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The Relationship of the HFE Gene H63A Mutant Type with Aerobic Exercise and A Bioinformatics Analysis

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Abstract

Objectives: Oxygen carrying capacity is important in endurance exercises. Iron is an important component of the oxygen transport mechanism. Impairment of iron metabolism can lead to the development of many diseases. Human homeostatic iron regulator protein (High Fe^{+2}: HFE) has a key role in the iron mechanism. Several mutations in HFE alter the function of iron metabolism. The H63A mutation causes more free iron to accumulate in the blood of sedentary individuals and decreases the uptake of iron into the cell, which is associated with various diseases. On the other hand, it is reported that long-term endurance exercise reduces blood serum iron level to normal level in sedentary individuals with HFE H63A mutation. Our study aims to model the three-dimensional structure of the HFE protein H63A mutation using the homology modeling technique, to analyze the hydrophobicity of the modeled structure, and the changes in volume and distance to the active site.

Methods: Homology modelling studies were created by using Swiss-Model. Homology models were analyzed by using UCSF Chimera.

Results: According to our results, a volumetric contraction of 64.6 $Å^3$ in the mutation region, a hydrophobic change of the neutral area with the large surface area, a hydrophobic change with the narrower surface area and a distance of 12.2 Å to the active site were found. The effects of the data obtained in the analysis on the iron mechanism are discussed.

Conclusions: While these results explain the possible cause of elevated serum iron levels due to the H63A mutation in the HFE gene, they highlight the need for more studies on balancing the serum iron level with long-term aerobic exercise and the transfer of iron into the cell.

Keywords: H63A polymorphism, HFE protein, endurance exercise, homology modelling

INTRODUCTION

Iron is an important element of hemoglobin and myoglobin protein complexes responsible for oxygen binding in metabolism. While hemoglobin provides oxygen transport in red blood cells, myoglobin stores oxygen in the muscle and facilitates oxygen transport to the mitochondria during muscle contraction. When iron passes into the blood, it is reduced to ferric iron (Fe^{+3}) and the free iron in the blood serum passes to the cells that store iron (Luck and Mason, 2012). Iron is stored in 65% of hemoglobins (Wallace, 2016). Iron deficiency in metabolism is characterized by fatigue, anemia, and low exercise performance (Della Valle 2013). Endurance athletes experience more iron deficiency due to training intensity and insufficient dietary iron intake, and iron levels fall below the physiological level (Hinton 2014). Iron homeostasis is associated with iron absorption, loss, and storage (Wallace, 2016).

Human homeostatic iron regulator protein (**H**igh **Fe**$^{+2}$: HFE) is a gene with a length of 9,609 bp and contains 7 exons on the chromosome 6p arm within the extended HLA class I region. Histone genes are present on both sides of the HFE. Exon 1 encodes the signal peptide and exon 2-4 encodes the α1, α2 and α3 domains, respectively, and exon 5 encodes the region within the cell membrane, and exon 6, which contains a natural stop codon, encodes the cytoplasmic tail region (Dorak, 2009).

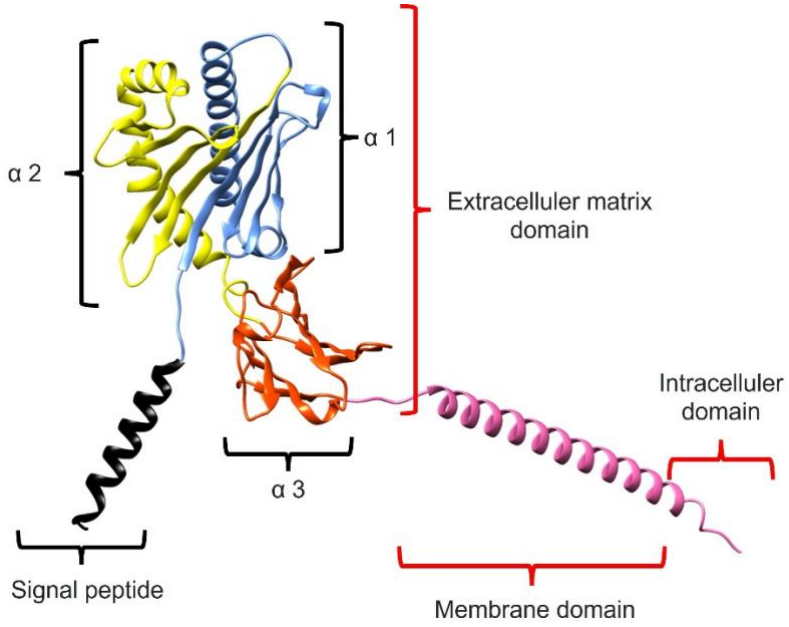

Figure 1. Structure image of the HFE protein. α 1 is blue, α 2 is yellow, α 3 is orange, and the signal peptide is shown in black. The extracellular, membrane, and intracellular regions are indicated.

HFE is a 343-amino acid protein that contains a signal peptide, an extracellular transferrin receptor binding site (α1 and α2), an immunoglobulin-like domain (α3), a

transmembrane domain, and a short cytoplasmic tail (Figure 1) (Feder et al. 1996). HFE contacts the iron-saturated transferrin on the cell surface and provides encrypted iron uptake in the crypt. When there is a malfunction in this mechanism, iron deficiency is observed in the crypt, causing excessive iron intake and iron accumulation in intestinal enterocyte cells. As a result of excess iron in the intestinal cell, the absorbed iron will be given to the plasma (Başol et al. 2007).

It is known that asparagine residues at positions 110, 130 and 234 are glycosylated during transport to the cell membrane. Glycosylation is important for normal intracellular traffic and function (Bhatt et al. 2010). HFE binds to Transferrin receptors (TFR1 and 2) (Salter-Cid, et al. 1999). TFR2 is a homolog of TFR1 and is required at both receptors for iron uptake into erythrocytes in the blood. It binds to the free transferrin of Fe^{+3} in the blood. Free transferrin in blood has two iron binding sites. When the pH of the environment is 7.5, one region has a higher Fe^{+3} affinity, while the pH of the environment is acidic (~ 5.5), the other region has a higher Fe^{+3} affinity (Luck and Mason, 2012). When free transferrin is not loaded with iron, the affinity of HFE and the TFR1 receptor is high. When free transferrin is loaded with iron, HFE leaves the TFR1 receptor and binds to TFR2. The HFE protein binds sequentially depending on the iron load of these two receptors (Goswami et al. 2006). It is stated that HFE and TRF2 proteins play a role in hepcidin hormone synthesis due to the high iron level in the medium (Gao et al. 2009). In hepcidin deficiency, iron accumulates in the blood serum, and in hepcidin excess, anemia develops due to iron deficiency (Nemeth and Ganz, 2006). Therefore, the HFE mutation, which plays a role in hepcidin regulation, is important.

Hepcidin production in the liver is triggered by high free iron level in serum and HFE. The hepcidin produced inhibits the release of more Fe^{+3} into the serum from the duodenum, bone marrow, red blood cells and macrophage, which are associated with the release of iron into the serum. In addition, decreased O_2 level and increased erythrocyte iron requirement prevent hepcidin production. In this way, the hepcidin amount is balanced with HFE, decreased O_2 level, and increased erythrocyte iron requirement (Figure 2). Iron metabolism is given in Figure 2.

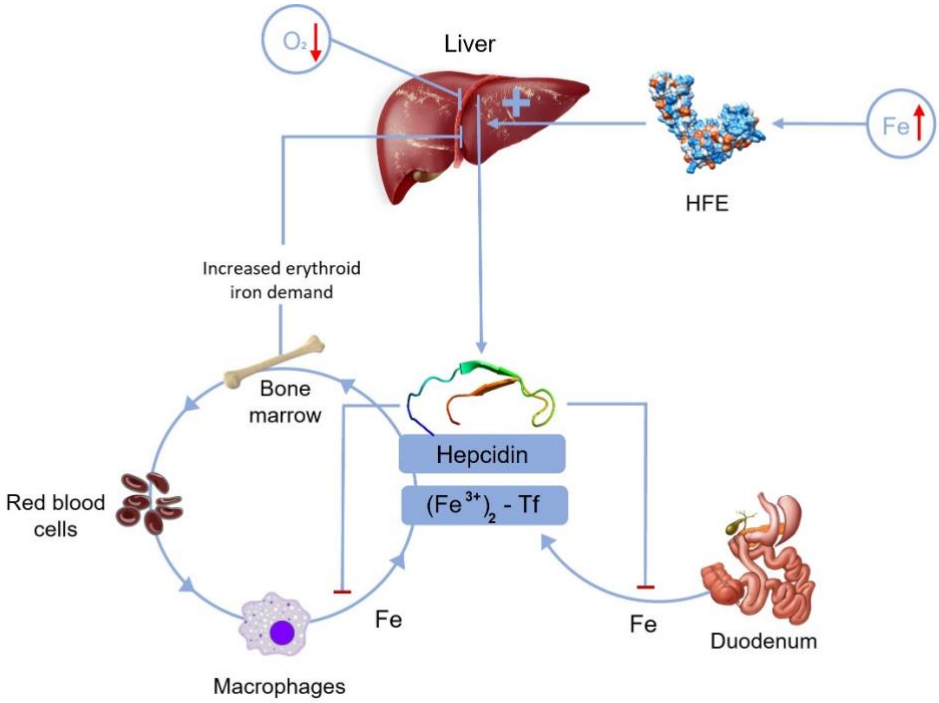

Figure 2. Normal iron metabolism. When iron rises in the blood serum, it triggers hepcidin production in the liver via HFE. Hepcidin inhibits the release of Fe^{+3} into serum from duodenum, bone marrow, red blood cells and macrophage, which are associated with iron release into serum. Decreased blood O_2 level and increased erythrocyte iron requirement inhibit hepcidin synthesis.

Fe: iron, Fe^{+3}: ferric iron, Tf: plasma free transferrin, O_2: oxygen, ↑: high level, ↓: decreased level, ⊥: inhibition

There is a higher amount of Fe^{+3} in the blood serum of sedentary individuals with the HFE protein with the H63A mutation compared to those without the mutation (Kortas et al. 2020). Although the high amount of iron in the serum causes some important disorders such as diabetes and cardiomyopathy, it is reported that the HFE protein with the H63A mutation can be advantageous in endurance athletes (Kortas et al. 2020). However, the mechanism has not been fully solved (Kortas et al. 2020). Kortas et al. stated in their study that there is HFE protein with H63A mutation in high-level endurance athletes. In addition, after 12 weeks of Nordic skiing exercise for the elderly with and without H63A mutation, the blood iron level decreased to normal in the elderly with H63A mutated HFE protein. While the H63A mutated HFE protein creates a disadvantage in sedentary individuals, the disadvantage disappears in individuals who do endurance exercise. A high positive correlation was determined between the various mutated HFE protein and the maximum oxygen consumption capacity (VO2max) in athletes (Semenova et al. 2020). Our study aims to prove that the negative effect of H63A mutated HFE protein on

iron metabolism, by using a bioinformatics analysis, decreases with long-term endurance exercise at the molecular level.

MATERIAL METHODS

Homology Modeling

Homology modeling is a method used to analyze and examine the three-dimensional structure of the protein, when the three-dimensional structure is unknown or the three-dimensional structure is changed as a result of polymorphism/mutation (Boissel et al., 1993). The three-dimensional structure of a protein is determined by the amino acid sequence and the physico-chemical properties of those amino acids. Physico-chemical analysis, on the other hand, is the examination of properties such as hydrophobicity, electrostatic properties, and protein stability of amino acids in a protein (Xia and Li, 1998).

Wild-Type and H63A mutant-type models were modeled with the Swiss-Model (Studer et al. 2021) bioinformatics tool. The modeling was based on the 1a6z.1.A template with a resolution of 2.60 Å, with a sequence similarity of 95.98% (wild type) to 95.56% (mutant type) and a GMQE of 0.75.

Model Visualization

Wild-type and mutant-type models were analyzed with the UCSF Chimera 1.15 program. The Histidine-Alanine change of amino acid 63 is atomically visualized by hydropathic surface representation of the overall protein. Hydropathic image of the mutation site was captured.

RESULTS

Our study provided the understanding of the HFE protein H63A mutant type mechanism, the creation of a three-dimensional model, and the interpretation of the exercise-associated mechanism.

The HFE was visualized and overlaid with wild-type and mutant-type ribbon-to-ribbon representation. When examined on a global scale, it was observed that there was no difference in the general structure of the protein, only an atomic change due to amino acid change in the mutation region (Figure 3). Due to the amino acid change, the large volume of histidine (153.2 $Å^3$) in the region was converted to the smaller volume alanine (88.6 $Å^3$). The region volume decreased by 64.6 $Å^3$.

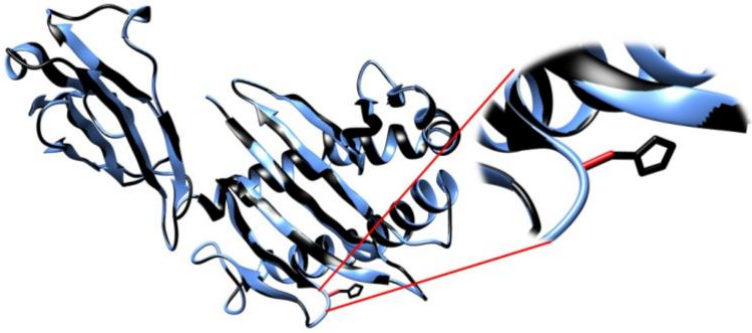

Figure 3. Overlay and visualization of HFE wild and mutant types with ribbon display. The wild type is shown in black, and the mutant type in blue. In the region indicated by the red arrow, mutant alanine red wild type histidine is shown in black.

According to the results of hydrophobic surface analysis, hydrophobicity increased because neutral histidine (-3.2 hydropathy index) was converted to hydrophobic alanine (1.8 hydropathy index) in terms of hydrophobicity in the exchange region. The neutral region with a large surface area has changed to a hydrophobic region with a narrower surface area (Figure 4).

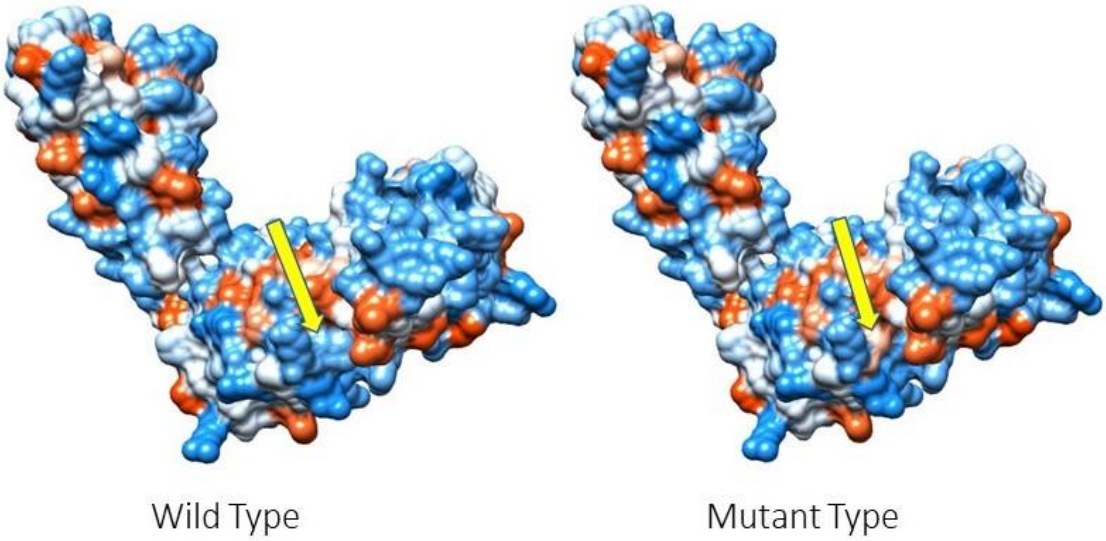

Figure 4. Visualization of wild and mutant type models with hydropathic surface display. The hydrophilic area is colored blue, the neutral area is colored white, and the hydrophobic area is colored red. The region indicated with the yellow arrow is the mutation region.

DISCUSSION

Functional impairment of iron metabolism and high serum iron levels are characterized by HFE gene mutations. It has been stated that this situation, which normally brings a disadvantage, may provide an advantage in Olympic endurance athletes. H63D HFE gene

mutation has been detected in athletes (Semenova et al. 2020). On the other hand, the H63A HFE gene mutation has been noticed in recent years and a study examining its relationship with exercise was found in the literature (Kartos et al. 2020). In this study, no difference was found between the serum iron levels at the end of 12 weeks of endurance exercise in the elderly with and without HFE protein with H63A mutation. Elderly people with HFE protein with H63A mutation at baseline have been reported to have high iron levels in blood serum. In our study, HFE gene homology models with H63A mutations have over 95% sequence similarity, have a resolution of 2.60 Å, and a GMQE value close to 1, which increases the accuracy of the protein homology model (Biasini et al. 2014).

The region of the HFE gene H63A mutation is directly in the TFR1 and 2 receptor binding regions on the erythrocyte cell membrane. It is known that this change in the binding region of the HFE gene increases the ferritin levels and the amount of iron in the plasma (Başol et al. 2007). In addition, the distance between the G93 region (Barton et al. 1999), which is thought to be associated with iron overload, and the mutation region was measured as 12.2 Å (Figure 5). It is thought that this proximity may reduce the iron overload capacity. Although it is not fully understood how HFE and TRF receptors work, it is thought that HFE with H63A mutation is related to the decrease in TRF binding affinity, and as a result, the affinity of TFR-iron-loaded transferrin changes and its entry into the cell decreases. This result supports our finding related to decreased TRF binding affinity with HFE with H63A mutation.

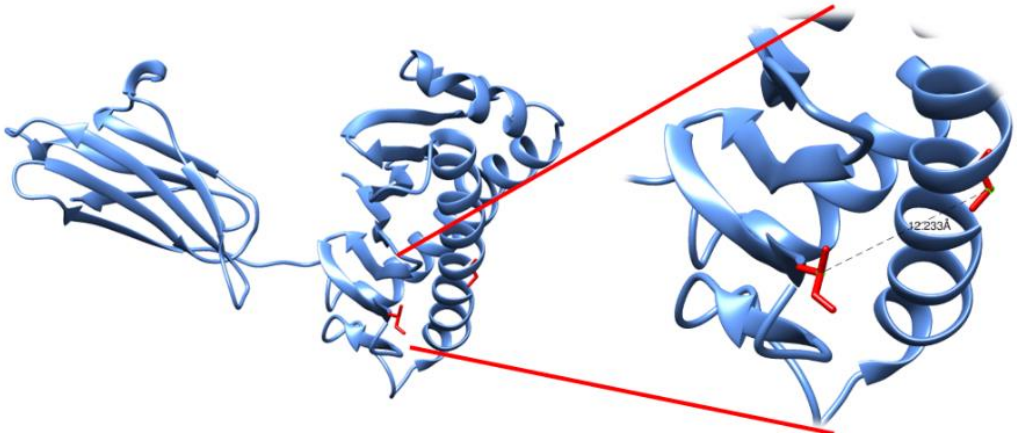

Figure 5. Distance of amino acids A63 and G93 in mutant type model.

Normally, when the serum level of iron is high, HFE and TRF-mediated hepcidin hormone secretion increases and hepcidin hormone decreases the intestinal iron uptake. Hepcidin levels are low in all known mutations of the HFE protein and in the H63A mutation (Kortas et al. 2020). The absence of an increase in hepcidin levels after Nordic skiing exercise

in the elderly with HFE protein with H63A mutation suggests that there may not be a decrease in the level of iron released from the intestine, old erythrocytes, and macrophages and transferred to the blood serum. Accordingly, it is considered that other than HFE transferrin-mediated iron uptake, another mechanism may also be effective in iron uptake into cells.

On the other hand, during the adaptation process to aerobic exercise, an increase in the number of blood erythrocytes and hemoglobin occurs (Kortas et al. 2020). Despite the high iron uptake into the blood serum due to hepcidin deficiency and the low iron uptake from the serum mediated by the H63A mutated HFE protein and TRF 1-2 to the erythrocytes and other iron-storing cells, the normalized serum iron level in the elderly who do Nordic discipline can be explained by increased erythrocyte and hemoglobin. The increased number of erythrocytes and the amount of hemoglobin in compliance with exercise may have created an opportunity for more free iron to be taken into the cell. This will allow more oxygen to enter the blood in the alveoli and more oxygen to the tissue cells. Semenova et al. (2020) found a relationship between HFE protein with H63D mutation and VO2max in professional athletes, which can be explained by increased erythrocyte and hemoglobin. In addition, iron metabolism disorder normally associated with mutated HFE protein is a factor in the mechanism of many diseases such as cirrhosis and Alzheimer's (Albayrak and Çürük 2009). It emphasizes that long-term aerobic exercise can stop and/or eliminate the effects of such a negative situation.

CONCLUSIONS

Our findings clarify the excess serum level of iron and the decrease in cellular iron stores in the H63A HFE mutation, as in various HFE mutations. In addition, it reveals that iron metabolism is balanced by long-term aerobic exercises. This situation once again emphasizes the importance of the protective and improving effect of exercise on health. On the other hand, our findings also indicate the need for further studies on the mechanism of iron uptake into the cell, which has not yet been fully elucidated, and the effect of exercise on this mechanism.

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