarer variants.
<i>Non HFE</i> -related	Rare pathogenic variants in “ <i>non-HFE</i> ” genes: <ul style="list-style-type: none"> - <i>HJV</i>-related - <i>HAMP</i>-related - <i>TFR2</i>-related - <i>SLC40A1</i> (GOF)-related 	Potentially, mutations in any hepcidin-regulatory gene may be causative (the effects of novel mutations should be confirmed through functional and epidemiological studies). Molecular subtypes characterization only at specialized centers, but the diagnosis of <i>non-HFE</i> related HC is sufficient to start phlebotomies at non-specialized centers*.
Digenic**	Double heterozygosity and/or double homozygosity/heterozygosity for mutations in two different genes involved in iron metabolism (<i>HFE</i> and/or <i>non-HFE</i>)	More commonly, <i>p.Cys282Tyr</i> mutation in <i>HFE</i> gene might coexist with mutation in other genes; rarely, both mutations involve <i>non-HFE</i> genes
Molecularly undefined	Molecular characterization (still) not available after sequencing of known genes (provisional diagnosis)	Patients should be referred (or DNA should be sent) to specialized centers

844 *Providing that iron overload is confirmed by MRI. If this is not accessible, close monitoring of Hb level is
845 needed to avoid the occurrence of anemia.

846 ** Caution is needed to interpret as digenic inheritance results from NGS outputs reporting several variants
847 in gene panels. Whenever possible, strict criteria for defining pathogenic variants should be adopted, and
848 corroborated by family segregation and/or functional studies.

850 **Figure 1. Heparin regulation by iron.** Increase in transferrin saturation induces
851 hepcidin transcription via the BMP/SMAD signaling pathway. Diferric transferrin binds to
852 TfR2, while BMP6 and BMP2 secreted by liver sinusoidal endothelial cells (LSECs) bind to
853 BMP receptors on hepatocytes. These events trigger phosphorylation of regulatory
854 SMAD1/5/8, recruitment of SMAD4, and translocation of the SMAD complex to the nucleus
855 for activating hepcidin transcription upon binding to BMP/SMAD responsive element in
856 the *HAMP* promoter. BMPs can be trapped by ERFE, leading to hepcidin inhibition in iron
857 loading anemias. Efficient iron signaling requires the BMP co-receptor HJV and the protein
858 HFE, and is negatively regulated by the transmembrane serine protease matriptase-2
859 (TMPRSS6). The complex molecular pathogenesis of HC reflects the numerous proteins
860 involved in regulation of the hepcidin-ferroportin axis.

861
862 **Figure 2. Proposal of an algorithm for the diagnosis of HC, from clinical/biochemical**
863 **and imaging studies to molecular confirmation.** Important note: in Caucasians, *HFE*
864 genotyping is indicated with the specific purpose of detecting *p.Cys282Y* homozygosity
865 and, if confirmed, to recommend appropriate preventive treatment by phlebotomies. Asian,
866 African, and native American subjects with defined HC phenotype could be directly
867 referred to second-level genetic test. In populations with a frequent component of northern
868 European ancestry, such as African-Americans and Hispanics, there may be still a role for
869 *HFE* genetic testing.

870
871 **Figure 3. Iron homeostasis in normal conditions (A) and mechanisms leading to iron**
872 **accumulation in HC (B) and in iron-loading anemias (C: non-transfusion dependent;**
873 **D: transfusion-dependent).** In HC, iron hyperabsorption through the portal vein leads to
874 iron accumulation in liver parenchymal cells, initially with a typical portal-central gradient
875 (see histology) and sparing of macrophages (Kupffer cells). In non-transfusion dependent
876 anemias with ineffective erythropoiesis hepcidin insufficiency is also central to the
877 pathogenesis of IO, but it is due to suppression by soluble factors (e.g. ERFE) produced by
878 ineffective/expanded erythroblasts, rather than to a genetic defect in pathways regulating
879 hepcidin synthesis. In transfusion dependent anemias, regular red blood cells (RBCs)
880 transfusions represent the major contributing factor to IO; in these conditions, hepcidin is
881 relatively upregulated by iron, but fluctuates in response to intermittent erythropoiesis
882 suppression by transfusions.

Figure 1

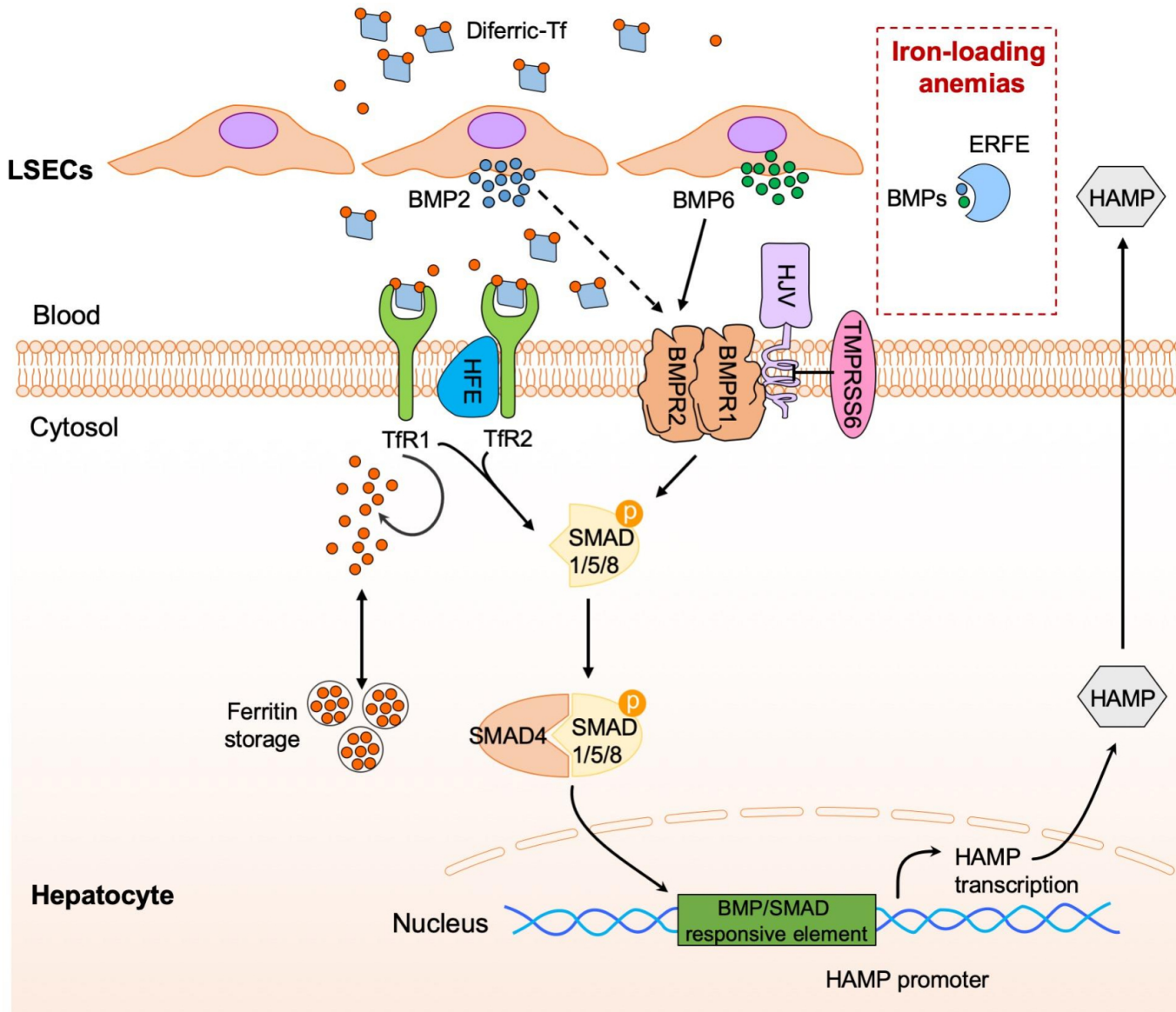


Figure 2

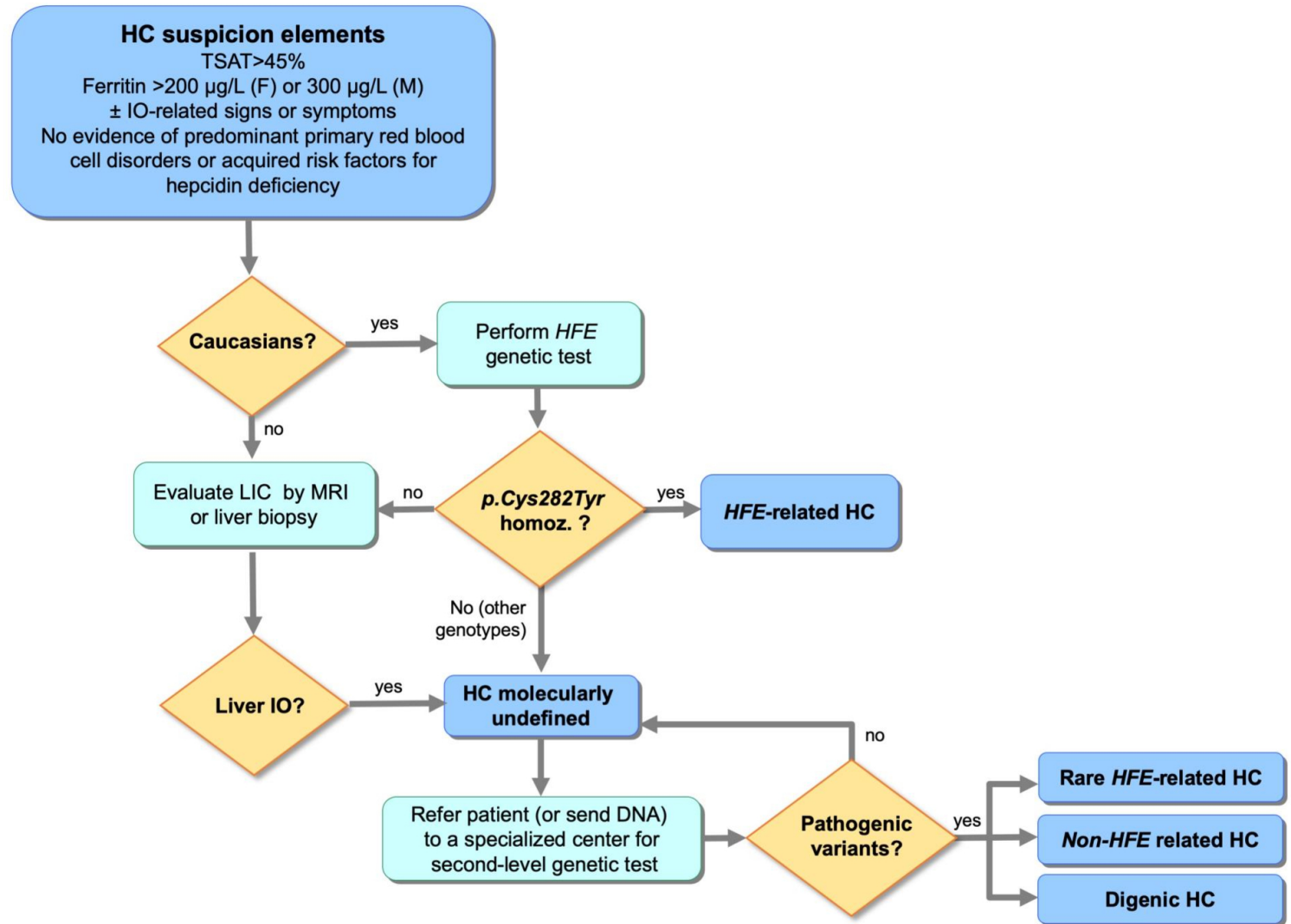


Figure 3

