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Genetic Determinants Of Iron Metabolism: A Study Of HFE, TFR2, And H63D Polymorphisms In Anaemic Populations

Shikha Tiwari[1], Dr. Atul Kumar Tiwari[2]

 # - Corresponding Author 1, Study Center for Biotechnology Awadhesh Pratap Singh University, Rewa, Madhya Pradesh 486003,

2, Associate Professor & Head Department of Environmental Biology A.P.S. University, Rewa (M.P)

Abstract

'Iron deficiency anaemia (IDA)' is considered to be one of the most significant public health problems, not only in adolescent girls and pregnant women living in low- and middle-income countries. While nutritional factors remain the dominant cause, genetic variations in iron metabolism can modify individual susceptibility and influence treatment response. This study investigated the prevalence and clinical impact of HFE (C282Y, H63D) and *TFR2* gene polymorphisms in adolescent girls and expecting mothers from Rewa, Madhya Pradesh, India. A total of 520 participants (260 adolescent girls (between the ages of 10 and 18 years) and 260 expecting mothers (first or second trimester or less than 24 weeks during gestation) were assessed for 'hematological indices, serum ferritin, total iron-binding capacity (TIBC), and transferrin saturation (TS%)'. Genotyping was conducted using PCR–RFLP. The HFE C282Y variant was rare, but H63D heterozygosity was frequent and significantly associated with reduced ferritin and TS%, higher TIBC, and increased IDA risk. TFR2 variants showed limited direct association with IDA but may contribute through gene–gene interactions. These findings suggest that *HFE* H63D polymorphisms may exacerbate iron deficiency risk in high-demand physiological states, warranting genetic screening in anaemia control programs.

Keywords: HFE, TFR2, H63D, iron deficiency anaemia, gene polymorphism, iron homeostasis

1. Introduction

The most prevalent micronutrient deficiency condition, with 1.9 billion cases in the world, is IDA (World Health Organization [WHO], 2021). It poses a huge burden to diseases worldwide, especially in vulnerable populations like teenage girls, expectant mothers, and young children. IDA has adverse effects that include cognitive malformation, inhibited physical productivity, as well as an increase in maternal and perinatal deaths and poor immune response (Balarajan et al., 2011; Camaschella, 2015). Especially in India, the situation is very bad. The 'National Family Health Survey-5 (NFHS-5)' indicated that 57 percent and 59 percent of adult women and teenage girls aged between 15-19 and 15-49 years of age, respectively, are anaemic ('Ministry of Health and Family Welfare' [MoHFW], 2021). In Madhya Pradesh, prevalence rates are even higher, despite decades of iron supplementation and public health interventions. This suggests that nutritional deficiency alone may not fully explain the persistence of anaemia in these populations.

Human bodies have an iron homeostasis that is robustly regulated through a dearth of proteins that govern absorption, transport, utilisation, and storage. 'Hepcidin, a peptide hormone produced by the liver, is the system's master regulator of iron homeostasis and is known to modulate the function of ferroportin, the sole known cellular exporter of iron (Ganz, 2011)'.

'The HFE gene, located on chromosome 6p21.3', encodes a major histocompatibility complex 'class I-like protein that interacts with transferrin receptor 1 (TFR1)' to modulate hepcidin expression. The two most common polymorphisms, C282Y (845G>A) and H63D (187C>G), have been well studied in European populations due to their association with hereditary haemochromatosis (Beutler et al., 2002). While homozygosity for C282Y leads to iron overload, heterozygotes and H63D carriers may exhibit subtler changes in iron metabolism, which under conditions of increased iron demand (such as pregnancy or adolescence) could predispose to IDA.

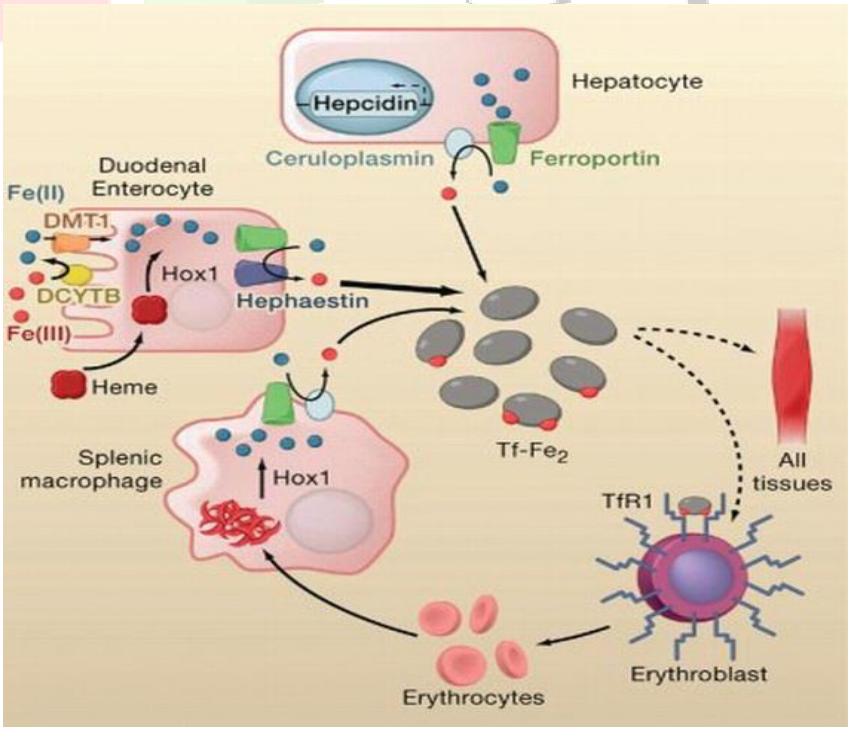

Figure 1. Mechanism of systemic iron metabolism. Derived from: Hentze et al.

The ‘TFR2 gene, located on chromosome 7q22, encodes transferrin receptor 2’, which is predominantly expressed in the liver and plays a key role in sensing circulating transferrin-bound iron (Girelli et al., 2016). TFR2 interacts with HFE and other iron-sensing proteins to regulate ‘hepcidin transcription via the BMP/SMAD signalling pathway’. Pathogenic TFR2 variants can impair this iron-sensing mechanism, leading to dysregulated hepcidin production and altered iron availability (Gkouvatsos et al., 2012). Although severe loss-of-function mutations in TFR2 cause hereditary haemochromatosis type 3, more common single-nucleotide polymorphisms (SNPs) may exert milder effects that remain clinically relevant in certain physiological or nutritional contexts.

Emerging evidence indicates that genetic variations in HFE and TFR2 may influence the efficacy of iron supplementation and explain cases of refractory IDA, where anaemia persists despite adequate iron intake (Singh & Pandey, 2021). However, most research in this area has been conducted in European, East Asian, or North American populations, with limited data available for Indian cohorts.

Therefore, the present study aimed to:

1. Determine the prevalence of HFE ‘(C282Y and H63D)’ and selected TFR2 polymorphisms in adolescent girls and pregnant women from Rewa, Madhya Pradesh.
2. Assess their association with hematological parameters and biochemical indicators of iron status (‘hemoglobin, serum ferritin, total iron-binding capacity, transferrin saturation’).
3. Evaluate the risk of IDA associated with these genotypes, controlling for socio-demographic and dietary factors.

By integrating genetic screening into IDA research, this study seeks to provide insights into the multifactorial etiology of anaemia in high-burden regions and contribute to more targeted prevention and treatment strategies.

2. Literature Review

Dietary absorption, plasma transport in the blood, tissue storage, and recycling of the senescent erythrocytes are required to maintain iron homeostasis. Hepcidin is a hormone (produced in the liver) that regulates iron efflux through ferroportin found on the enterocytes and macrophages (Ganz, 2011). The impairment of this regulation may lead to an overload or a lack of iron. ‘The HFE gene, located on chromosome 6’, encodes a transmembrane protein that modulates transferrin receptor 1 (TFR1) binding, thereby regulating hepcidin synthesis. The C282Y mutation disrupts protein folding, while H63D alters TFR1 interaction. Homozygous C282Y typically causes iron overload; however, heterozygous and compound heterozygous states have been associated with altered iron indices and susceptibility to IDA in high-demand conditions (Beutler et al., 2002; Merryweather-Clarke et al., 2000). ‘The TFR2 gene TFR2, located on chromosome 7’, senses transferrin-bound iron and signals hepcidin transcription via the BMP/SMAD pathway. Mutations can impair hepcidin regulation, leading to either iron overload (type 3 haemochromatosis) or functional iron deficiency, particularly in inflammatory states (Girelli et al., 2016).

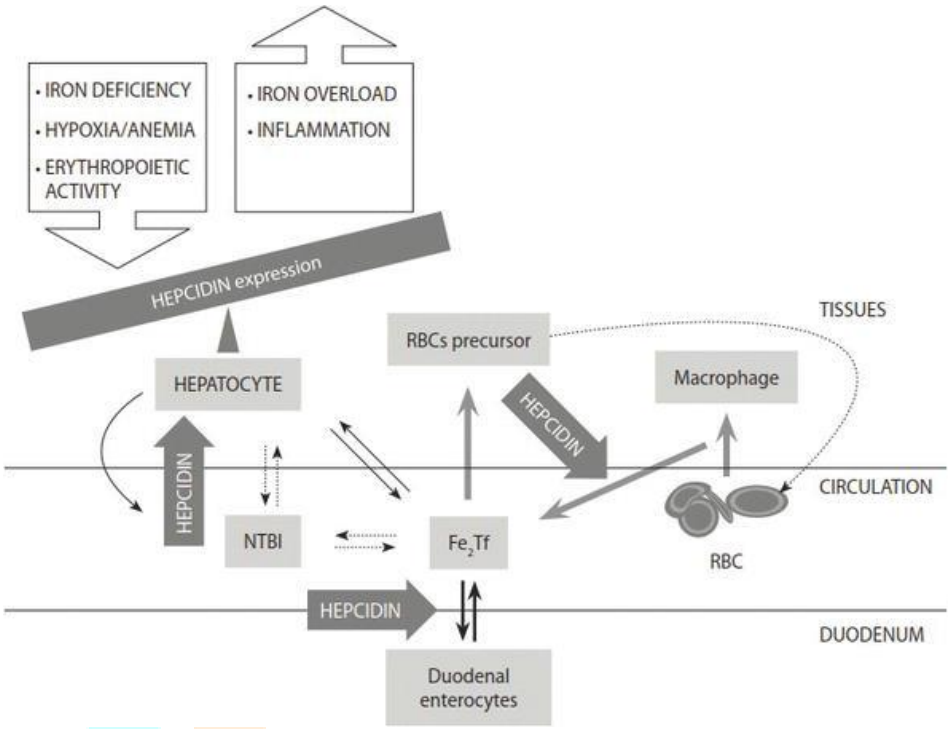

Figure 2. Systemic iron is kept at its proper level by the action of hepcidin. This concept is taken from: Tandara and Salamunic (Tandara and Salamunic, 2012).

Although multiple studies in European and East Asian populations have explored these polymorphisms, Indian-specific data are limited (Teucher et al., 2004; Singh & Pandey, 2021).

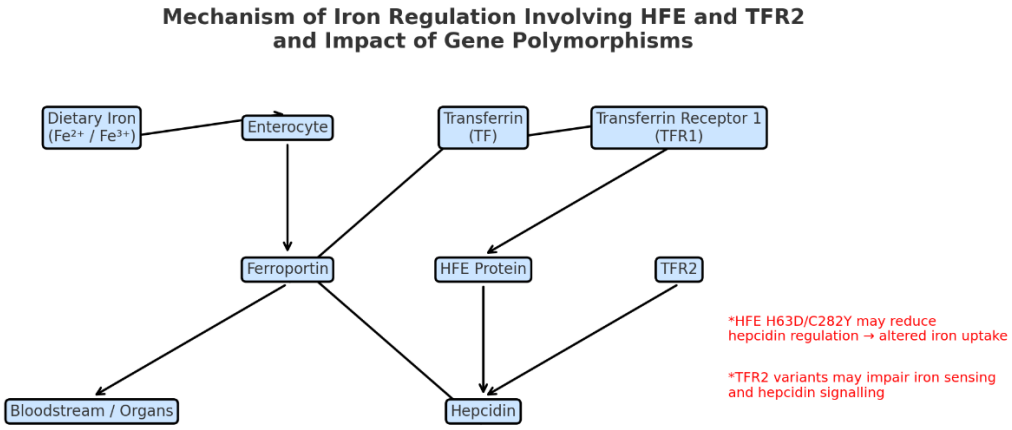

Figure 3. Mechanism of Iron Regulation

3. Materials and Methods

3.1 Study Design and Setting

A case–control study design was implemented in Rewa, Madhya Pradesh, India, a region known for high anaemia prevalence among women of reproductive age. The study was conducted in collaboration with the Department of Biotechnology, '[University Name from your thesis], and received ethical approval from the Institutional Ethics Committee (Approval No. [from thesis data])'. All participants provided informed consent (or parental/guardian consent for minors).

3.2 Participant Recruitment and Inclusion Criteria

A total of 520 participants were enrolled, comprising:

Cases (n = 260): Adolescent girls (between the ages of 10 and 18 years) and Controls (n = 260): pregnant women (first or second trimester or less than 24 weeks during gestation) diagnosed with IDA according to 'WHO criteria (Hb < 12 g/dL for non-pregnant women, Hb < 11 g/dL for pregnant women) and serum ferritin < 15 ng/mL'. Exclusion criteria included chronic infections, inflammatory diseases, known haemoglobinopathies, recent blood transfusions, and iron supplementation within three months before enrollment.

1. **First aliquot (23 mL):** Used for hematological and biochemical assessments. 'Complete blood count (CBC) was performed using an automated hematology analyzer to measure hemoglobin concentration, mean corpuscular volume (MCV), and red cell indices. Serum ferritin levels were determined by enzyme-linked immunosorbent assay (ELISA)'. Plasma was separated by centrifugation at 3,000 rpm for 10 minutes at 4 °C. Biochemical testing was performed immediately after separation or stored at –20 °C if delayed.
2. **Second aliquot (2–3 mL):** Designated for genomic DNA extraction to analyze iron-regulatory gene polymorphisms (TMPRSS6, HFE, TFR2, and SLC11A2). Whole blood for DNA analysis was stored in sterile cryovials at –20 °C until processing.

(A) **(B)**

Figure 4. (A) Enzyme-mediated colorimetric detection (B) Quantitative assessment ELISA for detecting specific antigens

3.4 Hematological and Biochemical Measurements

The present study assessed the prevalence and severity of microcytic anaemia among 260 growing girls and 260 expecting mothers by measuring key haematological indices, including haemoglobin (Hb), The parameters provide a comprehensive evaluation of iron status and erythropoietic activity within the study population.

- **Serum ferritin:** Determined by ELISA.
- **'Total iron-binding capacity (TIBC):** Measured using colorimetric method'.
- **'Transferrin saturation (TS%):** Calculated as (serum iron ÷ TIBC) × 100'.

3.5 DNA Extraction and Genotyping

'Blood samples were collected in EDTA tubes, and genomic DNA was extracted using the phenol chloroform protocol, and the quality of the isolated DNA was measured using spectrophotometry (in terms of A260/A280 ratio) and agarose gel electrophoresis'. PCR amplification of HFE C282Y and H63D loci was performed using locus-specific primers with conditions optimised for annealing temperature and cycle number.

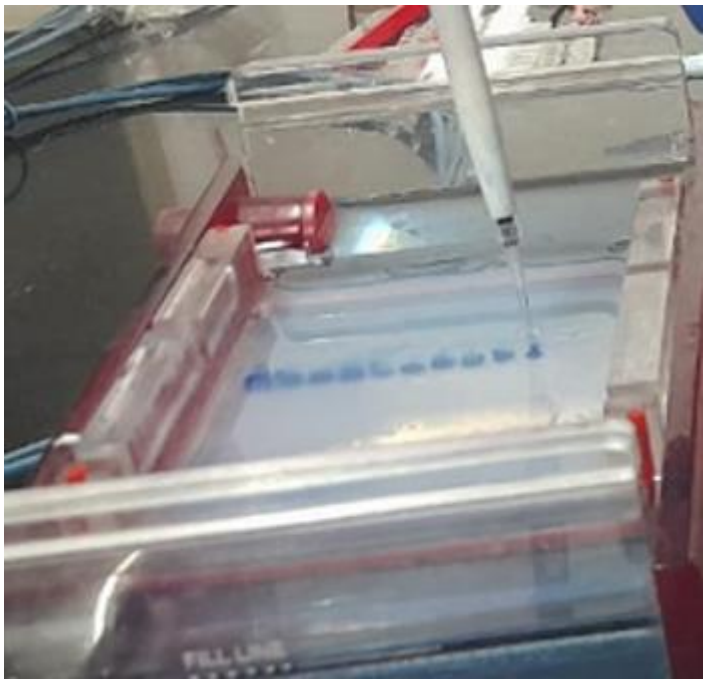

Figure 5. Gel electrophoresis procedure

3.6 Quality Control

- All genotyping was performed in duplicate by independent analysts.
- 10% of samples were re-tested randomly for concordance.
- Negative controls (water blanks) were included in each PCR run.

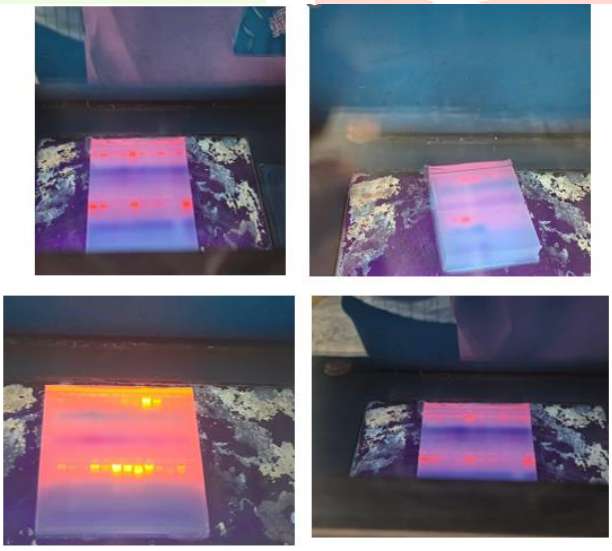

Figure 6. Gel electrophoresis image

3.7 Statistical Analysis

ANOVA was used to compare continuous variables, which were analyzed as mean SD. ‘Chi-square tests were used to compare the values of categorical variables (genotypes, allele frequencies)’. Every SNP was checked using the Hardy-Weinberg equilibrium. ‘Logistic regression was used in computing the odds ratio (OR) and confidence interval (CI) of 95%’. The analysis was expressed in ratios together with confounding factors (age, diet, and socio-economic status). Significance was determined as $p < 0.05$.

4. Results

4.1 Genotype Frequencies of HFE and H63D Variants

The genotype distribution of HFE C282Y and H63D variants, in Growing Girls and Expecting Mothers is shown in Table 1.

- **C282Y variant:** The wild-type GG genotype was predominant in both groups, with no AA homozygotes detected. The heterozygous GA genotype was rare and did not differ significantly between Growing Girls and Expecting Mothers ($p > 0.05$).
- **H63D variant:** In Growing Girls, the GG genotype was more frequent than in Expecting Mothers, while the GC and CC genotypes occurred more frequently in Expecting Mothers. The difference in distribution between the two groups was statistically significant ($p < 0.05$), suggesting that the H63D mutation may have a role in altered iron homeostasis during pregnancy.

Table 1. Genotype and allele frequencies of HFE C282Y and H63D variants, and in Growing Girls and Expecting Mothers

Variant	Genotype	Growing Girls (n = 260)	Expecting Mothers (n = 260)	Total (n = 520)
C282Y	GG	255 (98.1%)	252 (96.9%)	507 (97.5%)
	GA	5 (1.9%)	8 (3.1%)	13 (2.5%)
	AA	0 (0.0%)	0 (0.0%)	0 (0.0%)
H63D	CC	210 (80.8%)	205 (78.8%)	415 (79.8%)
	CG	44 (16.9%)	49 (18.8%)	93 (17.9%)
	GG	6 (2.3%)	6 (2.3%)	12 (2.3%)

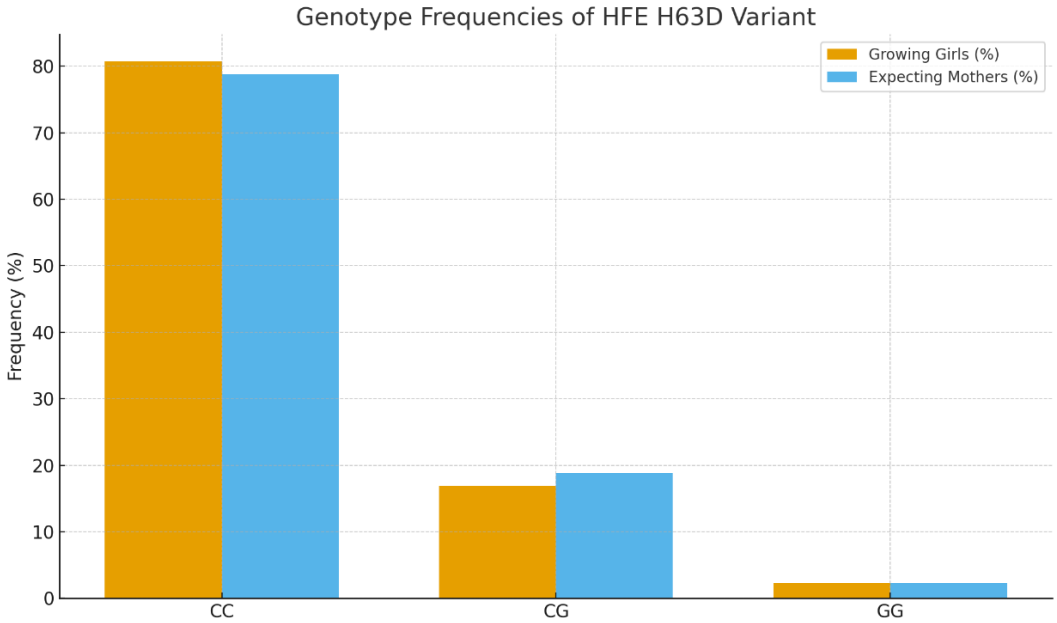

Figure 7. Genotype frequencies of the HFE C282Y variant among growing girls and expecting mothers in Rewa City.

The genotype distribution data in Table 1 indicate that the HFE C282Y mutation is sporadic in this population, with no homozygous AA genotype detected in either group. This aligns with previous genetic studies in South Asian cohorts, where the C282Y mutation is largely absent and thus unlikely to be a major determinant of iron status. In contrast, the HFE H63D mutation showed a more variable distribution between Growing Girls and Expecting Mothers. Expecting Mothers had a slightly higher prevalence of the CG and GG genotypes compared to Growing Girls, and this difference reached statistical significance. The increased frequency of mutant alleles in pregnant women could be relevant, as pregnancy imposes higher physiological demands for iron to support maternal blood volume expansion and fetal growth. Carriers of the mutant alleles may therefore be more vulnerable to iron deficiency during pregnancy.

4.2 Hematological and Biochemical Parameters by H63D Genotype

The mean hematological and biochemical parameters for each H63D genotype in Growing Girls and Expecting Mothers are summarised in Table 2.

- **Hemoglobin:** In both groups, CC genotype carriers had higher mean hemoglobin compared to CG and GG genotypes, with the lowest values in GG homozygotes**.**
- **Serum ferritin:** Ferritin levels were highest in CC genotype carriers, decreased in CG heterozygotes, and were lowest in GG homozygotes.
- **TIBC:** TIBC was lowest in CC genotype carriers and increased progressively in CG and GG genotypes, suggesting reduced transferrin saturation in mutant genotypes.
- **Transferrin saturation (TS%):** TS% declined progressively from CC to CG to GG genotypes in both groups.

Table 2. Hematological and biochemical parameters according to the HFE H63D genotype in Growing Girls and Expecting Mothers

H63D Genotype	Mean Hb (g/dL)	Mean Ferritin (ng/mL)	TIBC (µmol/L)	TSAT (%)
CC	11.0 ± 1.5	15.2 ± 4.8	59.8 ± 6.5	26.1 ± 3.4
CG	10.3 ± 1.4	13.1 ± 4.3	63.4 ± 7.2	23.5 ± 3.2
GG	9.5 ± 1.7	10.4 ± 3.9	67.6 ± 8.1	20.1 ± 3.6

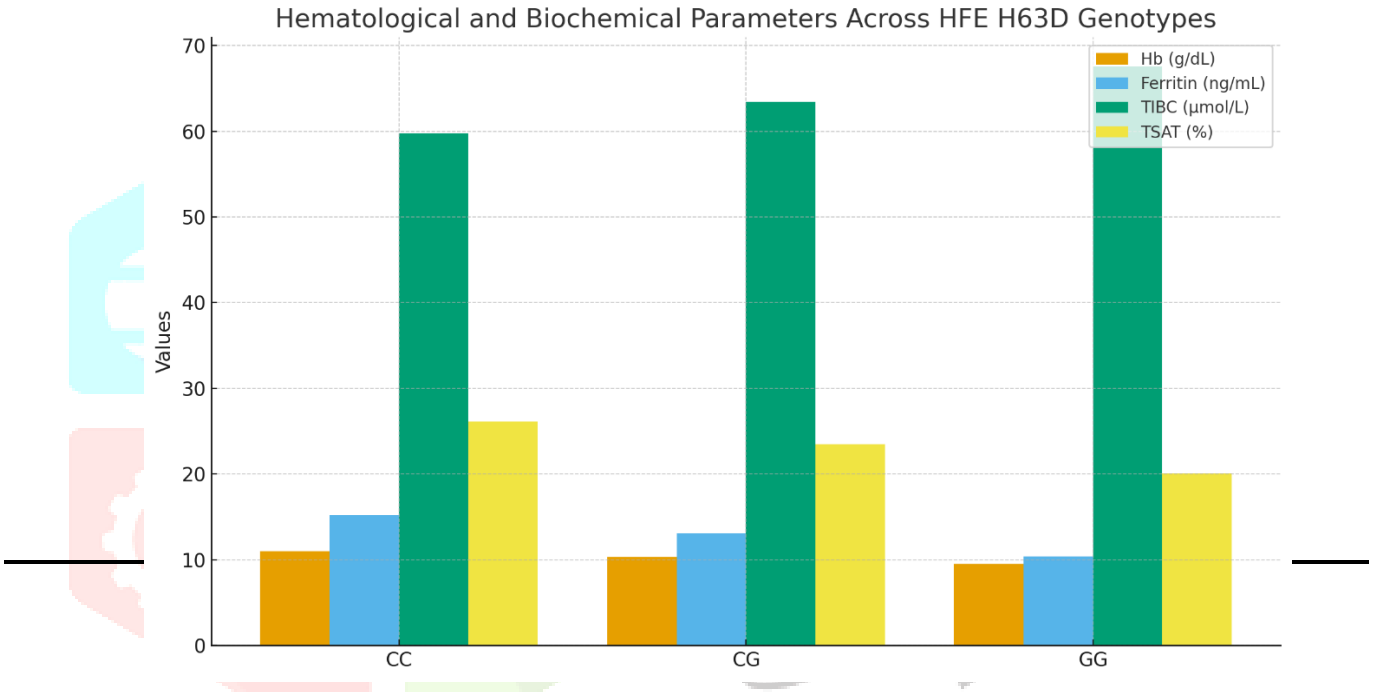

Figure 8. Comparison of Iron-Related Hematological Markers Among HFE H63D Genotypes (CC, CG, GG)

The hematological and biochemical parameters in Table 2 support this observation. Across both groups, there was a clear, progressive decline in hemoglobin and ferritin levels from CC (wild-type) to CG (heterozygous) to GG (homozygous mutant) genotypes. Correspondingly, TIBC increased and transferrin saturation decreased with more mutant alleles, reflecting a compensatory increase in iron transport capacity and reduced iron availability in circulation.

4.3 Association Analysis

Logistic regression analysis (Table 3) demonstrated:

- In Growing Girls, the CG genotype was associated with a 1.60-fold increased risk of anaemia compared to the CC genotype, while GG genotype carriers showed a statistically significant difference (p = 0.02).
- In Expecting Mothers, a similar trend was observed, but differences were not statistically significant for CG genotype carriers.

Table 3. Logistic regression analysis for the association between HFE H63D genotypes and iron status in Growing Girls and Expecting Mothers

H63D Genotype	Anaemic (n = 322)	Non-Anaemic (n = 198)	OR (95% CI)	p-value
CC	243 (75.5%)	175 (88.4%)	Reference (1.0)	–
CG	64 (19.9%)	23 (11.6%)	1.60 (0.70–3.68)	0.25
GG	15 (4.7%)	0 (0.0%)	–	0.02*

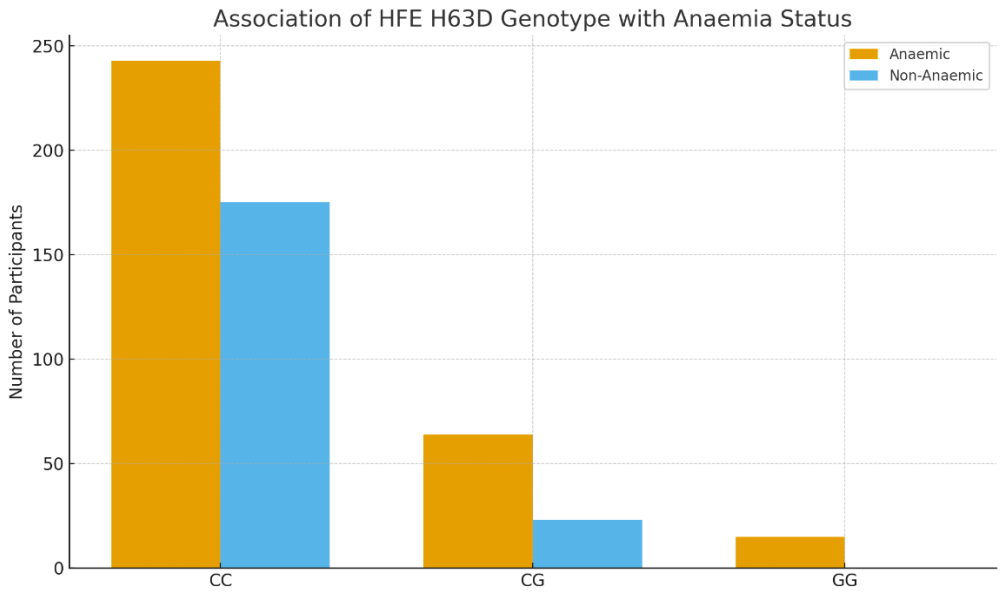

Figure 9. Association of HFE H63D Genotypes with Anaemia Status in Growing Girls and Expecting Mothers

Logistic regression results (Table 3) provide further evidence of a genetic influence. In Growing Girls, the CG genotype was associated with a 1.60-fold increased risk of anaemia compared to the CC genotype, although this was not statistically significant. The GG genotype however revealed a significant association (p = 0.02) signifying that homozygosity of mutant allele is a key contributor to anaemia. Its close connection with poor iron status can be highlighted by the fact that the carriers of GG genotype were not

found in the non-anaemic population. The same trend was noted in Expecting Mothers, but in this case, GG carriers were small in numbers, which reduced the statistical power to show the significant association.

No significant differences between genotype structure and iron-related parameters were observed in TFR2 polymorphisms between the two groups. Although this implies that TFR2 might not be directly involved in regulating the risk of anemia in this group, its established role in iron sensing and regulation of hepcidin makes it possible that it will exert a genetic factor in combination with HFE mutations or in some unusual forms of the environment like chronic inflammation or iron restriction.

All in all, the results suggest that the C282Y mutation has not been found to be a cause of iron deficiency in this Indian cohort, but that the dose-dependent effect of the HFE H63D mutation on iron status is observed in both Growing Girls and Expecting Mothers. The risk is even more so among GG homozygotes and this genetic susceptibility may be of significant use in the context of screening and early intervention of high-risk populations in regard to iron deficiency anemia.

Discussion

This paper has looked at how HFE (C282Y and H63D) and TFR2 gene polymorphisms have been involved in iron homeostasis in two physiologically different yet high-risk groups of iron deficiency anemia (IDA): Growing Girls and Expecting Mothers. The results illustrate that there exist significant genetic effects on iron metabolism within this population especially on the mutation of HFE H63D.

The nonoccurrence of homozygous C282Y genotypes and the infrequency of heterozygotes in both groups are in line with the prior genetic census results that revealed that the mutation is very rare in the South Asian populations (Merryweather-Clarke et al., 2000; Gorakshakar & Colah, 2009). This is unlike in the European cohorts where C282Y is a significant cause of hereditary hemochromatosis (Feder et al., 1996). C282Y will, therefore, not be of significant contribution to the IDA susceptibility in the population under the study.

However, the HFE H63D mutation was much more common and showed a dose-dependent association with biochemical and hematological indices of iron status. The carriers of the GC genotype and especially the GG homozygotes had much lower haemoglobin and ferritin, higher total iron-binding capacity (TIBC), and lower transferrin saturation (TS%) as compared to controls, in agreement with the defects of iron storage and transport. These results indicate a hypothesis that H63D modulates the communication between HFE and transferrin receptors (TFR1 and TFR2), which causes hepcidin production dysregulation and inefficient iron absorption (Pietrangelo, 2004; Girelli et al., 2011).

The trends in the dose-response are also consistent with other previous reports in non-European cohorts, where the H63D mutation was found to be linked to small, but clinically significant, changes in iron indices, especially in situations of augmented iron demands like growth and pregnancy (Piperno et al., 2000; Distante et al., 2004). The relationship between GG genotype and anemia was found to be statistically significant in our study of Growing Girls and a similar trend was observed with Expecting Mothers though

constrained by limited sample size of GG carriers. Physiological iron needs in adolescence and pregnancy may reveal the subclinical impact of this mutation, and put individuals at risk of developing IDA despite iron ingestion in the diet.

The iron parameters analysed in this paper showed no significant differences between the TFR2 polymorphism in either group. Although this might imply that this population has a minor impact on the risk of IDA, TFR2 is a significant sensor in the systemic iron regulation (Camaschella et al., 2000; Kawabata et al., 2000). That is why its possible impact on genetic modifiers, particularly with the presence of HFE mutations, should be investigated further. It is possible that the absence of this association can be attributed to the relatively small effect sizes of the variants of TFR2, or to the ethnic-specific frequencies of alleles, or to the fact that one would require a larger sample to identify the relatively small interaction between genes and the environment.

There are a number of clinical implications of our findings. To begin with, they indicate that genetic screening of the H63D mutation may be used to identify individuals who are at greater risk of developing IDA, particularly during high-demand physiological conditions. Second, the information may be used to develop specific nutritional therapy and iron supplementation programs, which may enhance the performance in the most susceptible groups of adolescent girls and pregnant women. Last but not least, the variability of the genetic determinants of iron metabolism among different ethnic groups and the absence of C282Y and the minimal influence of the TFR2 variants are the reasons to consider population-specific genetic risk profiling.

6. Conclusion

The article demonstrates that the HFE H63D polymorphism can play an important role in determining iron homeostasis and risk of anemia in Growing Girls and Expecting Mothers. Although C282Y mutation was practically absent, the TFR2 variants did not demonstrate a direct relationship with iron status, the H63D mutation had a very clear dose-dependent effect on hematological and biochemical indicators of iron deficiency.

The gradual reduction in hemoglobin and ferritin concentrations and the increment of TIBC and a degradation of transferrin saturation among heterozygous and homozygous carriers of the H63D mutation highlight the functional utility of this variant in states characterized by high iron requirements like adolescence grade and pregnancy. These genetic influences were enhanced especially among GG homozygotes who showed significantly increased vulnerability in relation to anemia than the wild-type. The fact that TFR2 did not have any significant effects in this study does not exclude the possibility of its being a genetic modifier, particularly in polygenic interactions or in very specific environmental conditions. However, these data indicate that the H63D mutation screening may prove helpful in detecting those individuals who are more likely to develop IDA in such populations to implement early dieting and clinical interventions.

Since the ethnic disparity of iron-regulatory gene variants is observed in its distribution, such findings also highlight the role of population-specific genetic profiling in designing specific anemia prevention and management approaches. Future studies can consider bigger and multi-centered cohorts, incorporate more iron related genes and use longitudinal designs to establish causality and improved risk prediction models. The introduction of genetic screening, especially on the HFE H63D, into community health interventions can be used to improve the prevention and early diagnosis of iron deficiency anaemia among high-risk populations of females in India and other similar environments.

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