Review

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Posted Date: 2 July 2024

doi: 10.20944/preprints202407.0167.v1

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Review

Iron Metabolism in Thalassemia and Sickle Cell Anemia: The influence of Genetic Modifiers

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Abstract: Iron metabolism plays a crucial role in the management of hemoglobinopathies, particularly in conditions such as β-thalassemia and sickle cell anemia (SCA). This paper describes the mechanisms of iron overload in patients with transfusion-dependent thalassemia (TDT), non-transfusion-dependent thalassemia (NTDT), and SCA, highlighting the distinct paths leading to iron accumulation in each condition. The primary focus of this review article is on the role of genetic modifiers influencing iron metabolism and the variability in iron overload presentation among patients. Genetic mutations in genes such as *HFE*, *HAMP*, *TFR2*, *SLC40A1*, and others significantly impact iron regulation. These modifiers can exacerbate or ameliorate iron overload, and understanding these genetic factors is important for explaining the diverse disease severity and differences in the mechanism and clinical presentation of iron overload in these patients. The ongoing INHERENT study aims to further elucidate the influence of these genetic modifiers through a comprehensive genome-wide association study, potentially leading to novel therapeutic interventions for managing iron homeostasis in hemoglobinopathy patients.

Keywords: iron; iron metabolism; iron overload; thalassemia; sickle cell anemia; hepcidin; hemojuvelin; erythroferrone; transferrin receptors; ferroportin

Introduction

Iron is an essential element for various physiological processes, particularly in respiratory function, as it is a critical component of hemoglobin. However, the delicate balance of iron homeostasis must be maintained, as both iron deficiency and iron excess can have detrimental effects on cellular function[1][2]. In the context of β thalassemia and Sickle Cell Anemia (SCA), a group of inherited hemoglobin disorders, patients are susceptible to iron overload, albeit through distinct mechanisms[3]. In β thalassemia, we recognize two groups of patients, transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT). In SCD there are also two groups of patients, patients homozygous for the Hgb S gene and patients with double heterozygous carriers like Sickle Cell β thalassemia and rarer combinations of the Sickle Cell gene and Hemoglobin C, D or O Arab genes. For the issue of iron overload, all the SCA patients are referred together in spite that there are some minor differences between them.

TDT patients develop iron overload initially by increased absorption due to the onset of dyserythropoiesis but mainly following blood transfusions, which become necessary to compensate for the inadequate production of functional hemoglobin. With each transfusion, these patients receive donor-derived red blood cells (RBCs) that contain significant amounts of iron [4] Over time, excess iron accumulation from transfused RBCs exceeds the body's capacity to utilize or eliminate it, which

 doi:10.20944/preprints202407.0167.v1

is minimal, resulting in iron overload[5] [6]. This chronic iron overload can lead to the deposition of iron in various critical organs and tissues, causing damage to their structure and function[7][8].[9]

In contrast, iron overload in NTDT patients arises primarily from the unique pathophysiology of ineffective erythropoiesis[3,10,11][12]. Ineffective erythropoiesis refers to the impaired production and premature destruction of RBCs in thalassemia, leading to increased iron absorption from the gastrointestinal tract. The underlying molecular mechanisms triggering this dysregulation are multifactorial and complex[13][14].[15] Insufficient production of normal hemoglobin chains in NTDT causes an imbalance in the α-to-non-α globin chain ratio, resulting in excess free α-globin chains. These free α-globin chains precipitate within the developing RBCs, leading to ineffective erythropoiesis, increased iron absorption, and subsequent iron overload[16][17][18]

Given the propensity for iron overload in both TDT and NTDT, iron chelation therapy is a vital component of thalassemia management. Iron chelators are pharmacological agents that bind to excess iron in the body, forming stable complexes that can be eliminated through urinary and fecal excretion [19]. These chelators help prevent the accumulation of iron in vital organs such as the heart, liver, and endocrine glands, mitigating the associated complications and improving patient outcomes.

On the contrary, patients with Sickle Cell Disease (SCD), either Homozygous SCD or double heterozygous Sickle Cell β thalassemia usually did not develop clinical features of iron overload [20,21].[22]

There is a group of SCA patients that require regular blood transfusions for several indications. Those patients may develop iron overload, and then they also require the use of iron chelators.

The Regulation of Iron Hemostasis:

Iron is absorbed from the intestine by active transport. In the gut, iron is in the ferric form (Fe^{3+}) and is reduced to the ferrous form (Fe^{2+}) by the enzyme ferric reductase. Then it is absorbed through the intestine epithelial cells by the DMT1 receptor. When iron is transported to the bloodstream, it is reverted again from Fe^{2+} to the Fe^{3+} form and then transported in the plasma conjugated to transferrin. The protein Hemojuvelin (HJV) have an active part in this process and the *HJV* gene encodes the HJV production. Afterwards, the iron molecule is incorporated into the cells and the transferrin protein is free to bind to other iron molecules. The incorporation of iron into the cells is carried out through transferrin receptors while the *TRF1 gene* encodes the transferrin receptors. Iron absorption is regulated by Hepcidin by blocking the iron exporter ferroportin. In conditions that require iron for erythropoiesis, hepcidin is downregulated and allows iron absorption, while in inflammation, iron absorption is inhibited by the increase of hepcidin production. Hepcidin is produced in the hepatocytes and regulated by the BMP/SMAD pathway; the *HAMPT1* gene encodes Hepcidin production. In β thalassemia, this process is influenced by the erythropoiesis requirement and not regulated by the iron overload conditions [23]. The hepcidin production is regulated by erythroferrone, a member of the tumor necrosis factor superfamily of cytokines. Erythroferrone is produced by the erythroblasts and encoded by the *ERFE* gene.

Evaluation of Iron Overload:

The most frequently used analysis for iron overload evaluation is serum ferritin, with all the known limitations of this analysis, principally being ferritin affected by inflammatory processes. Serum transferrin saturation over 70 or 80% can predict the appearance of serum-free iron, the non-transferrin bind iron (NTBI), that is absorbed into the cells and stored as ferritin. Direct analysis of free serum iron can be evaluated by the level of NTBI or Labile plasma iron (LPI) [24]

Nowadays, the gold standard for iron overload evaluation is T2* Magnetic Resonance Imaging. This procedure can assess iron deposition in the heart, liver and pancreas.

Genetic Modifiers in Hemoglobinopathies

Genetic modifiers exert a significant influence on the variable manifestations of iron overload among patients with hemoglobinopathies[25] Unraveling these modifiers is of utmost importance as

 doi:10.20944/preprints202407.0167.v1

it can aid in identifying individuals who are at a higher risk of developing iron overload and guide personalized treatment strategies[26]. The severity of anemia and the extent of iron overload can vary widely among those patients, even within the same subtype. This variability is attributed, at least in part, to genetic modifiers that influence iron metabolism.

Several genes involved in iron metabolism including those encoding human homeostatic iron regulator protein (*HFE*), transferrin (*TF*), transferrin receptor 2 (*TFR2*), ferroportin (*SLC40A1*), hepcidin (*HAMP*), hemojuvelin (*HJV*), glutathione S-transferases (*GST*) and ceruloplasmin (*CP*) have been described as genetic modifiers of metabolic iron disorders [21–23]. By leading to hemochromatosis, which is characterized by excessive dietary iron absorption and iron accumulation, mutations on the *HFE*, *HJV*, *HAMP*, *TRF2* and *SLC40A1* could aggravate already existing iron overload in thalassemia patients[27][28],[29]. Genetic variations of the *GST* genes, however, exhibited a high association with the deposition of iron specifically in cardiac tissue[30],[31],[32],[33]. Variations on *TMPRSS6*, *TF*, *CF*, and L-ferritin (*FTL*) genes were also associated with either high serum ferritin levels or iron accumulation in specific tissues such as the liver, pancreas, heart, and basal ganglia[34],[35],[36],[37],[38],[39]

HFE Gene

The *HFE* gene, located on chromosome 6p21.3, encodes a protein involved in regulating iron homeostasis[40],[41],[42]. The High FE2+ (*HFE*) protein interacts with transferrin receptor 1 (TfR1) and plays a crucial role in the sensing and regulation of iron absorption from the intestinal epithelium[43]. Through its interaction with *TfR1*, the *HFE* protein modulates the expression of hepcidin, a key hormone that regulates iron absorption and recycling[44]. Mutations in the *HFE* gene are known to cause hereditary hemochromatosis, a disorder characterized by excessive iron absorption and deposition in various organs, including the liver, heart, and pancreas. The most common *HFE* mutations are C282Y and H63D, which have been extensively studied in various populations[45],[46].

The presence of *HFE* gene mutations in thalassemia patients has important clinical implications. It can serve as a predictive marker for the development of iron overload and help identify individuals who may require more aggressive iron chelation therapy. Regular monitoring of iron parameters, such as serum ferritin levels and transferrin saturation, along with genetic testing for *HFE* gene mutations, can aid in risk stratification and personalized management of iron overload in thalassemia patients. The effect of *HFE* mutations on iron metabolism in thalassemia patients is complex and may depend on several factors, including the type of thalassemia, the age of the patient, and the frequency and duration of transfusions[27],[28],[29]. The presence of *HFE* mutations was associated with increased serum ferritin levels, a marker of iron overload, in beta-thalassemia carriers and a higher risk of developing hepatic iron accumulation in NTDT patients, whereas literature shows mostly no significant association between *HFE* mutations and serum ferritin levels in thalassemia major patients who were regularly chelated[27],[47],[28].

The mechanisms by which *HFE* gene mutations contribute to iron overload in thalassemia are not fully understood. However, it is believed that these mutations affect the regulation of hepcidin, the master regulator of iron homeostasis[42],[43],[44]. The C282Y mutation has been shown to disrupt the interaction between HFE and TfR1, leading to reduced hepcidin production[42] Decreased hepcidin levels result in enhanced iron absorption from the gastrointestinal tract, leading to iron overload in thalassemia patients with *HFE* gene mutations[48],[49],[50] A recent study that analyzed the impact of the *HFE* gene on SCD patients showed that patients who received chelation therapy had more liver iron deposition compared to patients without the *HFE* mutation[51].

Hepcidin Gene (_HAMP_)

Hepcidin is a key regulator of iron homeostasis. It controls body iron levels by modulating iron absorption in the small intestine and iron release from macrophages[48],[50],[52] The *HAMP* gene, located on chromosome 19q13, encodes pre-prohepcidin, which is processed into the biologically active hormone hepcidin[53] Hepcidin acts by binding to ferroportin, the iron exporter protein,

 doi:10.20944/preprints202407.0167.v1

leading to its internalization and degradation, thereby inhibiting iron release from enterocytes and macrophages [49,50]. Dysregulation of hepcidin production[23], often associated with genetic variations in the *HAMP* gene, can disrupt iron balance and contribute to iron overload in thalassemia patients[54].

Several *HAMP* gene variants have been identified and associated with alterations in hepcidin levels and iron metabolism[55],[56],[57] One such variant is the *HAMP* -582 A>G polymorphism, which has been linked to decreased hepcidin expression and increased iron absorption[58]. This polymorphism is more prevalent in thalassemia patients with iron overload, suggesting its role in influencing disease severity and iron burden[59]. In the study conducted by Andreani et al., it was revealed that the c.-582 A > G variant was correlated with liver iron overload and elevated serum ferritin levels in individuals with β-thalassemia major who had irregular chelation therapy[58]. Furthermore, Island et al. demonstrated that the c.-153C > T variant, located within a BMP-responsive element, decreased the basal expression of the hepcidin gene by impairing its response to BMPs and IL-6[60].

In one study on SCD patients, serum hepcidin levels were found to be normal in all the patients (17-286 ng/ml), irrespective of serum ferritin levels or the number of blood transfusions [61].

Low levels of hepcidin were found in transfusion-dependent β thalassemia patients but not in SCD. Increased intestinal absorption may contribute to iron overload and high transferrin saturation present in transfusion-dependent β thalassemia but not in SCD. [62]

Therapeutic strategies aimed at modulating hepcidin levels and restoring iron balance are being explored. One potential approach is the use of hepcidin agonists, which mimic the action of hepcidin and promote iron sequestration, thereby reducing iron overload. Clinical trials investigating the efficacy and safety of hepcidin agonists in thalassemia patients are underway, with promising preliminary results [63],[64]

Transferrin Receptor 2 Gene (*TFR2*)

TFR2, encoded by the *TFR2* gene located on chromosome 7q22, is a transmembrane glycoprotein expressed predominantly in hepatocytes[65],[66] It functions as a key regulator of iron homeostasis by interacting with transferrin and modulating iron uptake into cells. TFR2 forms a complex with the transferrin receptor 1 (TfR1) on the cell surface, facilitating the internalization of transferrin-bound iron into hepatocytes. Through its association with hemojuvelin (HJV) and the bone morphogenetic protein (BMP) signaling pathway, TFR2 influences the production of hepcidin, a hormone that regulates iron absorption and recycling[49],[50],[67],[68].

Studies have shown that *TFR2* mutations or dysregulation are associated with iron overload[69],[70],[71],[72]. Disruption of TFR2 function affects the interaction with HJV and impairs the BMP signaling pathway, resulting in decreased hepcidin production. Reduced hepcidin levels lead to increased iron absorption from the intestine and enhanced release of iron from macrophages, exacerbating iron overload in thalassemia[49],[50]

A recent murine model of *TFR2* deletion in TDT significantly ameliorated anemia and improved erythroid differentiation and RBCs morphology, leading to an overall reduction of transferrin saturation and serum iron in bone marrow, likely due to increased iron consumption by improved erythropoiesis[73],[74]. Iron content in the liver, spleen, kidney, and heart, along with hepatic expression of TFR1 and iron-responsive bone morphogenetic protein 6, however, remained unchanged. Another study showed no significant difference in ferritin levels and transferrin saturation between NTDT patients with and without *TFR2* polymorphisms (Exon 5 I238M C>G, IVS16 +251 CA deletion)[75].

Despite the evidence on *TFR2* mutations leading to hemochromatosis, literature is scarce on the role of these mutations in thalassemia and SCD, and it is unclear if they could exacerbate or, on the contrary, ameliorate existing iron overload in these patients.

 doi:10.20944/preprints202407.0167.v1

Ferroportin Gene (*SLC40A1*)

SLC40A1 has a role in regulating iron homeostasis by encoding the transmembrane protein ferroportin (also known as solute carrier family 40 member 1 (SLC40A1) or iron-regulated transporter 1 (IREG1)), which is responsible for exporting iron from cells into the bloodstream[26],[76]. In thalassemia, disruptions in the function of ferroportin can contribute to iron overload. Studies have reported that certain mutations or variants in the ferroportin gene are associated with altered iron transport, leading to increased iron accumulation in various tissues[77],[78]. These genetic modifications can impact ferroportin expression, stability, or activity, resulting in impaired iron export and subsequent iron overload in thalassemia patients. A small study on thalassemia intermedia and S/β-thalassemia patients, on the other hand, reported no association between Val 162del mutation of the *SLC40A1* gene and serum ferritin levels[79].

A ferroportin *Q248H* variant, a variant that causes intracellular iron accumulation was found in a group of fourteen SCD patients. Those patients had significantly higher levels of interleukin-6 and C-reactive protein compared to a group of patients that did not show this mutation. This finding may suggest that this variant may affect not only iron overload in SCD patients but also the known inflammatory status present in SCD[80].

Considering the importance of ferroportin in iron regulation, modulating its activity has emerged as a potential therapeutic strategy in thalassemia[26]. By targeting ferroportin modulation, these therapeutic interventions aim to restore proper iron balance and mitigate iron overload complications in thalassemia patients. While the role of ferroportin in iron metabolism is evident and the potential of ferroportin modulation as a therapeutic target in thalassemia shows promise, the evidence regarding the association between *SLC40A1* expression and iron overload remains limited.

Furthermore, other genetic modifiers have also been implicated in iron overload in thalassemia. Variations in genes encoding proteins involved in iron transport and storage, such as hemojuvelin (*HJV*), glutathione S-transferase (*GST*), and ceruloplasmin (*CP*) have been associated with altered iron metabolism and an increased risk of iron overload in thalassemia patients. These genetic modifiers affect iron absorption, cellular iron export, and the breakdown of heme, collectively influencing the overall iron balance.

Mutations in the *HJV* gene have been associated with juvenile hemochromatosis, a rare form of iron overload disorder[81],[82],[83]. In thalassemia, certain *HJV* gene polymorphisms have been linked to altered hepcidin levels and iron overload. For example, the *HJV* I222N (AA) genotype is associated with significantly higher cardiac iron overload in thalassemia patients[78]. These polymorphisms may contribute to the dysregulation of hepcidin and disruption of iron homeostasis in thalassemia.

Glutathione S-transferases are a family of enzymes involved in the detoxification of xenobiotics and oxidative stress[84],[85]. *GST* polymorphisms have been implicated in the susceptibility to various diseases, including iron overload in thalassemia[85]. *GST* gene polymorphisms may influence the antioxidant capacity and detoxification processes, leading to imbalanced iron homeostasis and iron accumulation in thalassemia. Certain *GST* gene variants have been associated with higher serum iron and ferritin levels, as well as liver and cardiac iron deposition in thalassemia patients[31],[32],[33],[86].

Ceruloplasmin is a copper-binding protein involved in iron metabolism and oxidative stress regulation[87],[88],[36]. Genetic variations in the *CP* gene have been associated with alterations in ceruloplasmin activity and iron overload[36],[89]. These gene variants may affect the antioxidant properties of ceruloplasmin and disrupt its role in iron metabolism, contributing to iron overload in thalassemia.

Understanding the impact of these gene polymorphisms on iron metabolism in thalassemia patients has important clinical implications. Identification of these genetic variations can help predict disease severity, assess the risk of iron overload, and guide personalized treatment strategies. Therapeutic interventions aimed at modulating the expression or activity of these genes may offer potential avenues to restore iron homeostasis and mitigate iron-related complications in thalassemia. Further research is needed to elucidate the functional significance of these gene polymorphisms and

 doi:10.20944/preprints202407.0167.v1

their precise role in iron metabolism dysregulation in thalassemia. Despite an abundance of genes known to be associated with iron metabolism and iron-related disorders, there is not much evidence of their implication in thalassemia except for several *HFE* polymorphisms. In SCD, studies of the effect of those genes on iron metabolism are even more rare. This, in fact, necessitates a GWAS study of a large caliber to reveal existing associations specifically in patients with hemoglobinopathies.

Clinical Studies Related to Iron Overload in SCD Patients:

Few studies analyzed the clinical features of iron overload in SCD patients. All those studies showed that even regularly transfused SCD patients did not develop severe iron overload clinical features as in transfusion-dependent β thalassemia. This issue required more research in larger cohorts. We found it as important to describe some studies reported in the literature.

In a study published by Vichinsky et al, organ dysfunction in β thalassemia and SCD was compared. In that study, no cardiac or endocrine disease was found among SCD patients despite similar ferritin levels. In another study by the same group, no significant difference in clinical complications of iron overload was found between transfused and non-transfused SCD [90,91]

In a more recent study performed by Badaway et al, moderate to severe transfusional iron overload in SCD was not associated with aberrations in other measures of cardiac function based on echocardiogram or serum biomarkers [92]

Iron overload Imaging Studies in SCD Patients:

Cardiac iron measurements in SCD, studied by T2*MRI showed no indication of significant myocardial siderosis and no correlation between serum ferritin and cardiac iron content [93],[94].

In another study, six out of ten patients with sickle/β^{o}-thalassemia demonstrated evidence of liver iron overload, with variable severity (<6.3ms). No heart iron deposition was found in those patients[95].

Iron Metabolism Studies in SCD:

Few laboratory studies analyzed the iron overload metabolic status in SCD.

The results of our study (Koren et al.) indicated that none of the 36 SCD patients had clinical symptoms of iron overload despite no continuous or regular iron chelator treatment. In this study, only two SCD patients had NTBI values in the grey zone (0.4 units) and none had positive values. Only one SCD patient had positive LPI levels. The parameters of iron status in SCD patients even after frequent transfusions were different when compared to patients with thalassemia. The low NTBI and LPI levels found in patients with SCD were in keeping with the absence of clinical signs of iron overload in this disease [22].

In another study, there were no measurable levels of LPI or NTBI in any of the ten sickle/β^{o}-thalassemia patients studied [95].

In the study by Badaway et al, SCD patients receiving chronic transfusions did not demonstrate significant cardiac iron loading irrespective of ferritin levels, liver iron content assessed by T2*MRI and erythropoiesis suppression [92].

Discussion:

The parameters of iron status in SCD patients, even after frequent transfusions are different when compared to patients with β thalassemia[22].

Pippard has suggested that subjects with SCD may be protected from iron-related toxicity because of their chronic inflammatory state resulting in the potentially less toxic accumulation of iron in the macrophages[96].

In a study performed by Eduard J. van Beers et al in 2015, several genes related to iron-regulated gene expression were analyzed, and an association with inflammasome complex pathway genes like NLRP3, NLRC4, and CASP1, was demonstrated. This study supports Pippard's theory that in SCD, inflammation may prevent iron overload[97].

 doi:10.20944/preprints202407.0167.v1

In summary, analyzing different genes that are involved in iron hemostasis and inflammation in a very large group of SCD patients, and comparing them with the expression of the same genes in NTDT and TDT β thalassemia patients can add new data and understanding for the interpretation of iron hemostasis and probably for the development of new drugs that may prevent or treat iron overload in those patients.

The ongoing INHERENT study (NCT05799118) intends to analyze the genetic modifiers in patients with hemoglobinopathies through a large, multi-ethnic genome-wide association study (GWAS) [98]. Such modifiers may explain not only diverse disease severity but also the differences in the iron overload or iron metabolism, not only between different diagnosis but also between patients with the same initial genetic diagnosis as TDT, NTDT, SCD and other hemoglobin disorders.

Table 1. Summary of the genetic modifiers of iron metabolism in Thalassemia and SCD.

Genetic Modifier	Chromosome Location	Reported Mutations/ Variants	Function/ Association	Implication in Thalassemia/SCD
HFE (Homeostatic Iron Regulator)	6p22.2	C282Y (rs1800562) H63D (rs1799945) S65C (rs1800730) 5569G-A (rs1800758) V53M (rs28934889) V59M (rs111033557) Q127H (rs28934595) R330M (rs111033558) I105T (rs28934596) G93R (rs28934597) Q283P (rs111033563)	Regulates iron homeostasis, interacts with TfR1, influences hepcidin expression	Mutations or variants can lead to hereditary hemochromatosis, associated with *increased serum ferritin and hepatic iron accumulation*
HAMP (Hepcidin)	19q13.12	1-BP DEL, 93G (rs1189025914) R56X (rs104894695) 4-BP DEL, ATGG (rs142287964) G71D (rs104894696) +14G-A, 5-PRIME UTR (rs944843686)	Key regulator of iron homeostasis, controls iron absorption and release from macrophages	Genetic variations associated with *altered hepcidin levels* and *iron overload*, potential target for therapeutic strategies
TFR2 (Transferrin Receptor 2)	7q22.1	Y250X (rs80338880) 1-BP INS, 84C (rs8033887) M172K (rs80338879) R455Q (rs41303501) Q690P (rs80338889)	Regulator of iron homeostasis, interacts with transferrin, modulates iron uptake into cells	Mutations or dysregulation associated with *iron overload*, impacts hepcidin production
SLC40A1 (Ferroportin)	2q32.2	N144H (rs104893662) A77D (rs28939076) VAL162DEL (rs878854984) D157G (rs104893663) Q182H (rs104893670) G323V (rs104893671) D181V (rs104893672) G80V (rs104893673) G267D (rs104893664)	Encodes transmembrane protein responsible for exporting iron from cells into the bloodstream	Mutations or variants associated with altered iron transport, may contribute *to iron overload*
HJV (Hemojuvelin)	1q21.1	G320V (rs74315323) R326X (rs74315324) I222N (rs74315325) I281T (rs74315326) C80R (rs28940586) L101P (rs74315327) C321X (rs121434374) 4-BP DEL, NT980 (rs786205063) R54X (rs121434375)	Involved in iron metabolism, associated with juvenile hemochromatosis	Polymorphisms linked to altered hepcidin levels and *iron overload*, impact on *cardiac iron overload*
CP (Ceruloplasmin)	3q24-q25.1	IVSAS, G-A, -1 (rs386134142) 5-BP INS (rs386134145)	Copper-binding protein involved in	Genetic variations associated with

		W858X (rs121909579) 1-BP INS, 184A (rs386134143)	iron metabolism and oxidative stress regulation	alterations in ceruloplasmin activity and *iron overload*
GST (Glutathione S-transferases)	11q13.2	*miscellaneous*	Enzymes involved in detoxification, implicated in antioxidant capacity and detoxification processes	Gene polymorphisms associated with *higher serum iron and ferritin levels, liver and cardiac iron deposition*

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