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HFE Gene Variants Affect Iron in the Brain[1–3]

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Abstract

Iron accumulation in the brain and increased oxidative stress are consistent observations in many neurodegenerative diseases. Thus, we have begun examination into gene mutations or allelic variants that could be associated with loss of iron homeostasis. One of the mechanisms leading to iron overload is a mutation in the HFE gene, which is involved in iron metabolism. The 2 most common HFE gene variants are C282Y (1.9%) and H63D (8.9%). The C282Y HFE variant is more commonly associated with hereditary hemochromatosis, which is an autosomal recessive disorder, characterized by iron overload in a number of systemic organs. The H63D HFE variant appears less frequently associated with hemochromatosis, but its role in the neurodegenerative diseases has received more attention. At the cellular level, the HFE mutant protein resulting from the H63D HFE gene variant is associated with iron dyshomeostasis, increased oxidative stress, glutamate release, tau phosphorylation, and alteration in inflammatory response, each of which is under investigation as a contributing factor to neurodegenerative diseases. Therefore, the HFE gene variants are proposed to be genetic modifiers or a risk factor for neurodegenerative diseases by establishing an enabling milieu for pathogenic agents. This review will discuss the current knowledge of the association of the HFE gene variants with neurodegenerative diseases: amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and ischemic stroke. Importantly, the data herein also begin to dispel the long-held view that the brain is protected from iron accumulation associated with the HFE mutations. J. Nutr. 141: 729S–739S, 2011.

Introduction

Iron plays a significant role in many biological functions essential for life. The citric acid cycle and electron transport chain of mitochondria contain several iron-dependent enzymes and complexes such as cytochromes, succinate dehydrogenase, NADH-dehydrogenase, and aconitase. The activity of ribonucleotide reductase, which catalyzes the essential step of DNA synthesis, is dependent on iron (1). Iron is also an indispensable component for neurotransmitter synthesis and myelinogenesis (1,2). Iron deficiency affects the composition and the amount of myelin in white matter by altering the proliferation and differentiation of oligodendrocytes. In addition to brain morphology, iron deficiency also causes an alteration in dopamine and norepinephrine metabolism, which affects neurochemistry and may delay central nervous system (CNS)[4] development (3).

Though iron is an essential cofactor for many proteins in the CNS, free or unbound iron can serve as a pro-oxidant. Ferrous iron (Fe^{2+}) catalyzes the conversion of reactive oxygen species (ROS) to highly reactive hydroxyl radical (·OH) via Fenton reaction, while ferric iron (Fe^{3+}) can react with superoxide ($O_2^{\cdot-}$) and generates Fe^{2+}, leading to ·OH formation via the Heber-Weiss reaction. Excess iron can cause protein peroxidation, lipid peroxidation, and DNA oxidation, which eventually can lead to cellular and neuronal damage or death (4,5). Therefore, iron content in the body and in the CNS is strictly regulated via expression of several proteins (6,7).

One iron regulatory protein that is receiving increased attention in neuroscience is the HFE protein. Mutations in the HFE gene are commonly associated with the iron overload genetic disorder hereditary hemochromatosis (HH) (8,9). Be-

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[4] Abbreviations used: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; FAD, familial Alzheimer's disease; HH, hereditary hemochromatosis; MCI, mild cognitive impairment; NFT, neurofibrillary tangle; PD, Parkinson's disease; ROS, reactive oxygen species.

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cause of their association with iron accumulation, the HFE gene mutations are being investigated as genetic risks for neurodegenerative disorders (4,7,9–11). In this review, we will discuss iron physiology in the brain in relation to HFE structure and function and describe the relationship between HFE gene variants and 4 neurodegenerative disorders: amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and ischemic stroke.

Iron in the brain

The iron concentration in the brain is second to the liver among the organs in the body (12). Iron is distributed heterogeneously throughout the brain, with the highest concentration found in the globus pallidus, followed by the red nucleus, substantia nigra, putamen, and the dentate nucleus of the cerebellum (12,13). In the cortical fields, the highest iron concentration was found in the motor cortex, followed by the occipital cortex, sensory cortex, and parietal cortex (12). Brain iron content increases with advancing age (2,12,14) and iron content rapidly increases in all brain regions except medulla oblongata during the first 2 decades of life (12). It is noteworthy that functionally, the regions of the brain that have the highest iron content are all involved in motor functions. In addition to its high iron content, the brain generates a large amount of ROS as a consequence of its high oxygen consumption (20% of the body's total resting oxygen consumption) to meet its high metabolic rate (1,4). Moreover, it is rich in lipids with unsaturated fatty acid and has only a moderate amount of antioxidant enzymes (5). Therefore, the brain is more vulnerable to iron- and ROS-induced toxicity.

Because both iron overload and iron deficiency cause neuronal dysfunction, the brain expresses several iron management proteins, which are involved in the uptake, export, storage, and utilization of iron (11), to regulate its iron content. The proteins involved in the brain iron homeostasis system include HFE (9), ferritin, transferrin (Tf), transferrin receptor (TfR) (15), iron regulatory protein, divalent metal transporter 1, and cerruloplasmin (4,7,10,16,17). The expression of these iron management proteins can be altered in accordance with iron availability in an attempt to maintain the iron content in the brain. For example, iron deficiency results in decreased expression of iron storage protein, ferritin, and increased expression of iron transport protein, TfR (18). The end result would be to reduce iron storage and at the same time increase iron uptake during iron deficiency. When iron is in excess, ferritin expression would be increased, while TfR expression would be decreased, resulting in limiting iron uptake and increasing iron storage (6,10).

Traditionally, the brain was thought to be protected from iron overload by the blood brain barrier (BBB), which separates and restricts the exchange of iron and iron management proteins between the brain tissue and the blood. Thus, a paradigm became established that the brain was protected from iron-related genetic disorders such as HH. This traditional notion came from the histochemical stains for iron in late 1930s and 1940s (19,20). However, these studies did report that besides circumventricular regions, where the BBB is absent, the deposition of iron was observed in other brain regions, including the cerebral cortex, hypothalamus, lentiform nucleus, and the dentate nucleus that are behind the BBB (19,20). More recently, MRI studies have reported an iron accumulation in the basal ganglia of patients with HH (21–23) as well as in the substantia nigra, the red nucleus (21), and the dentate nucleus (21,23). However, the notion that the brain is protected from iron overload in HH somehow became established in neuroscience despite evidence to the contrary. Iron overload observed in HH is primarily caused by mutations in the HFE gene (8). The HFE protein resides on the brain microvasculature and choroid plexus where it can affect iron uptake by the brain (24); therefore, it should not be surprising that HFE mutations are associated with increased brain iron and have recently been proposed to be the genetic modifiers for risk of developing neurodegenerative disorders (7,9,25).

The HFE gene

Simon et al. (26) first demonstrated in the late 1970s that the gene responsible for HH is closely linked to the human leukocyte antigen (HLA) locus on a short arm of chromosome 6. Twenty years later, this gene was identified and termed as HLA-H gene by Feder et al. (27). The HLA-H gene, now renamed as the HFE gene, is comprised of 7 exons and is expressed widely or at low level in most tissues, including brain.

Two common polymorphisms in the HFE gene associated with HH

A G-to-A transition at nucleotide 845 changes cysteine to tyrosine at amino acid 282 (C282Y) and a C-to-G transition at nucleotide 187 results in a histidine to aspartic acid substitution at amino acid 63, i.e., H63D (27). Eighty-five to 90% of HH patients are homozygous for the C282Y variant and 5% of patients are compound heterozygous for the C282Y and H63D variants (27–29). HH is an autosomal recessive disorder characterized by an excessive absorption of dietary iron leading to abnormal iron accumulation, with secondary tissue damage in various organs such as liver, pancreas, and heart. The clinical consequences of HH include cirrhosis, hepathomegaly, diabetes mellitus, and cardiomyopathy (30,31). HH is the most common inherited disorder in individuals of Northern European descent (1 in every 200–400 individuals), with even higher prevalence in Ireland (1:100) (28). Although the C282Y HFE variant is more commonly associated with HH, the H63D HFE variant (8.1%) is more frequently present in the general population than the C282Y variant (1.9%). Similar to the distribution of HH, the C282Y HFE variant is more abundantly present in those of Northern European descent. The H63D HFE variant has a more general and broader distribution with a higher frequency in Europe (14.9%) and moderate frequency in Asia, Africa, Middle East, and America (28,32). The product of the HFE gene is a 343 residue type 1 transmembrane glycoprotein named HFE (27,33,34).

The structure of HFE protein

The HFE protein is a member of the major histocompatibility complex class-1 (MHC-1)-like family. Like other MHC class-I like molecules, a single polypeptide HFE protein contains a transmembrane region, short cytoplasmic tail, and 3 extracellular domains (α1, α2, and α3) (27,34). Two peptide-binding domains (α1 and α2) consist of 8 antiparallel β strands and 2 antiparallel α helices, and are positioned on the top of an immunoglobulin-like domain (α3) (34). The HFE protein contains 4 cysteine residues forming a disulphide bridge in α2 and α3 domains (27), which is 1 of the important conserved features of the MHC class-I family required for noncovalent interacting with β_2 microglobulin (35) and for a transport from the endoplasmic reticulum to the cell surface (36,37). However, the peptide binding groove in the HFE protein is narrower by the translation of α1 helix toward α2 helix and the HFE protein has only 2 of 4 tyrosine residues in the peptide-binding region, which are important for peptide binding. Therefore, unlike other MHC-1 proteins, the HFE protein does not function as an antigen-presenting molecule (27,33).

Function of HFE protein

The major function of the HFE protein is to regulate iron homeostasis. The HFE protein interacts with β_2 microglobulin in the endoplasmic reticulum and is transported to the plasma membrane (37,38) where the HFE protein forms a stable complex with the TfR (39). The binding of the HFE protein to the TfR is pH dependent, with a tight interaction at pH 7.5 but very weak or no binding at acidic pH (33,40). The HFE-TfR interaction lowers the affinity of the TfR for holotransferrin, i.e. Fe-Tf (39). Lebrón et al. (41) later reported that HFE bound to TfR at or near the Fe-Tf binding site where it could competitively inhibit Tf binding to the TfR. In the absence of the HFE, TfR homodimers bind 2 Fe-Tf molecules, while HFE-bound TfR binds only 1 Fe-Tf molecule by forming a ternary complex consisting of 1 HFE, 2 TfR polypeptide chains, and 1 Fe-Tf (33,41). Therefore, the HFE protein functions in the regulation of iron homeostasis by binding to the TfR and reducing the transport of Fe-Tf molecules.

The cysteine residue in α3 domain that is altered in the C282Y HFE variant is one of the conserved residues important for forming disulphide linkages and interacting with β_2-microglobulin for cell surface expression (27,37,38). Therefore, the C282Y HFE variant is located primarily intercellular (37,38) and does not bind to the TfR to limit transferrin-mediated iron uptake (39). The functional consequence of the H63D HFE variant was first reported by Feder et al. (39). Similar to the wild-type HFE protein, the H63D HFE interacts with β_2-microglobulin and is transported to the plasma membrane (37,38); however, the interaction of the H63D HFE with the TfR does not limit transferrin-mediated iron uptake (39). Because the H63D mutation is present in the α1 domain of the peptide-binding region (27,41), the functional consequence of this variant is reduction of the affinity of the H63D protein for TfR. Thus, both C282Y and H63D HFE are associated with an increased iron accumulation compared with expression of the wild-type HFE.

In addition to its important role in iron homeostasis, the HFE protein affects a range of cellular functions, including innate immunity (42,43). Lee et al. (44) developed stable human neuroblastoma cell lines (SH-SY5Y) carrying the wild-type, C282Y, or H63D HFE and demonstrated that HFE mutations were associated with iron accumulation and increased oxidative stress. Neuroblastoma cells carrying HFE mutations, in particular the H63D HFE, also increase intracellular calcium levels, have greater glutamate secretion and reduced uptake (45), and increase production of monocyte chemoattractant protein-1, i.e. MCP-1 (46). Each of the above mechanisms, including oxidative stress and iron accumulation, has been considered as an underlying mechanism contributing to the pathogenesis of neurodegenerative disorders (2,4,5,10,47).

HFE and neurodegenerative disorders

In order for the HFE protein to affect brain iron accumulation, it should be found in the brain. Indeed the HFE protein is expressed in choroid plexus epithelial cells, endothelial cells of the microvasculature, and ependymal cells lining the ventricle in the brain along with TfR, where it can influence iron uptake into the brain (24,48). Nevertheless, the relationship between HFE mutations and CNS diseases has not received much attention until recently. Because brain iron concentration increases with age (2,12) and HFE gene mutations are associated with excess iron accumulation (49–51) in different organs, it is logical that individuals who carry a HFE variant are at higher risk for brain iron accumulation and the accompanying neurological sequelae (31). A recent study of Bartozokis et al. (52) demonstrated that the presence of the H63D HFE gene variant and/or C2 allele of transferrin gene was associated with increased brain ferritin iron in older men compared with noncarriers. Given the presence and location of the HFE protein at the interface between the brain and the vasculature and the cerebrospinal fluid (CSF) where it can influence brain iron uptake (24,48), it is not a surprise that the mutant forms of HFE could contribute to iron overload in neurodegenerative disorders (4,10,17,53–56). Thus, it was a logical quest to examine HFE genotypes and how they influence the course of neurodegenerative disorders.

HFE and ALS. The H63D HFE was present in as many as 30% of ALS patients (57), which was higher than that reported for the superoxide dismutase (SOD1) mutation (58) and represented the second most frequent genetic variation found in ALS (57). Four other groups in the UK (59), Italy (60), The Netherlands (61), and most recently China (62) have also reported an increased incidence of the H63D HFE in patients with ALS. There is 1 study that did not find the association between the H63D HFE and ALS (63). This study was limited by a low number of ALS patients and ~10% of those ALS patients represented ethnic backgrounds in which HFE mutations are absent or present at lower frequency than the control population (32). A meta-analysis including 66,000 cases revealed that homozygosity for the H63D HFE was associated with a 4-fold risk of developing ALS (25). In contrast to the H63D HFE, the C282Y HFE has not been identified as being associated with ALS (25,57,59,61,63) (**Table 1**).

Iron misregulation caused by mutations in the HFE gene is likely to contribute to the relationship between the H63D HFE and ALS. A number of studies have reported increased iron levels in the CNS of ALS patients (53,54,64,65). In the ALS animal model, Jeong et al. (66) recently reported that iron chelation treatment extended survival by 5 wk, suggesting iron contributed to the disease process. Sporadic ALS patients have elevated serum ferritin (67), which was even more increased in ALS patients with the H63D gene variant (68). Current hypotheses for the underlying mechanisms contributing to motor neuron degeneration in ALS include glutamate excitotoxicity, oxidative stress, dysregulation of iron homeostasis, mitochondria dysfunction, cytoskeletal abnormalities, and protein aggregation (69,70). Cell culture models developed to identify the relationship of HFE gene variants to pathogenic factors in ALS have also identified oxidative stress (44), glutamate release and uptake (45), increased MCP-1 secretion suggesting an alteration in innate immunity (46), and increased tau phosphorylation (71) in H63D carrying cells compared with the wild-type. It is probable that these mechanisms converge to mediate motor neuron toxicity in ALS and we have proposed that the H63D HFE gene variant creates a permissive milieu for ALS pathogenic factors.

In addition to being a risk factor for ALS, the HFE gene variant could also affect treatment of ALS. For example, dysphagia is a common symptom in ALS patients, leading to increased risk of malnutrition, weight loss, and dehydration; therefore, patients will receive an enteral nutrition treatment (69,72). The iron content of enteral formulas ranges from 13 to 24 mg/L and most patients will receive at least 1 L/d, which is higher than the recommended daily iron intake (10 mg/d) (73). Because the HFE mutation is associated with iron overload, the long-term enteral nutrition treatment in ALS patients with HFE mutations may worsen iron accumulation. Thus, stratification by HFE genotypes should be considered before initiating the long-term enteral nutrition treatment in ALS patients.

TABLE 1 Association between HFE mutations and ALS

Participants, *n*	Location	ALS,[1] %	Control, %	Comments	References
51: ALS 47: Control	Texas	H63D: 25.5 C282Y: 3.9	H63D: 23.4 C282Y: 4.3	No association between HFE genotypes and the age at onset, rate of progression in sporadic ALS (Small samples and ethnically diverse cases and controls)[2]	Yen et al. (63)
121: ALS 133: Control	Pennsylvania	H63D: 29.75 C282Y: 0.83	H63D: 14.29 C282Y: 0	Higher frequency of H63D in sporadic ALS patients	Wang et al. (57)
379: ALS 400: Control	Birmingham and Ireland, UK	H63D: 34.3 C282Y: 18.2	H63D: 22 C282Y: 19	Overrepresentation of H63D in sporadic ALS	Goodall et al. (59)
149: ALS 168: Control	Torino, Italy	H63D: 28.8 C282Y: 3.3	H63D: 14.8 C282Y: 1.8	Increased incidence of H63D in sporadic ALS patients	Restagno et al. (60)
289: ALS 5886: Control	Utrecht, The Netherlands	H63D: 27.8 C282Y: 8.3	H63D: 26.7 C282Y: 11.5	Homozygosity for H63D predisposed to sporadic ALS and higher age at onset in H63D carriers	Sutedja et al. (61)
195: ALS 405: Control	China	H63D: 10.3	H63D: 3.2	Higher risk for ALS in H63D carriers	He et al. (62)

[1] % represents the percentage of HFE mutations among ALS patients and controls, respectively.
[2] Comments in parentheses represent study methods that may explain results.

Moreover, ALS patients with HFE mutations may respond differently to therapeutic interventions compared with those without HFE mutations. For example, minocycline, an antibiotic with iron chelation, antioxidant, antiinflammatory, and mitochondria-protective properties, had no benefit in ALS patients (74) despite its beneficial effect in rodent ALS models (75–78). Mitchell et al. (46) demonstrated that minocycline reduced MCP-1 release in H63D, but not in wild-type HFE, expressing neuroblastoma cell lines (SH-SY5Y). Therefore, patients' heterogeneity for HFE alleles may explain the lack of benefit of minocycline in ALS patients. Because the H63D HFE variant is present in ~30% of ALS patients (57), HFE genotypes should be considered when assessing treatment strategies or therapeutic interventions for ALS. We have recently found that a mouse line carrying the H67D HFE gene variant (the equivalent of the human H63D variant) when crossed with the SOD1 mouse model for ALS results in a shorter life span and shorter disease duration (W. Nandar, E. Neely, Z. Simmons, and J. Connor, unpublished data). This exciting observation extends the cell culture observations to an in vivo model.

HFE and AD. Excessive iron accumulation in neuritic plaques and neurofibrillary tangles (NFT) and oxidative stress (9,10, 24,79–82) are consistent observations in the pathogenesis of AD. Pathological features associated with AD such as senile plaque formed by amyloid β peptide, NFT, and hyperphosphorylation of tau protein can be influenced by iron. For example, iron modulates the ability of α-secretase to cleave amyloid precursor protein (APP) (83), promotes A-β toxicity (84) and aggregation, and can directly regulate the synthesis and expression of APP via the iron responsive element at 5′ untranslated region of APP mRNA (82,85,86).

Given the involvement of the HFE protein in iron regulation and oxidative stress, mutations in the HFE gene can be expected to affect AD pathogenesis. This statement is supported by studies demonstrating that the HFE protein is expressed in glial and neuronal cells associated with neuritic plaques and NFT (9,24). In addition, HFE can be induced by stress factors such as serum deprivation and β-amyloid in mouse microglia BV-2 cells. Induction of HFE decreases the labile iron pool, which may be a protective response to limit iron uptake during cellular stress (87). The mutant forms of HFE protein, however, may not effectively limit iron uptake, which would result in intracellular iron overload and increased cell vulnerability to oxidative stress.

Recent studies by Hall et al. (88) suggest an association between the H63D HFE with tau phosphorylation and Prolyl-peptidyl isomerase (Pin1), an enzyme responsible for phosphorylation of APP and tau. The expression of the H63D HFE upregulated the tau phosphorylation in neuroblastoma cell lines by decreasing Pin1 activity (88) and increasing glycogen synthase kinase-3β activity. The H63D effect appears to be associated with iron and subsequent oxidative stress, because iron exposure increased tau phosphorylation while antioxidant Trolox, a vitamin E analogue, treatment decreased tau phosphorylation (71). Iron chelation with desferroxamine and Trolox exposure decreased Pin1 phosphorylation. Consistent with the cell model study, a H67D knockin mouse line (mouse homologous to H63D in human population) also exhibited increased Pin1 phosphorylation (88). All of the above data suggest that the basic biochemistry of cells is altered in the presence of the H63D variant and provide a compelling proposition that HFE gene variants are modifying risk factors for AD.

During the past 10 y, multiple studies have addressed the association of the HFE gene mutations with AD (**Tables 2**, **3**). Moalem et al. (89) were the first to report that the HFE mutations were overrepresented in males with familial AD (FAD) and among noncarriers of the ApoEε4 allele, a well-known genetic risk factor for AD. The HFE mutations predisposed males to FAD but were somewhat protective in females. A study conducted by Percy et al. (90) found that in a folate-supplemented Ontario population, the presence of both ApoEε4 and H63D HFE predisposed females, but not males, to AD. Robson et al. (91) showed that bi-carriers of C282Y HFE and C2 allele of transferrin, both involved in iron metabolism, had a 5 times greater risk of AD. Pulliam et al. (92) included individuals with mild cognitive impairment (MCI) and nondemented controls with AD-like pathology (HPC) in addition to AD patients in their study and reported that the proportion of homozygous or compound heterozygous for HFE mutations was higher in AD/MCI and HPC patients. They extended their study by evaluating the ventricular (CSF) fluid F2-isoprostane level, which hallmarks brain lipid peroxidation, and found an association of HFE mutations with increased oxidative stress in AD patients (92).

Sampietro et al. (93) examined the effect of HFE mutations on age at onset in patients with sporadic AD and found that patients who were either heterozygous or homozygous for the H63D HFE developed AD on average 5 y earlier than those with

TABLE 2 Studies showing an association between HFE mutations and AD

Participants, *n*	Location	AD,[2] %	Control, %	Comments	References
26: FAD	Toronto	H63D: 26.9	H63D 26.8	Higher frequency of HFE mutations in males with FAD	Moalem et al. (89)
41: Control	Ontario	C282Y: 15.4	C282Y: 9.8		
107: SAD[1]	Milan, Italy	H63D: 20.6	H63D: 25.3	H63D mutation was more frequent in SAD	Sampietro et al. (93)
99: Control		C282Y: 3.7	C282Y: 4	patients with an earlier disease onset	
133: AD	Kentucky	HFE variants (homozygous or compound	HFE variants (homozygous or compound	More homozygous or compound heterozygous HFE	Pulliam et al. (92)
5: MCI		heterozygous for H63D, C282Y, and S65C): 47.1	heterozygous for H63D, C282Y, and S65C): 55.2	mutations in AD/MCI	
67: Control				Increased oxidative stress in AD patients with HFE mutations	
328: SAD	Northern Spain	H63D: 48.2		Lower age at AD onset in H63D/ApoE ε4	Combarros et al. (94)
191: AD	Oxford	H63D: 27.8	H63D: 27.9	Bi-carriers of C282Y and C2 allele of transferrin are	Robson et al. (91)
69: MCI		C282Y: 15.7	C282Y: 11.9	5 times greater risks of AD	
269: Control					
54: SAD	Toronto,	Male	Male	Presence of both H63D and E4 alleles predisposed	Percy et al. (90)
58: Control	Ontario	H63D: 27.3	H63D: 52	females to AD	
	27: SAD	C282Y: 9.1	C282Y: 4.0	H63D by itself appeared to protect males against AD.	
	58: Control	Female	Female	(Low frequency of H63D homozygotes in the	
		H63D: 37.5	H63D: 18.2	study partly due to small sample sizes)[3]	
		C282Y: 18.8	C282Y: 15.2		
	Kingston, Ontario	Male			
	27: SAD	H63D: 22.2			
		C282Y: 0			
		Female			
		H63D: 27.8			
		C282Y: 11.1			
113: AD	Porto, Portugal	H63D: 17.2	H63D: 20.3	Association of H63D with earlier AD onset	Correia et al. (95)
82: Control		C282Y: 1.3	C282Y: 5.8	Negative association of C282Y with AD	
211: AD	Basque Country, Spain	H63D: 18.0	H63D: 29.9	Higher frequency of H63D in AD patients	Blazquuez et al. (100)
167: Control		C282Y: 4.5	C282Y: 3.3		

[1] Abbreviations: SAD, sporadic AD.
[2] % represents the percentage of HFE mutations among AD patients and controls, respectively.
[3] Comments in parentheses represent study methods that may explain results.

TABLE 3 Studies showing no association between HFE mutations and AD

Participants, *n*	Location	AD,[1] %	Control, %	Comments	References
108: AD	Spain	H63D: 42.6	H63D: 34.5	Trend toward an increased frequencies of H63D variants in AD	Lleo et al. (96)
110: Control		C282Y: 3.7	C282Y: 3.6		
123: AD	Northern Italy	H63D: 23.6	H63D: 19.5	HFE variants did not influence age at onset or the risk of AD	Candore et al. (98)
152: Control		C282Y: 1.6	C282Y: 0.7		
213: AD	Montreal, Canada	H63D: 33	H63D: 34	Trend of an accelerated rate of MCI-to-AD conversion in	Berlin et al. (97)
106: MCI		C282Y: 5	C282Y: 10	H63D homozygotes	
63: Control				No effect on age at onset or diagnosis or onset of cognitive symptoms	
130: AD/MCI	Coimbra, Portugal	H63D: 34.6	H63D: 35.6	HFE genotypes did not contribute to age at onset or	Guerreiro et al. (99)
115: Control		C282Y: 4.6	C282Y: 4.3	the risk of AD. (Low "n" or genetic background)[2]	
105: AD	Colombia	H63D: 32.4	H63D: 29.6	H63D did not contribute to the risk or age at onset of FAD	Avila-Gomez et al. (101)
220: Control		C282Y: 0	C282Y: 0.45		
268: AD	Rotterdam, The Netherlands	Male	Male	Age at onset tended to be earlier in males homozygous	Alizadeh et al. (102)
2079: Control		H63D: 32.0	H63D: 28.6	for H63D (no effect of HFE variants on the risk of AD)	
		C282Y: 6.9	C282Y: 11.5		
		Female	Female		
		H63D: 23.9	H63D: 26.5		
		C282Y: 8.7	C282Y: 13.0		

[1] % represents the percentage of HFE mutations among AD patients and controls, respectively.
[2] Comments in parentheses represent study methods that may explain results.

the wild-type HFE. In that same study, in patients who developed disease symptoms before 70 y old, the H63D variant was twice as frequent as in patients who developed AD between 70 and 80 y old and 5 times more frequent in patients who developed AD after 80 y old (93). Sampietro et al. (93) also suggested that the effect of HFE alleles on age at onset was independent of ApoEε4 genotypes in Italian AD patients, but Combarros et al. (94) found the synergistic effect of the H63D HFE and ApoEε4 on age at onset in Spanish AD patients. Combarros et al. (94) reported that the presence of 1 or 2 copies of the H63D allele in ApoEε4 homozygotes significantly reduced the age at onset compared with ApoEε4 heterozygotes or noncarriers of ApoEε4. Similarly, Correia et al. (95) found an association of the H63D HFE with earlier age at onset in Portuguese AD patients. However, their study showed the negative association between C282Y HFE with AD, suggesting the protective role of C282Y HFE in AD. A meta-analysis including 66,000 cases and 226,000 controls showed a weak association (1.1-fold risk) with AD in individuals with H63D/H63D (25).

In contrast to the above studies, some studies found no association of HFE alleles with AD (Table 3). Lleo et al. (96) first reported that neither the C2 nor HFE alleles were associated with an increased risk for AD; however, there was a trend toward a higher frequency of the H63D HFE in males. A study by Berlin et al. (97) showed a similar frequency distribution for HFE alleles among AD and control groups. Although HFE alleles had no significant impact on age at onset or diagnosis, age at onset of cognitive symptoms, or severity of neuropsychological deficits, individuals homozygous for H63D HFE displayed a trend toward accelerated conversion from MCI to AD (97). Candore et al. (98) found neither a significant difference in frequencies of H63D and C282Y HFE nor the effect of HFE alleles on age at onset between sporadic AD and controls from Northern Italy. Guerreiro et al. (99) included both AD and MCI patients in their study and found that HFE alleles did not contribute to the risk of developing AD or MCI. In addition, they extended their study by conduction a meta-analysis of both H63D and C282Y HFE in the 5 published studies of AD (89,91,93,97,98). They found no significant association between HFE variants and the development of AD (99). Blaquez et al. (100) were reported the negative association between H63D HFE and AD, suggesting the protective role of this variant in AD, but they did not observe any difference between AD and controls regarding C282Y HFE. Avila-Gomez et al. (101) found that neither the allelic frequencies of H63D HFE mutations nor their effect on age at onset differed between FAD patients with a E280A mutation in presenilin-1 gene (PSEN-1) and nondemented controls. Alizadeh et al. (102) reported no association of HFE alleles with AD, although they did suggest that H63D homozygotes tended to have earlier age at onset compared with noncarriers.

Population differences in the frequencies of HFE alleles and the interaction with environmental factors, age, gender, and/or other genes may all attribute to these discordant findings regarding the association of the HFE gene variants with complex diseases like AD. Animal models involving mutations in the HFE gene are required to further elucidate the association between HFE gene variants and AD independent of environmental conditions, which are significant confounding variables in human studies. The effect of the gene-environmental interaction on the neurological consequences of HFE gene variants and the importance of animal models involving HFE mutations will be discussed later in this review.

HFE and PD. Excess accumulation of iron in the substantia nigra, detected by postmortem examination of brain tissues from PD patients and MRI (103), is a consistent observation in PD (10,17,82,104). Free iron promotes Parkin and α-synuclein aggregation, thus enhancing the generation of Lewy-body, a pathological hallmark of PD (10,17,104). Nielsen et al. (21) reported the relationship between iron accumulation in the basal ganglia and development of a Parkinsonian syndrome in a HH patient. In addition, a recent case study reported on 4 patients with concurrent HH and idiopathic PD, and the authors suggested that increased iron levels in the basal ganglia could be associated with symptoms of idiopathic PD (105). These studies all suggest the involvement of iron in the pathophysiology of PD, and the HFE gene mutations associated with iron accumulation are genetic risk factors for PD.

The studies that have sought to identify a more direct link between HFE mutations and PD have mixed results (**Table 4**). Dekker et al. (106) determined the role of HFE mutations in PD and Parkinsonism in 2 population-based series derived from Rotterdam and southwestern Netherlands. They found an increase in frequency of the C282Y variant in PD compared with controls. Moreover, more patients with Parkinsonism were carriers for the C282Y variant in both populations. The frequency of the H63D variant did not differ between individuals with PD and controls (106). A study in a Portuguese population also found an increased frequency of C282Y carriers in PD, but its presence did not affect age at onset (99). A study in Australia, however, reported that the presence of the C282Y HFE was protective against the development of PD (107).

Although this review is focused on the H63D and C282Y HFE polymorphisms, a study by Borie et al. (108) found those 2 HFE variants were similar between PD and controls but that the G258S transferrin polymorphism was present at a higher frequency in PD patients. A group from Germany analyzed the entire coding region of the HFE gene in PD patients (109). Prior to the analysis of the HFE gene, patients in this study were chosen not only by the clinical symptoms but also by transcranial sonography examination for the subtantia nigra hyperechogenicity, which suggests increased iron levels. They identified 2 novel variants of the HFE gene in exon 2 and 4 (K92N and I217T) present only in PD patients but no association of the 2 more common HFE variants, C282Y and H63D, with PD (109). Similarly, the frequencies of the C282Y and H63D HFE genes were not different between PD and controls in Norwegians (110) nor in the population of the Faroe Islands, where the prevalence of PD and HFE variants are higher than expected (111). Biasiotto et al. (112) also reported that the HFE variants, particularly the H63D allele, did not contribute to the development and clinical features of PD in an Italian population.

Conflicting findings from the above studies concerning the role of the HFE mutations in PD are most likely the result of an interaction between genes and environmental factors such as diet. It has previously been reported that high dietary intake of iron was associated with risk for PD (113,114). Moreover, the smaller sample size and an inappropriate control or patient samples may also contribute to divergent results of previous studies. For example, a study in the French population (108) included a large proportion of patients with positive family history of PD. In patients with family history of PD, genes other than HFE may have a stronger effect on the development of PD; therefore, including patients with a positive family history of PD in a study may underestimate the effect of the HFE mutations on PD.

HFE and ischemic stroke. Following ischemic-anoxic insult and subsequent resuscitation, increased iron accumulation was

TABLE 4 Associations between HFE mutations and PD

Participants, *n*	Location	PD,[2] %	Control, %	Comments	References
216: PD 193: Control	Paris, France	H63D: 36.4 C282Y: 5.0	H63D: 33.9 C282Y: 5.0	No association between HFE genotypes and PD. (Large proportion of patients in the study had positive family history of PD)[3]	Borie et al. (108),
438: PD 485: Control	Queensland, Australia	C282Y: 10.7	C282Y: 16.5	Protective effect of C282Y in PD. (Controls included patients' siblings)	Buchanan et al. (107),
137: PD 47: non-PD PS 2914: Control 60: PD 25: non-PD PS[1]	Rotterdam, The Netherlands	PD H63D: 26.3 C282Y: 10.9 Non-PD PS H63D: 19.1 C282Y: 25.5	H63D: 26.7 C282Y: 11.9	Increased frequencies of C282Y homozygotes in PD and increased C282Y carriers in non-PD PS	Dekker et al. (106)
2914: Control	Southwest Netherlands	PD H63D: 26.7 C282Y: 10.0 Non-PD PS H63D: 20.0 C282Y: 32.0	H63D: 26.7 C282Y: 11.9	Increased frequencies of C282Y carriers in non-PD PS (Small samples for C282Y/C282Y in both studies)	
132: PD 115: Control	Coimbra, Portugal	H63D: 32.6 C282Y: 13.6	H63D: 35.6 C282Y: 4.3	Higher prevalence of C282Y carriers in PD patients	Guerreiro et al. (99)
278: PD 280: Control	Germany	H63D: 16.0 C282Y: 4.7	H63D: 14.1 C282Y: 5.8	Identified rare HFE variants (K92N and I217T) in PD No association of C282Y and H63D with PD	Akbas et al. (109)
388: PD 505: Control	Central Norway	H63D: 18.8 C282Y: 14.9	H63D: 21.6 C282Y: 15.2	No difference in HFE genotypes between PD and controls	Amadt et al. (110)
475: PD 99: Control 1 152: Control 2 2100:Control 3	Milan, Italy	H63D: 14.53 C282Y: 1.7	1: H63D: 13.13 C282Y: 2.0 2: H63D: 11.2 C282Y: 0.3 3: H63D: 13.3 C282Y: 1.6	HFE mutations did not influence the development of the clinical features of PD	Biasiotto et al. (112)
79: PD 153: Control	Faroe Islands, Denmark	H63D: 31.6 C282Y: 15.2	H63D: 28.1 C282Y: 18.3	No association between HFE genotypes and PD. Cannot exclude weak association. (Small sample size)	Halling et al. (111)

[1] Abbreviations: Non-PD PS, Parkinsonism.
[2] % represents the percentage of HFE mutations among PD patients and controls, respectively.
[3] Comments in parentheses represent study methods that may explain results.

detected in the basal ganglia, thalami, and periventricular and subcortical white matter by MRI (115). Also, a correlation between iron concentration in the basal ganglia of acute ischemic stroke patients detected by T2* MRI and stroke lesion growth has been reported (47). The population-based study in The Netherlands found a significant association between higher serum ferritin concentrations, an indication of high body iron stores, and increased risk for ischemic stroke in postmenopausal women (116). Similarly, plasma and CSF ferritin levels were higher in patients with progressive stroke compared with those with stable stroke (117) and a high baseline serum ferritin level was associated with the poor clinical outcome in patients with acute ischemic stroke (118,119). In vivo studies also suggest that brain ischemia disrupts the iron homeostasis system, resulting in an excess iron deposition in the brain that contributes to lipid peroxidation of the cell membrane and neuronal death (47). The catalytic role of iron in the production of reactive hydroxyl radical via a Fenton reaction may explain the link between iron and ischemic stroke.

Because deregulation of iron homeostasis is associated with neuronal damage following ischemic stroke and iron plays a role in platelet activation, atherosclerosis, diabetes mellitus, and hypercholesterolemia, which are predisposing factors to ischemic stroke (47), the HFE gene mutations associated with iron overload could be expected to influence the risk of ischemic stroke. However, the relationship between the HFE gene and ischemic stroke has received little attention and studies concerning the role of HFE variants in ischemic stroke are inconclusive (**Table 5**).

Two prospective studies have reported the association between the HFE gene variants and stroke. The first study reported an increased risk of cerebrovascular death in women who were carriers for the C282Y HFE. The mortality rate for cerebrovascular death in C282Y carriers was further increased by smoking and hypertension (120). This finding suggests a potentially critical connection between HFE gene mutations and other risk factors of ischemic stroke, such as smoking and hypertension (120,121). The HFE gene mutation may set up a permissive milieu that lowers the threshold for risk factors associated with diseases. The second study reported that homozygosity for H63D HFE increased the risk of ischemic cerebrovascular disease and ischemic stroke by 2- to 3-fold (122).

In contrast to the above studies, Njajou et al. (121) reported that the C282Y and H63D HFE were not related to the risk of stroke, although the presence of HFE mutations could modify the relationship between stroke and its risk factors, such as smoking and hypertension. A group from Sweden conducted the prospective study in which the roles of both iron status and HFE genotypes in ischemic stroke were simultaneously studied and they found that neither the C282Y and H63D HFE nor high iron stores influenced the risk of ischemic stroke (123). The association of HFE mutations and ischemic stroke is still controversial and further studies are needed to clarify whether HFE mutations are genetic modifiers of other risk factors for the risk of ischemic stroke.

Animal models

Although there is some evidence that HFE mutations are genetic risk factors for ALS and AD, the role of the HFE mutations in other neurodegenerative disorders such as PD and ischemic stroke discussed in this review are still uncertain. Because the HFE protein is involved in iron regulation, environmental factors such as diet, alcohol, and phlebotomy treatment in the case of HH can be expected to influence the neurological consequences of HFE mutations (29,124). These gene-environmental interactions may contribute to inconclusive findings from previous human population studies concerning the relationship between the HFE and neurodegenerative diseases. To evaluate the neurological consequences of HFE mutations independent of environmental risk factors that can influence the outcomes of the human population studies, in vivo models are required for study.

The mouse homolog for the human HFE gene has been identified by Hashimoto et al. (125) and it has ~66% amino acid sequence homology to the human HFE gene. An HFE knockout mouse line was generated by Zhou et al. (126) to evaluate whether the deficiency or loss of the function of HFE gene product is an underlying mechanism for HH. Similar to the biochemical abnormalities in HH patients, the HFE knockout mice exhibited excessive hepatic iron concentrations and higher transferrin saturation than the wild-type mice (126); brain iron accumulation was not detected by histopathology in HFE knockout mice (127). Tomatsu et al. (128) later generated the HFE knockin mice, H67D (mouse homolog for H63D in humans) and C294Y (mouse homolog for C282Y in humans). The hepatic iron concentration in H67D and C294Y knockin mice was higher than in the wild-type but lower than in HFE knockout mice. Because the consequences of the HFE mutations may result from gain of function of HFE mutations, in vivo models carrying analogous HFE mutations are likely to be better models for evaluating the neurological consequences of the HFE mutations.

Understanding the role of the HFE mutations in neurodegenerative diseases has an important clinical implication. Because HFE mutations can influence iron level, inflammatory response, and oxidative stress, individuals who carry HFE mutations may have higher baseline stress, iron concentration, and inflammation than individuals with wild-type HFE. Thus, it

TABLE 5 Associations between HFE mutations and ischemic stroke

Participants, *n*	Location	Stroke,[2] %	Control, %	Comments	References
79: Cardiovascular cases[1]	Utrecht, The Netherlands	C282Y: 10.7	C282Y: 7.7	Increased risk of cerebrovascular death	Roest et al. (120)
153: Control				in female C282Y carriers	
202: Stroke	Rotterdam, The Netherlands	H63D: 31.2	H63D: 26.5	HFE mutations increased risk of stroke only when other	Njajou et al. (121)
2730: Control		C282Y: 14.1	C282Y: 12.2	risk factors, smoking, or hypertension were present	
393: Stroke	Denmark	H63D: 25.7	H63D: 23.6	Homozygosity for H63D predicts a 2- to 3-fold risk of	Ellervik et al. (122)
8577: Control		C282Y: 10.2	C282Y: 10.9	ischemic cerebrovascular diseases and ischemic stroke	
231: Stroke	Sweden	H63D: 19.1	H63D: 18.9	No association between HFE mutations and the	Ekblom et al. (123)
550: Control		C282Y: 8.2	C282Y: 8.9	risk of ischemic stroke	

[1] Cardiovascular diseases were defined by the International Classification of Diseases, Ninth Revision; ICD.
[2] % represents the percentage of HFE mutations among stroke patients and controls, respectively.

can be expected that the HFE genotype will influence responses to antioxidants, metal chelators, and antiinflammatory treatments. Therefore, stratification of patient populations by HFE genotypes should be included when evaluating treatment strategies. The HFE knockin mice may also prove useful in testing how intervention strategies may be affected by HFE genotypes.

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