

# Pathophysiological consequences and benefits of *HFE* mutations: 20 years of research

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## ABSTRACT

Mutations in the *HFE* (hemochromatosis) gene cause hereditary hemochromatosis, an iron overload disorder that is hallmarked by excessive accumulation of iron in parenchymal organs. The *HFE* mutation p.Cys282Tyr is pathologically most relevant and occurs in the Caucasian population with a carrier frequency of up to 1 in 8 in specific European regions. Despite this high prevalence, the mutation causes a clinically relevant phenotype only in a minority of cases. In this review, we summarize historical facts and recent research findings about hereditary hemochromatosis, and outline the pathological consequences of the associated gene defects. In addition, we discuss potential advantages of *HFE* mutations in asymptomatic carriers.

## Introduction

Iron plays a key role in various physiological pathways. All cells of the human body contain iron as an integral part of FeS-proteins. These are essential for oxidation-reduction reactions that occur, for example, in the mitochondrial respiratory chain where biochemical energy is generated. In red blood cells, iron binds oxygen in the hemoglobin molecule to enable its transport from respiratory organs throughout the human body. Sufficient amounts of serum iron are mainly ensured by macrophages, which recycle approximately 25 mg of iron a day from aging or damaged erythrocytes. Iron losses resulting from bleeding or skin desquamation are compensated for by dietary iron absorption, which amounts to approximately 1-2 mg/day.<sup>1</sup> Duodenal iron absorption is a highly regulated process. If duodenal iron uptake exceeds the requirements for physiological iron consuming processes, such as erythropoiesis, excess iron accumulates in parenchymal organs, including the liver, heart, and pancreas. Iron overload causes oxidative damage in these organs and, ultimately, the iron-overload disease hereditary hemochromatosis (HH) may develop when genetic and environmental risk factors are present.<sup>2</sup>

Ever since it was first described in the 19<sup>th</sup> century, scientists and clinicians have been increasing their efforts to unravel the causes and consequences of HH. Modern research has been successful in identifying HH-causing gene mutations and unraveling molecular mechanisms responsible for elevated dietary iron uptake. HH is mainly caused by mutations in the *HFE* (hemochromatosis) gene. The most pathologically influential *HFE* mutation, p.Cys282Tyr (C282Y), is frequently inherited in a heterozygous state (overall carrier frequency approx. 1:16) in the Caucasian population, but accounts for a phenotype only in a minority of cases.<sup>3</sup> This observation has led to the hypothesis that *HFE* mutations may confer a genetic advantage to asymptomatic hemochromatosis 'patients' and have, therefore, continued to spread.<sup>4</sup>

## A history of hereditary hemochromatosis

Hemochromatosis was first described in the mid-1800s by French physicians who referred to the disease as "bronze diabetes" and "pigmented cirrhosis".<sup>5-7</sup> A few years later, the German pathologist Friedrich Daniel von Recklinghausen linked the syndrome to iron metabolism after he had observed an excess of iron in the tissues of patients and introduced the present-day term hemochromatosis.<sup>8</sup> In

Haematologica 2017

Volume 102(5):809-817

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Received: November 24, 2016.

Accepted: March 1, 2017.

Pre-published: March 24, 2017.

doi:10.3324/haematol.2016.160432

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: [www.haematologica.org/content/102/5/809](http://www.haematologica.org/content/102/5/809)

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those days, scientists and physicians believed that the syndrome was exclusively caused by environmental factors and conditions such as diabetes, alcohol abuse, metabolic disturbances, or infections. The English gerontologist Sheldon rejected this hypothesis in 1935 and postulated that hemochromatosis was a hereditary disease.<sup>9</sup> This assumption was experimentally verified in the 1970s in a study that associated hemochromatosis with the *HLA* (human leukocyte antigen) gene locus and identified it as an autosomal recessive disease that mainly affects men.<sup>10</sup>

The discovery of the intracellular iron storage protein ferritin<sup>11</sup> and the iron-binding blood plasma protein transferrin<sup>12</sup> in the first half of the 20<sup>th</sup> century gave researchers the first molecular hints about the biological mechanisms regulating iron metabolism. Those and subsequent mechanistic studies have provided an understanding of the molecular processes involved in hemochromatosis. Twenty years ago, the genetic factor responsible for HLA-linked HH was finally identified as a mutation in the gene encoding the non-classical MHC class I-like molecule HFE.<sup>13</sup> Subsequently, rare non-HFE-related HH subtypes were discovered, hallmarked by mutations in newly identified iron-related proteins such as hemojuvelin (HJV; hemochromatosis type IIA),<sup>14</sup> hepcidin, the master regulator of systemic iron homeostasis, (HAMP; hemochromatosis type IIB),<sup>15</sup> transferrin receptor 2 (TfR2; hemochromatosis type III),<sup>16</sup> or ferroportin (FPN or SLC40A1; type IV or ferroportin disease).<sup>16,17</sup>

Large-scale population studies have revealed that most *HFE* mutation carriers with a mild iron overload-phenotype lack any clinically relevant disease symptoms.<sup>19</sup> This has led to a debate about the potential benefits of *HFE* gene defects and has been motivating the scientific community to identify potential advantages of these mutations.

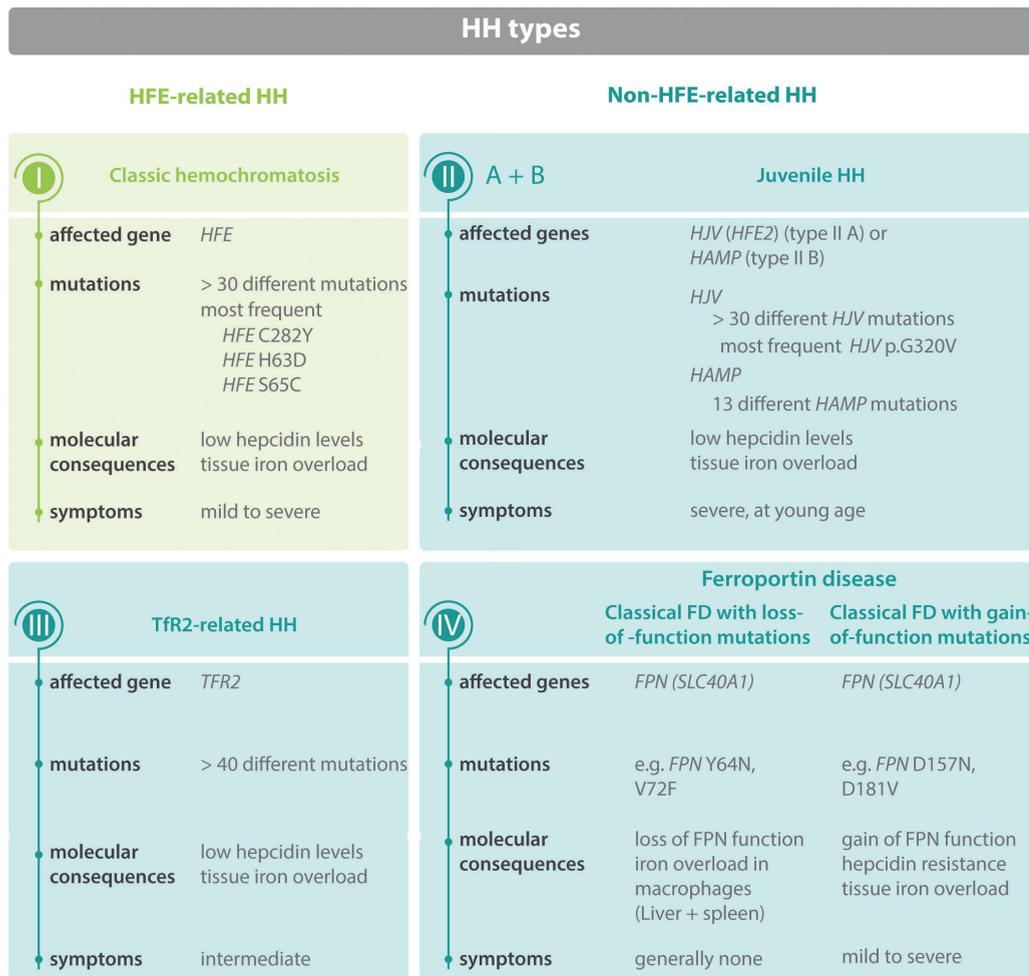
### The molecular and genetic causes of hereditary hemochromatosis

The iron-regulated hormone hepcidin is of central importance to the pathogenesis of HH. It is mainly produced in hepatocytes and secreted into the blood stream. It binds to and degrades its target receptor, the iron exporter ferroportin, to inhibit iron release from duodenal enterocytes, iron-recycling macrophages, and hepatocytes. Any defect that ultimately impairs hepcidin function and inhibits the hormone's ability to monitor and regulate serum iron levels may provoke an iron overload phenotype. In HH patients, hepcidin levels are reduced, causing excess ferroportin-mediated iron export and, as a consequence, increased dietary iron uptake and release from macrophages.<sup>20</sup> Elevated iron levels in the blood stream subsequently lead to the saturation of the binding capacity of the iron transporter transferrin. Above a transferrin saturation of approximately 75%<sup>21</sup> highly reactive, non-transferrin bound iron species (especially labile plasma iron, LPI) appear in the blood, which will preferentially be taken up by parenchymal cells of the liver, the pancreas and other organs,<sup>22</sup> and ultimately provoke an HH phenotype.

Mutations in either the hepcidin gene itself, in genes affecting upstream activators of hepcidin expression (*HFE*, *TFR2*, *HJV*), or in ferroportin (the iron exporter which acts as the hepcidin receptor) can cause different classes and subtypes of HH<sup>20</sup> (Figure 1). Most cases of HH are due to mutations in the *HFE* gene. The most prevalent disease-

causing *HFE* mutation in the general population is the 845G polymorphism, which causes a p.Cys282Tyr amino acid substitution (C282Y) in the HFE protein. Today, approximately 0.4% of Caucasians carry a homozygous<sup>23,24</sup> and approximately 6% a heterozygous *HFE* C282Y mutation.<sup>24</sup> HFE is a non-classical major histocompatibility (MHC) protein<sup>13</sup> located on the cell surface. The C282Y mutation disrupts the formation of a disulfide bond in the HFE protein and impairs its capability to bind  $\beta$ 2-microglobulin.<sup>25</sup> As a consequence, HFE is unable to reach the cell surface and aggregates intracellularly.<sup>25,26</sup> This causes impaired signaling leading to reduced hepcidin mRNA expression, decreased plasma hepcidin levels, and excessive systemic iron accumulation in adults (aged over 40 years).<sup>20</sup> The molecular mechanism by which HFE regulates hepcidin expression is not yet fully understood. It has been proposed that HFE plays a regulatory role in the sensing of serum TF-Fe concentrations, involving protein-protein interactions between HFE, the ubiquitous transferrin receptor TfR1 and the liver-specific transferrin receptor TfR2. High concentrations of iron-bound transferrin dissociate HFE from TfR1<sup>27,28</sup> which have been found to strengthen the interaction between HFE and TfR2.<sup>29</sup> Increased iron levels further stimulate the expression of BMP6 (bone morphogenetic protein 6), a member of the TGF $\beta$  superfamily, whose genetic impairment causes severe iron overload in mice<sup>30,31</sup> and in patients.<sup>32</sup> BMP6 binds to the GPI-anchored receptor hemojuvelin (HJV) at the cell surface,<sup>33</sup> as well as to type 1 (ALK2 and ALK3<sup>34</sup>) and type 2 (BMPR2 and ACTR2a<sup>35</sup>) serine threonine kinase receptors. As a result, SMAD1/5/8 is phosphorylated and binds to SMAD4, which translocates to the nucleus to induce hepcidin transcription.<sup>36</sup> Stimulation of hepcidin expression by BMP6 has recently been reported to also take place in an HJV-independent manner.<sup>37</sup> HFE has been found to prevent the ubiquitination and proteasomal degradation of the BMP6-receptor ALK3,<sup>38</sup> as well as to engage in a tertiary complex with the BMP6-receptor HJV and TfR2.<sup>39</sup> These observations suggest a role of HFE in BMP/SMAD signaling and provide a first mechanistic explanation for the impairment of BMP/SMAD signaling in patients with HFE-hemochromatosis.<sup>40</sup>

*HFE* gene sequencing approaches have identified additional *HFE* mutations with different pathological impact. These include the amino acid alteration S65C, which is not considered clinically meaningful,<sup>20,41,42</sup> or H63D, which may, in rare cases, contribute to abnormal iron parameters in H63D/C282Y compound heterozygote individuals.<sup>43-45</sup> By contrast, individuals carrying one H63D and one healthy allele are asymptomatic as long as no additional risk factors are present.<sup>41</sup> In addition, researchers discovered a deletion (p.Y231del) in an Huh7 hepatoma cell line derived from a Japanese HH patient<sup>46</sup> which prevents HFE cell surface expression. The identical mutation has more recently been discovered in another Japanese patient,<sup>47</sup> showing, for the first time, that *HFE*-associated HH can also occur in Asians. Furthermore, a few Sardinian individuals show deletions of the entire *HFE* gene.<sup>48,49</sup> Additional *HFE* mutations have been detected to influence iron levels when co-inherited with heterozygous C282Y mutations, such as the p.Arg226Gly (R226G) mutation<sup>50</sup> or the nonsense mutations *HFE*-Brianza and *HFE*-Ossola, termed after the Italian provinces they were detected in.<sup>51</sup> Although the prevalence of such mutations is low, they show that the presence of genetic factors may contribute



**Figure 1. Hereditary hemochromatosis (HH).** Three different HH classes (HFE-related HH, non-HFE-related HH, and ferroportin disease) are subcategorized into different subtypes (I, II A + B, III and IV) depending on mutations in iron-related genes (*HFE*, *HJV*, *HAMP*, *TFR2*, *FPN/SLC40A1*) and related pathophysiology. FD: ferroportin disease.

to the clinical manifestation of hemochromatosis in C282Y heterozygotes.

Non-HFE-hemochromatosis (commonly referred to as hemochromatosis type II to IV) is much rarer than HFE-HH. In contrast to HFE-hemochromatosis, it also occurs in individuals of non-European descent, in both adult and juvenile onset forms.<sup>52</sup> Mutations causing non-HFE-HH are detected in genes encoding hepatocytic membrane proteins [*HJV* (hemojuvelin), *TFR2*], which play a role in the monitoring of iron levels and signaling to hepcidin (see above), or *HAMP* itself. Mutations in *HJV*<sup>14</sup> and *HAMP*<sup>15</sup> cause juvenile non-HFE-HH forms (HH types II A and II B) which are characterized by very low circulating hepcidin levels. Patients usually develop a severe iron overload phenotype before the age of 30 years, including cardiovascular, liver, and endocrine complications.<sup>53</sup> Adult onset non-HFE-HH forms (HH types III and IV) are either caused by mutations in *TFR2* encoding transferrin receptor 2,<sup>16</sup> or the hepcidin receptor ferroportin (*FPN/SLC40A1*), generally referred to as HH type IV.<sup>55</sup> Gain-of-function mutations within ferroportin confer resistance to hepcidin binding and thus prevent ferroportin internalization and degradation (HH type IV, classical ferroportin disease with gain-of-function mutation). As a consequence, uncontrolled iron export from cell types expressing ferroportin, such as duodenal enterocytes or macrophages, causes high levels

of iron overload.<sup>56</sup> By contrast, loss-of-function mutations in ferroportin diminish the ability of ferroportin for iron export (HH type IV, classical ferroportin disease with loss-of-function mutations), which is characterized by iron accumulation in macrophages of the spleen and the liver, and is associated with low serum iron levels.<sup>2</sup>

### Symptoms, diagnosis and treatment of HH

Elevated liver enzymes and/or iron parameters indicative of iron overload (serum ferritin and transferrin saturation levels) usually precede a symptomatic manifestation of hemochromatosis.<sup>20</sup> Individuals with elevated body iron levels despite fully functional erythropoiesis are thus commonly diagnosed with hemochromatosis, regardless of whether or not they show disease symptoms.<sup>57</sup> Genetic and biochemical screening of the general population for *HFE* mutations has been discussed but is currently not recommended because of its high costs and the low penetrance of the disease.<sup>20,58</sup>

Depending on the degree of iron accumulation, cytotoxic hydroxyl and lipid radicals will be produced causing various organ pathologies, including those of the liver, heart, and pancreas, and ultimately lead to organ failure in affected individuals who remain untreated.<sup>2</sup> The symptoms of hemochromatosis range from chronic fatigue, hyperpigmentation, joint and bone symptoms to diabetes, and liver

diseases, such as fibrosis, cirrhosis, and hepatocellular carcinoma<sup>20</sup> (Table 1 and Figure 2). The correlation between increased ferritin levels and transferrin saturation with organ damage is poor,<sup>59,60</sup> highlighting the need for better markers for iron-induced organ damage. Despite this, symptoms develop especially in men with homozygous p.Cys282Tyr mutations and serum ferritin concentrations greater than 1000 µg/L.<sup>61</sup> Liver biopsies may thus be performed to test for liver damage in HH when serum ferritin levels exceed 1000 µg/L, while diverse arthropathies may also be observed in C282Y mutation carriers without severe iron overload.<sup>60</sup> Patients diagnosed with clinical HH face a higher risk of developing liver cancer (when cirrhosis is present at diagnosis) compared to controls.<sup>62</sup> Although highly debated, several studies have suggested a direct association of *HFE* mutations with the progression of various cancers including colon, breast, prostate, and epithelial ovarian cancer.<sup>63-66</sup> Patients with iron overload are also more susceptible to some infections, such as those caused by *Listeria monocytogenes*, *Vibrio vulnificus*, or HIV.<sup>67,68</sup> Consistently, studies in mice with iron overload resulting from a hepcidin deficiency showed decreased survival of animals infected by *Vibrio vulnificus*<sup>69</sup> or the malaria-causing *Plasmodium berghei*.<sup>70</sup> In addition, Hfe-deficiency in mice prevents a proper response to infection in that recruitment of pulmonary neutrophils upon an inflammatory stimulus is reduced.<sup>71</sup>

Until today, traditional phlebotomy (venesection) has remained the standard treatment for hemochromatosis. It was introduced in 1950 and has been applied ever since to improve the survival of HH patients.<sup>72</sup> Phlebotomy removes large amounts of iron localized in red blood cells, which stimulates erythropoiesis and mobilizes iron stored in peripheral tissues. It is a relatively simple and inexpensive treatment but has limited effects on specific HH-associated pathologies, including musculoskeletal symptoms.<sup>73</sup> Whether unaffected mutation carriers should undergo phlebotomy has been highly debated, especially because evidence for a beneficial effect of phlebotomy in these individuals has not been documented.<sup>20</sup> More recent insights into the molecular mechanisms maintaining iron homeostasis led to the development of alternative therapies, including the use of iron chelators, (eg. the most recent available oral chelator deferasirox, which effectively removes excess iron in HFE-HH patients<sup>74</sup>), and mini-hepcidin,<sup>75</sup> which might be applicable for patients with HH in the future.<sup>76</sup>

### Prevalence and penetrance of HFE-HH

The *HFE* mutation C282Y occurs in approximately 6% of Caucasians<sup>77</sup> and thus represents the most common genetic variant among this population. The frequency of the C282Y allele decreases from Northern to Southern Europe suggesting that it initially occurred as a 'master' mutation in the Neolithic Age in the Celtic population and then spread throughout the rest of Europe later on.<sup>78</sup> Immigration from Europe to the US and Australia led to the widespread distribution of HH among Caucasian adults living in those geographic areas.<sup>79</sup>

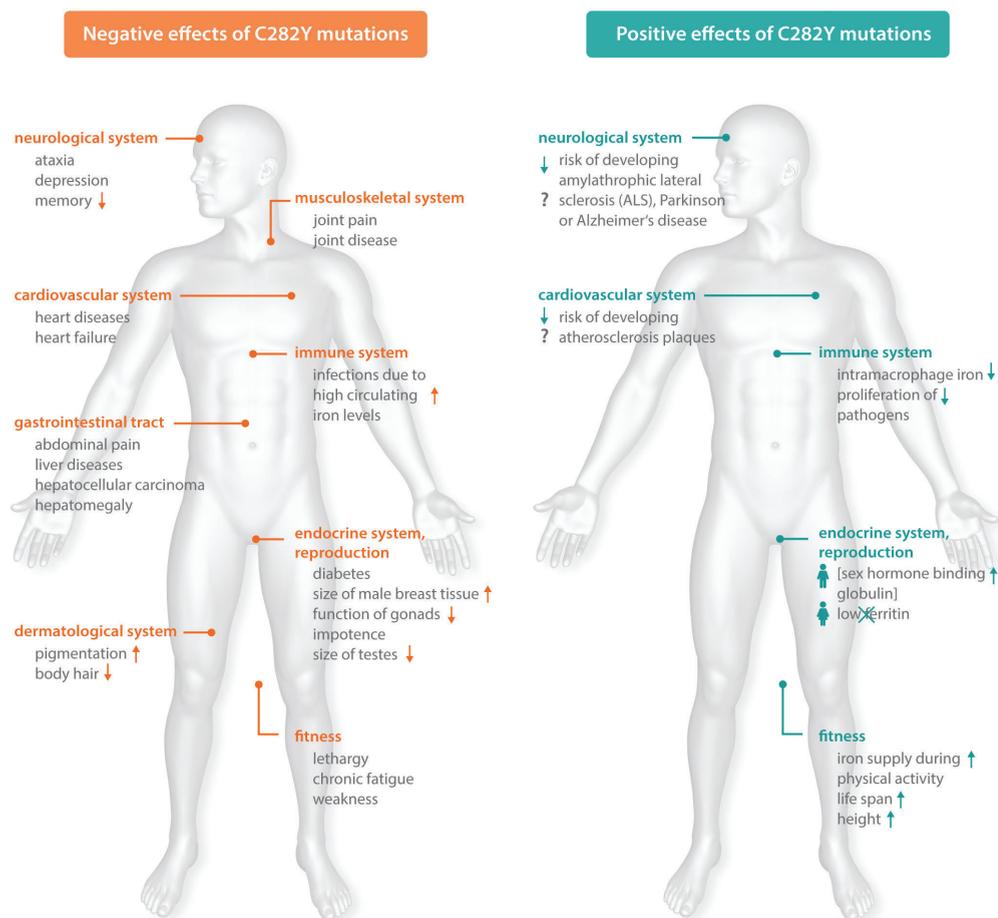
Despite the high prevalence of the C282Y allele, numerous population screening studies provided evidence that the mutation clinically manifests in hemochromatosis in only a subgroup of carriers (Table 2). Differences in study design, inclusion criteria, and the definition of disease penetrance make it difficult to confirm an exact number for

**Table 1. Symptoms of hereditary hemochromatosis.**

Neurological	ataxia (lack of voluntary co-ordination of muscle movements) depression impaired memory lethargy, chronic fatigue weakness
Gastrointestinal	abdominal pain cirrhosis hepatocellular carcinoma hepatomegaly (enlarged liver)
Musculoskeletal	arthralgia (joint pain) arthritis chondrocalcinosis
Dermatological	hyperpigmentation loss of body hair
Endocrinal	diabetes gynecomastia (non-cancerous increase in the size of male breast tissue) hypogonadism (diminished functions of gonads) impotence testicular atrophy (diminished size of testes)
Cardiovascular	cardiomyopathy ("heart muscle disease") heart failure adrenal insufficiency

the overall penetrance of the C282Y mutation. A meta-analysis taking into account the data from 16 independent studies reported a penetrance of C282Y homozygosity of 14%.<sup>24</sup> It was shown that homozygous men face a much higher risk of developing an iron overload phenotype than women,<sup>61</sup> which may be explained by the recurrent physiological blood loss in women or by the frequency disparity of certain *HLA* haplotypes that occurs between male and female patients.<sup>80</sup> The penetrance of the heterozygous C282Y mutation is even lower, with only approximately 3% of mutation carriers showing disease symptoms.<sup>3</sup> Despite the fact that *HFE* mutations are not always pathogenic *per se*, but rather represent polymorphisms that predispose to iron overload, murine disease models with Hfe-deficiency<sup>81</sup> or engineered mutations corresponding to the human C282Y mutation show an iron overload phenotype. Like in humans, the genetic background of the mouse strain strongly affects the severity of tissue iron accumulation.<sup>82</sup> The disease manifestation of other *HFE* mutations, such as H63D in combination with C282Y, is negligible<sup>41</sup> (Table 2). In rare cases, H63D mutations can lead to phenotypic expression of HH when additional risk factors such as heavy alcohol consumption or hepatitis virus infections are present.<sup>83</sup>

The discrepancy between the frequency of *HFE* mutations and their phenotypic expression indicates that additional environmental or genetic factors contribute to the manifestation of HH in affected individuals. Despite significant efforts to determine these parameters, only a few risk factors have been identified, including alcohol abuse, being heavily overweight, liver disease, or viral infections.<sup>57</sup> The consumption of more than 60 g of alcohol per



**Figure 2. The positive and negative effects of *HFE* mutations.** Hereditary hemochromatosis (HH)-associated mutations affect the health of their carriers in various ways. Besides provoking classical HH disease symptoms (left), these mutations can provide benefits for affected individuals (right). Some of the benefits associated with HH have remained speculative to date and need further experimental validation (marked with a question mark).

day leads to higher serum iron and ferritin concentrations, contributing to the progression of cirrhosis in C282Y homozygotes.<sup>84</sup> Certain dietary habits, such as heme iron intake and meat consumption, are also associated with increased iron loading.<sup>85</sup> In addition, recent genome-wide association studies have identified SNPs in so-called 'modifier' genes that are associated with iron-related phenotypes. These include polymorphisms in genes with known roles in iron metabolism, such as those encoding transferrin receptor 1 (*TFR1*) and ferroportin (*FPN/SCL40A1*),<sup>86</sup> but also in novel genomic loci, eg. in transmembrane protease, serine 6 (*TM6SP6*)<sup>87</sup> and in the vicinity of the genes *FADS2*, *NAT2*, *ABO* and *TEX14*.<sup>86</sup> SNPs in transferrin (*TF*),<sup>86,88</sup> transferrin receptor 2 (*TFR2*),<sup>86</sup> bone morphogenetic protein 2 (*BMP2*),<sup>89</sup> aryl hydrocarbon receptor nuclear translocator-like protein 1 (*ARNTL*),<sup>20</sup> glyceronephosphate O-acyltransferase (*GNPAT*),<sup>90</sup> and cytochrome B reductase 1 (*CYBRD1*)<sup>91</sup> were further found to influence iron-related parameters in C282Y homozygotes. In addition, a large cohort study reported an association between common variants in the genes coding for bone morphogenetic protein 4 (*BMP4*) and hemojuvelin (*HJV*) and increased serum ferritin levels in homozygous C282Y mutation carriers.<sup>89</sup> Moreover, a polymorphism in the *PCSK7* (proprotein convertase subtilisin/kexin type 7) gene has been linked with liver cirrhosis and advanced fibrosis in C282Y homozygotes.<sup>92</sup> These variants are extremely rare and may thus determine disease development on an individual basis. Although carriers of heterozygous *HFE* mutations mostly

lack a hemochromatosis phenotype, enhanced iron loading and manifestation of HH may occur in persons with heterozygous *HFE* mutations and additional mutations in the hepcidin (*HAMP*)<sup>93,94</sup> *HJV*,<sup>93,95</sup> or *TFR2*<sup>96</sup> genes. Additional HH modifiers, such as haptoglobin (*HP*)<sup>97</sup> and ceruloplasmin (*CP*) have been identified in mice. *Hfe* knock-out mice (*Hfe*<sup>-/-</sup>) with a heterozygous R435X nonsense mutation in the *CP* gene and *Hfe*(+/-) mice with a homozygous R435X nonsense mutation showed lower liver iron levels compared to single mutant animals, revealing a protective effect of this specific ceruloplasmin variant in mice.<sup>98</sup> In line with this, ceruloplasmin levels were found to be lower in HH patients when compared to control subjects.<sup>99</sup>

#### Do *HFE* mutations offer any advantage to asymptomatic patients?

The observation that C282Y *HFE* mutations are frequent but only cause a disease-related phenotype in a subgroup of carriers led to the hypothesis that this *HFE* gene variant may be of an environmental or genetic advantage to asymptomatic carriers and that this is the reason for which it has been inherited with such a high frequency. Increased iron uptake, a hallmark of HH, may have helped humankind, and especially women of reproductive age, to better cope with the iron-reduced cereal grain-based diet which replaced the paleo diet rich in red meat in Europe in the Neolithic Age, at the time when the first *HFE* mutation occurred.<sup>100</sup> Over centuries, the mutation may have

Table 2. Prevalence and penetrance of *HFE* (hemochromatosis) gene mutations.

	C282Y/+	C282Y/ C282Y	H63D/+	H63D/ H63D	S65C/+	S65C/ S65C	H63D/ C282Y	S65C/ C282Y
Prevalence	10% <sup>23</sup>	0.4% <sup>24</sup>	~21% <sup>23</sup>	~1% <sup>23</sup>	~3% <sup>29,42</sup>	NA	~1% <sup>23</sup>	~0.3% <sup>29,42</sup>
Penetrance	3% <sup>3</sup>	controversial: 14% (in meta analysis) <sup>24</sup>	n.s. <sup>41</sup>	n.s. <sup>41</sup>	n.s. <sup>42</sup>	NA	n.s. <sup>44</sup>	~0.6% <sup>42</sup>

NA: not available; n.s.: not significant.

spread throughout the Caucasian population because it actively promoted the health of its carriers. Recent studies have provided evidence that the *HFE* C282Y variant as well as the H63D and S65C variants can positively influence the immune system, the general fitness and reproductive status of mutation carriers, and might even diminish the risk of developing diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease, and atherosclerosis (Figure 2).

Micro-organisms heavily depend on the availability of iron for their proliferation. Iron levels in macrophages of carriers of the C282Y allele are reduced, which makes them less susceptible to those bacteria that depend on macrophage iron, such as *Mycobacterium tuberculosis*,<sup>101</sup> *Leishmania amazonensis*,<sup>102</sup> and *Chlamydia* or *Legionella* species.<sup>103</sup> In line with this, proliferation of *Salmonella Typhimurium* in macrophages of mice with hetero- and homozygotic *Hfe*-deficiencies is strongly attenuated.<sup>104</sup> In addition, in a mouse model with an *HFE* H63D mutation, increased survival over wild-type mice was observed following an infection with *Plasmodium falciparum*, a parasite that causes cerebral malaria.<sup>105</sup> Besides being critical for micro-organismal growth, intramacrophage iron levels influence the inflammatory state of macrophages. While reduced iron levels attenuate the inflammatory response of macrophages to *Salmonella* infection or LPS-stimulation,<sup>106</sup> increased iron levels trigger a phenotypic switch of macrophages towards a pro-inflammatory state.<sup>107</sup> *HFE* mutations may thus interfere with the inflammatory response of macrophages by reducing iron levels in macrophages in a hepcidin-dependent manner and protect carriers from infectious diseases. This may provide an explanation of why *HFE* mutations were inherited with such a high frequency.

In addition to influencing the host immune system, mutations in the *HFE* gene have also been linked to the increased fitness of affected individuals. Intravenous and oral iron supplementation can treat fatigue in non-anemic women with low iron stores,<sup>108,109</sup> showing that elevated iron levels positively influence fitness. A recent study demonstrated that 80% of successful French athletes carry a heterozygous *HFE* mutation (C282Y, H63D or S65C) suggesting that the resulting enhanced iron supply during physical activity accounts for the superior physical performance of these sportsmen.<sup>110</sup> Large-scale studies among the Sicilian population further showed that *HFE* C282Y heterozygous individuals, particularly women, have a significantly increased life expectancy compared to controls.<sup>111,112</sup> A trend towards an extended life span has also been observed in Sardinian women carrying the H63D mutation.<sup>113</sup> Treated C282Y homozygotes with serum ferritin concentrations less than 1000 µg/L further exhibit a lower mortality related to cardiovascular events and extra-

hepatic cancers when compared to the general population.<sup>114</sup> Interestingly, people diagnosed with *HFE*-HH and verified iron overload are also taller on average, potentially because augmented iron absorption has a beneficial effect on growth.<sup>115</sup>

Unlike the carriers of homozygous *HFE* C282Y mutations, who may suffer from hypogonadism, heterozygous individuals may also have a reproductive advantage. This is supported by a study that reported higher levels of the sex hormone-binding globulin in the blood of C282Y heterozygous men.<sup>116</sup> Women of reproductive age carrying a heterozygous C282Y mutation were found to suffer less commonly from low serum ferritin concentrations compared to control subjects, which might positively affect their fertility.<sup>117</sup>

*HFE* C282Y mutations have been associated with the attenuation of various disease states. For example, a lower incidence rate of atherosclerosis in *HFE* C282Y carriers has been reported. Possible explanations include lower serum cholesterol and low-density lipoprotein cholesterol levels,<sup>118,119</sup> or iron deficiencies of macrophages that may contribute to a diminished inflammatory response<sup>106</sup> and a decreased tendency to form atherosclerotic plaques.<sup>120</sup> However, other studies failed to establish a link between hemochromatosis and atherosclerosis.<sup>121-124</sup>

Screening studies and corresponding meta-analyses further associated mutations in the *HFE* gene with a decreased risk of developing neurodegenerative diseases. So far, experimental validations for these findings are lacking and they are still considered highly controversial. Screening among patients with AD detected an association with the C282Y allele,<sup>125</sup> a result in line with data from a meta-analysis that revealed a correlation between AD and the C282Y but not the H63D polymorphism.<sup>126</sup> Similarly, two different meta-analyses reported an association of the C282Y mutation and sporadic ALS<sup>127</sup> or Parkinson's disease,<sup>128</sup> both excluding a role for the H63D polymorphism in disease manifestation.

Taken together, several studies suggest that *HFE* mutation carriers might benefit from mutation-associated increased iron levels as long as the iron overload phenotype is mild. This demonstrates that we clearly need to distinguish between hemochromatosis 'patients' who suffer from the pathophysiological complications associated with iron overload and individuals who carry HH-associated mutations without showing any disease symptoms. In fact, it is possible that the beneficial effects of the mutations observed in heterozygotes might also manifest in homozygote individuals during growth and early adulthood before significant organ iron overload develops. Future clinical and biochemical studies may reveal more detailed information about the impact of *HFE* mutations on the health of their carriers and may provide an

answer to the long-standing question as to why HFE mutations have so far been inherited with such a high frequency.

### Acknowledgments

The authors would like to thank Prof. Peter Nielsen, Prof.

Pierre Brissot and Prof. Graça Porto for their critical reading of the manuscript. MUM acknowledges funding from the Deutsche Forschungsgemeinschaft (SFB1118 and SFB1036) and the Dietmar Hopp Stiftung. We would like to apologize to all colleagues whose work could not be cited because of space constraints.

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