

REVIEW

Open Access



TMPRSS6 gene polymorphisms associated with iron deficiency anaemia among global population

Farah Nur Elina Mohd Atan¹, Wan Asmuni Wan Mohd Saman², Yuhaniza Shafinie Kamsani^{3,5},
Zalilah Khalid² and Amirah Abdul Rahman^{1,4*} 

Abstract

Iron deficiency anaemia (IDA) has been recognised as a common global health problem that affects more than 1.2 billion people worldwide, particularly in high-risk individuals such as young children, pre-menopausal women, and pregnant women. In most cases, IDA arises due to the prolonged effect of iron deficiency (ID). On the other hand, it has been estimated that iron deficiency without anaemia is more frequent nowadays. Apart from the lack of nutrients, infections and inflammatory diseases, genetic factors can also be another factor that drives iron instability in the blood, leading to IDA. Previous studies, including genome-wide association studies, have identified multiple *trans-membrane protease serine 6 (TMPRSS6)* genetic variants associated with different iron parameters, especially variants contributing to an increase in hepcidin level, low blood, and iron status. Despite multiple studies on *TMPRSS6* gene polymorphisms, fewer studies are reported among the Asian population. Thus, further association studies of *TMPRSS6* genetic polymorphisms between ID and IDA are warranted among the Asian population. This review provides a comprehensive summary of the causative *TMPRSS6* genetic variants and their roles associated with iron deficiency among the global population.

Keywords: Iron deficiency, Iron deficiency anaemia, Hepcidin, *TMPRSS6* gene and single nucleotide polymorphisms

Introduction

One of the most crucial micro-elements required in various metabolic processes is iron. Iron plays two prominent roles: it has the properties of transition metals and can form protein complexes that are essential for body metabolism. For example, its ability to exist in ferric and ferrous states supports its function in essential enzyme activities involving oxygen and electron transport and cellular energy production. Iron's ability to bind and form physiologically active iron compounds such as heme iron and iron–sulphur clusters

is predominantly acquired for body iron absorption, iron transport and storage, DNA repair and replication, mitochondrial respiration, host defence and cell signalling [1]. In contrast, iron also has its disadvantages. In the presence of hydrogen peroxide and oxygen, it can produce harmful free radicals that have detrimental effects on DNA and cells, thus can disrupt the body's metabolism. Due to the human body's lack of iron excretion pathways, such as through the shedding of intestinal cells and blood loss, tight dietary iron uptake and systemic delivery regulations are crucial in maintaining body iron's bioavailability [2]. Its importance towards human life has led to identifying essential proteins involved in body iron homeostasis, such as transferrin, ferritin, ferroportin (FPN), and divalent metal-ion transporter 1 (DMT1). The discovery of hepcidin as the master regulator of iron homeostasis has

*Correspondence: amirahar@uitm.edu.my

¹ Institute For Medical Molecular Biotechnology, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia

Full list of author information is available at the end of the article

led to further discoveries on the body iron's primary transport and regulatory processes that lack iron excretion pathway [1, 2].

Normal body iron ranges from approximately 3–5 g [2]. A slight deviation from the normal range may lead to iron overload or iron deficiency (ID), resulting in pathologic consequences [2]. This review will focus more on ID and iron deficiency anaemia (IDA), a condition resulting from the prolonged effect of ID. An inadequate iron supply may impede the production of essential iron-containing proteins that are required for optimal cellular physiology, resulting in various adverse repercussions, and indirectly affecting erythropoiesis, the production of red blood cells. It shows that IDA may arise from the uncontrollable situation of iron inadequacy. However, the development of IDA is slow over the months or even years, depending on the availability of dietary iron and the effectiveness of the gastrointestinal function [3]. Iron deficiency anaemia is persistent among human populations and is the leading factor of anaemia worldwide. Anaemia is a condition in which the body's supply of red blood cells and haemoglobin is insufficient to meet the physiological body requirements. A systematic analysis of global anaemia data ranked IDA as the most common cause among the other cause-specific attribution for 17 conditions related to anaemia [4].

Global Burden of Disease Study reported that IDA was described as one of the leading causes of years of living with a disability (YLDs) in 2016, in which about 34 million people worldwide were affected [5]. Children under five presented 42% with anaemia, whereas reproductive-age and pregnant women represented 39% and 46% with anaemia in 2016, respectively. Women were consistently at higher risk of anaemia than men across all geographic regions and age groups. The elderly are also at risk as the prevalence of anaemia increase with age, although the evidence is scarce [6]. Gardner and Kassebaum reported a decrease in the prevalence of anaemia approximately by 4.2% in 2019 globally. Even though the prevalence has decreased, the total number of anaemia cases persisted, in which 1.74 billion individuals were diagnosed with anaemia in 2019. Central Sub-Saharan Africa, Western Sub-Saharan Africa, and South Asia had the highest burden across all age groups and both sexes [6, 7]. A systematic analysis of global burden anaemia reported that Sub-Saharan Africa contributes to 23.9% of worldwide anaemia. Meanwhile, South Asia accounted for approximately 37.5% of global anaemia [4] and studies have found that almost all of the countries in Southeast Asia have a significant prevalence of anaemia and IDA [8]. Even though there is still no concrete figure of individuals with ID and IDA, the high number of reported cases

of anaemia indicates that many people are suffering from ID [9].

The aetiology of IDA during puberty could be due to decreased iron intake or increased iron loss, iron malabsorption, chronic blood loss, pregnancy, or even a parasitic infection. Poor physical, mental, and cognitive performance in children with strong IDA relationships may continue until adulthood, resulting in low work efficiency and impacting economic production [10]. Frequent blood donations are also another little-known cause of IDA [11]. The Retrovirus Epidemiology Donor Study II (REDS-II) under the National Heart, Lung and Blood Institute (NHLBI) conducted a programme on the REDS-II Donor Iron Status Evaluation (RISE) reported that among 2425 individuals, 27.1% and 66.1% of frequent female donors had iron-restricted erythropoiesis and lack of iron stores, respectively, compared to 16.4% and 48.7% for male donors [12]. In the elderly, IDA is more difficult to cure, and it only accounts for about 30% of anaemic cases, as other kinds of anaemia may exist. Although oral iron supplementation can help iron-deficient patients to treat them, not all patients respond to the treatment provided, including intravenous (IV) iron treatment. This may be due to the individual's genetic factors that cause the effectiveness of the treatment given to be different from that of normal patients [13, 14].

Although definitive statistics of the prevalence of genetic variants of IDA are scarce, it is estimated that iron refractory iron deficiency anaemia (IRIDA) accounts for less than 1% of all instances of IDA seen in clinical practice [11]. Studies have shown that variability in iron concentrations with a heritability estimation of 20% to 30% is partly genetically determined [15]. The recognition of hepcidin and its role in iron metabolism has led scientists to study more on iron-related genes that caused the dysregulation of hepcidin to induce low iron status. Several genetic polymorphisms within the iron regulatory genes have been attributed to the disturbances in iron homeostasis, resulting in iron deficiency or overload [15–17]. Genetic variants leading to iron insufficiency are mainly found in *transferrin (TF)*, *solute carrier family 40 member 1 (SLC40A1)* and *transmembrane protease serine 6 (TMPRSS6)* genes. Among these three genes, loci on the *TMPRSS6* gene are commonly associated with low iron and low level of haematological indices such as haemoglobin and erythrocytes volumes [18–20]. The *TMPRSS6* gene encodes matriptase-2 (MT-2), one of the inhibitors of hepcidin expression. As mentioned earlier, hepcidin is the master regulator of iron homeostasis that reduces the amount of iron in the circulation, whereas the *TMPRSS6* gene plays a role in the hepcidin suppressive pathway to prevent further iron insufficiency.

Finberg et al. [21] found that normally *TMPRSS6* modifies or cleaves proteins such as hemojuvelin (HJV) that function as the hepcidin-activating pathway in the hepatocytes. Due to the polymorphisms arising in the *TMPRSS6* gene, these variants affect the catalytic activity domain of MT-2 and, thus, unable to suppress the hepcidin expression, causing a high level in the circulation, thus leading to anaemia due to lack of plasma iron [21]. Recent studies demonstrated that *TMPRSS6* gene polymorphisms were associated with elevated hepcidin levels in end-stage renal failure [22] and chronic kidney disease patients with IDA [23]. Mutations in the *TMPRSS6* gene were also reported in patients with IRIDA, a rare autosomal recessive disorder in which the hepcidin levels were shown to be elevated [24, 25]. Therefore, it is critical to understand the metabolic mechanism of the normal *TMPRSS6* gene and how the polymorphisms in the *TMPRSS6* gene cause IDA. Differences in the frequency and the trends of linkage disequilibrium of risk alleles may account for the limited replication of association results among global populations. As a result, there is a need to study the population-specific genetic variations that may influence iron status. This review provides a comprehensive summary of the causative genetic variants and their roles in iron deficiency among the global population, especially focused on the *TMPRSS6* gene. This paper also sought to summarise the association of *TMPRSS6* genetic variants on underlying diseases with IDA. The findings could assist in the design of future genetic association studies aimed at identifying population-specific genetic risk factors for iron deficiency and, eventually, directing population-specific iron intervention strategies.

A brief introduction to genetic polymorphism

The difference in DNA sequence between individuals, families, and populations is referred to as genetic polymorphism [26]. The most common genetic variation among humans is the single nucleotide polymorphism (SNP), which highlights this review. Single nucleotide polymorphism is defined as different nucleotides (specifically the nitrogenous base) found on the same locus in the same gene among different individuals. For instance, in a population, the DNA molecules for some individuals may have a T-A base pair at a specific nucleotide site, while others may have a C-G base pair at the same site. On average, SNP can be found at every 300–1000 bp in the genome [26]. Moreover, the SNP occurrence can be at multiple locations in the genome, such as at the coding, non-coding gene, promoter region, and exon–intron region. Due to the dispersion of SNP locations in the genome, this can affect the regulation of the genes and

affect the gene's production, which causes different responses to the treatment given among the diseased individuals.

Nonetheless, non-causative SNPs are also found close to a disease-causing region. In this case, the presence of an SNP that correlates with the presence of the disease may increase the chance of developing the disease. Although not causal, these SNPs are useful in diagnostics and disease propensity screening. Finding genetic variants in the iron regulatory gene *TMPRSS6* that may be associated with different responses to IRIDA and IDA treatment is therefore critical for understanding the underlying mechanism of these disorders and developing new interventions [19, 24].

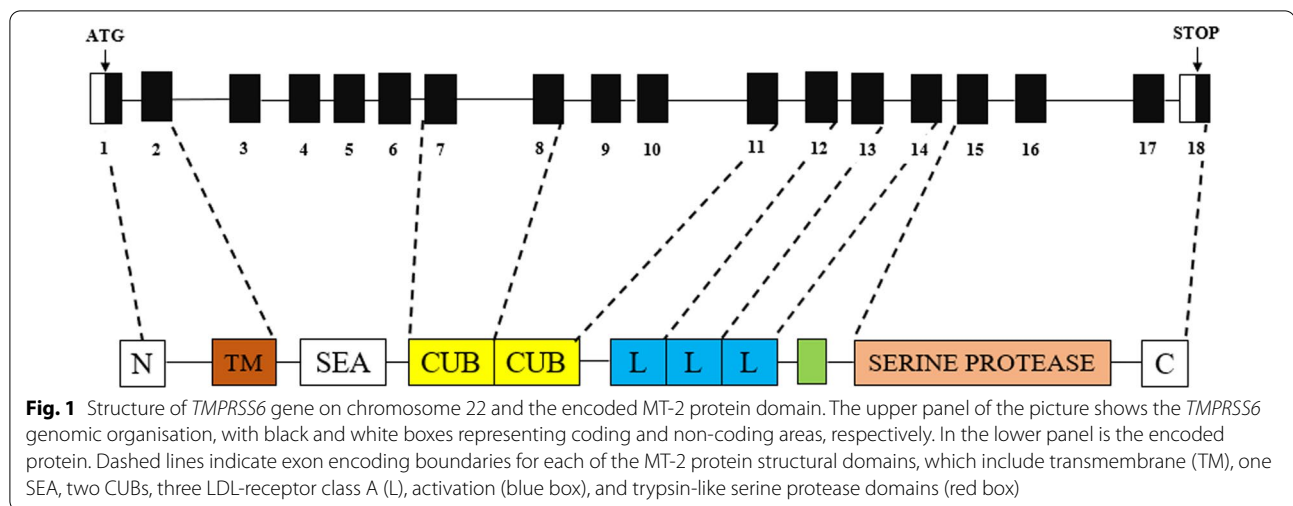
TMPRSS6 gene

The *transmembrane protease serine 6* (*TMPRSS6*) gene is highly conserved across mammalian species, ranging from 29 kb in mice to 40 kb in chimps [27]. The *TMPRSS6* gene located on chromosome 22 consists of 18 exons with 17 intervening introns, where the MT-2 protein domain boundaries correspond to the intron/exon junctions across all species. The first CUB domain is encoded by exons 7 and 8, whereas the second CUB domain is encoded by exon 9 until exon 11. Exons 12, 13 and 14 encode MT-2's three LDLR domains. Finally, exons 15 to 18 encode the serine protease domain, including the activation domain as shown in Fig. 1, which illustrates the structure of the *TMPRSS6* gene on chromosome 22 and the encoded MT-2 protein domain.

Accordingly, the structural features are conserved between humans, macaques, dogs, cows, mice, and rats, with the human protein sharing 95.6%, 91.1%, 85.6%, 80.1%, and 80.4% identical to MT-2 from these species, respectively [27]. Consistently, previous research used Western blot analysis of lysates from transiently transfected cells to show that both human and mouse MT-2 migrated close to the anticipated molecular mass of ~90 kDa [28]. This showed that MT-2 of mice mirrored human MT-2 protein. Furthermore, MT-2 mRNA expression was restricted to hepatocytes in the liver, predominant in glandular columnar epithelial cells in the uterine, and widespread throughout the kidney [28].

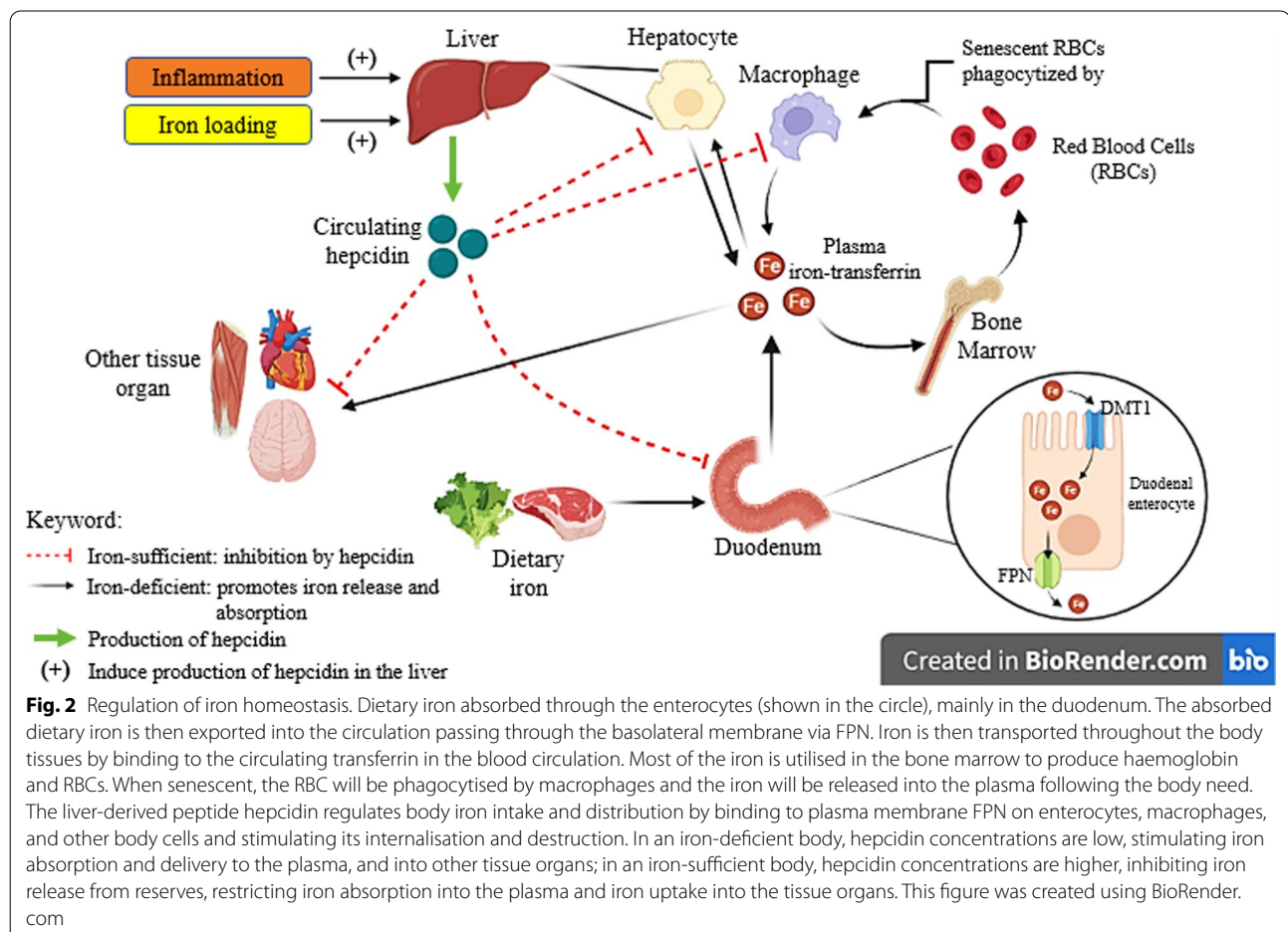
Regulation of systemic iron by Matritase-2

The biological process in humans that requires a high demand of iron is erythropoiesis, the production of erythrocytes. The primary component of circulating erythrocytes is haemoglobin, which has four heme groups and temporarily binds to oxygen molecules before releasing them throughout the body. When senescent, macrophages phagocytise the erythrocytes, making the iron available for reutilisation. To overcome the scarcity



of biologically available body iron and non-specific bodily iron losses such as through the shedding of intestinal cells and blood loss, tight dietary iron uptake by the enterocytes and its release from storing macrophages

and hepatocytes are crucial in maintaining the level of body iron [2]. Commonly, at the whole-body level, these processes are regulated by hepcidin (Fig. 2).



Hepcidin, a liver-derived hormone encoded by the *hepcidin antimicrobial peptide (HAMP)* gene plays an important role in regulating bodily iron supply in response to its needs. Hepcidin regulates iron entrance into the plasma by triggering the internalisation and degradation of the iron export molecule ferroportin (FPN), which is found on the surface of intestinal enterocytes, macrophages, and hepatocytes [29]. Hence, the release of iron into the circulation is prevented (Fig. 2). Iron status, inflammation and erythropoiesis are the major regulators of the hepcidin expression. Qiao et al. [29] reported that hepcidin binding on HEK293 cells that stably express FPN promotes the ubiquitination of FPN and the inhibition of FPN ubiquitination, preventing the FPN internalisation. In the non-classical ferroportin disease, the truncated form of FPN due to mutation inhibits the binding of hepcidin, thus causing the cells to be resistant towards hepcidin, eventually leading to iron overload [30]. Aschemeyer et al. [30] found that hepcidin binding not only caused FPN endocytosis but functioned in occluding the transporter. Meanwhile, the dysregulation of hepcidin expression may result in iron disorders, such as hemochromatosis, IRIDA, and IDA.

The bone morphogenetic protein (BMP) signalling pathway promotes hepatic hepcidin expression [31–33]. Hepatocytes produce hemojuvelin (HJV), a membrane GPI-linked protein that acts as a co-receptor for BMP2, BMP4 and BMP6. The main activator of hepcidin expression is BMP stimuli transmitted by the son of the mother against decapentaplegic (SMAD) proteins. Ideally, BMP2, BMP4, and BMP6 bind to the co-receptor membrane hemojuvelin (m-HJV) and the BMP receptor extracellularly in the normal regulation of hepcidin (BMPR). This causes phosphorylation of SMAD1, SMAD5, and SMAD8 and the formation of heteromeric complexes with the shared mediator SMAD4. Following nuclear translocation, the heteromeric SMAD complexes promote transcription of the hepcidin antimicrobial peptide (HAMP) gene. The produced hepcidin will then further inhibit the release of iron into circulation. Meanwhile, MT-2 will cleave the hemojuvelin on the plasma membrane in the event of an iron deficit, inactivating the hemojuvelin BMP-SMAD complex. As a result, hepcidin expression is reduced, which facilitates the iron absorption [27] as shown in Fig. 3.

The underlying mechanism for the MT-2 function is debatable. Previous studies, however, revealed that MT-2 reduces hepcidin expression by cleaving HJV via its proteolytic activity [34, 35]. Through in vitro findings showing MT-2 proteolytically processes membrane hemojuvelin, drastically lowering hepcidin transcription in response to BMP2 stimulus, the hemojuvelin/hepcidin regulatory system has been causally linked to the

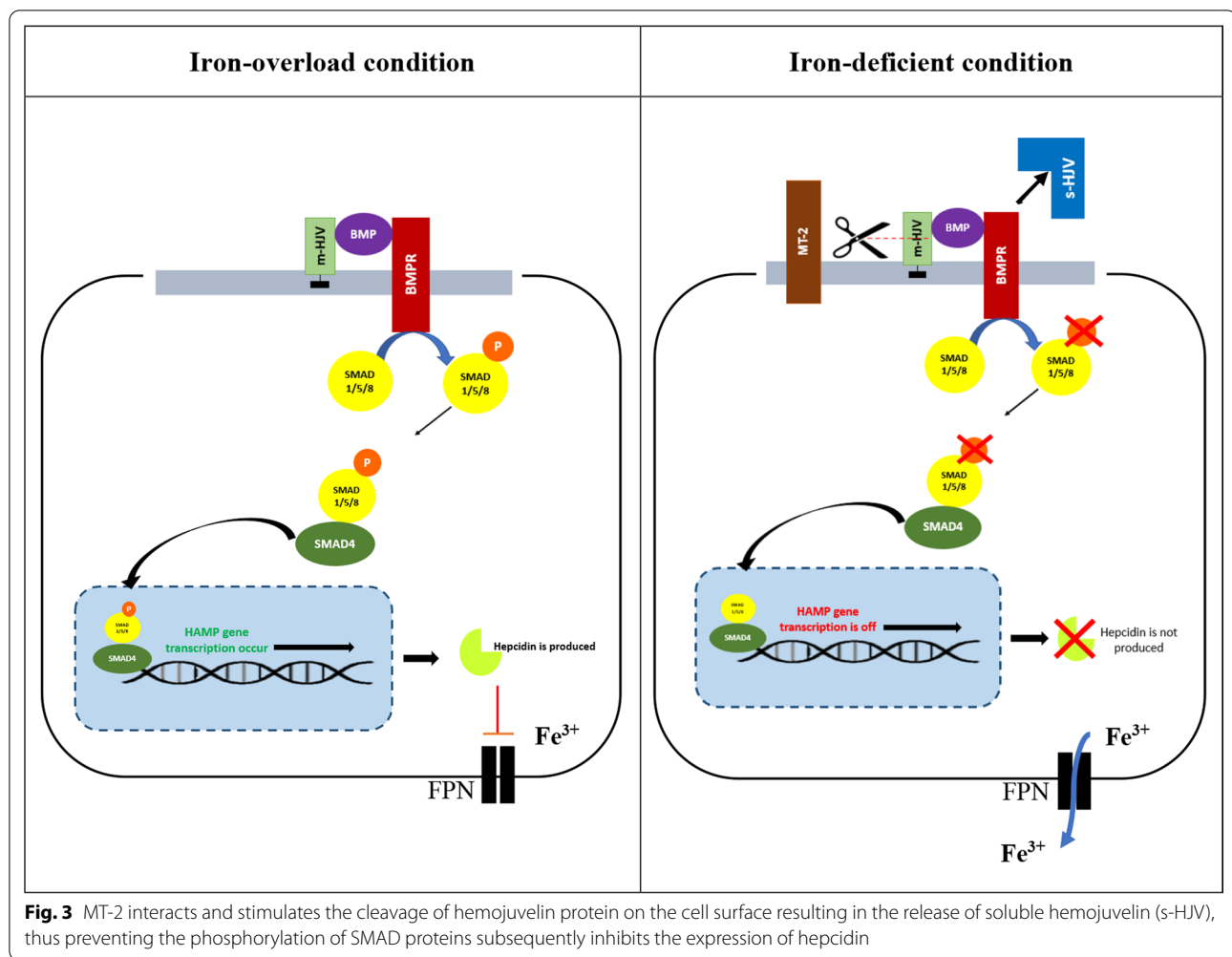
mouse *TMPRSS6*^{-/-} and mask phenotypes [34]. Recent evidence suggests that MT-2 acts independently of HJV in vivo, cleaving numerous components of the hepcidin induction pathway in HEK293 cells [36]. Enns, Jue, and Zhang, 2020, discovered that MT-2 could cleave HJV, Alk3, ActRIIA, and HFE in hepatoma cells. Validation of these in vitro and in vivo demonstrations is critical for further understanding of the mechanisms of MT-2 in systemic iron control.

Hepcidin-deficient mice and humans with hepcidin mutations consistently develop severe iron overload disorders [37–39]. Meanwhile, mice with a high level of transgenic hepcidin expression in the liver, on the other hand, suffer severe iron deficiency anaemia [40]. As a result, modulating hepcidin expression is a critical checkpoint for iron balance. A study performed by Wahedi and colleagues reported that MT-2 functions as a negative regulator of hepcidin expression [41] supporting theories demonstrated by Du et al. [42] in which mask mice (*TMPRSS6*^{-/-}) exhibit reduced dietary iron absorption due to the splicing defect on *TMPRSS6* gene, resulting in the high level of hepcidin production in the plasma. Conversely, mice lacking the *TMPRSS6* gene increase hepcidin transcription, overt alopecia phenotype, and severe iron deficiency anaemia [41, 43].

Furthermore, *TMPRSS6*^{-/-} mice had lower ferroportin protein levels on the basolateral membrane of duodenal enterocytes, resulting in iron retention inside these cells. Subcutaneous iron dextran administration efficiently recovers the phenotype, eliminating the hematologic deficits and restoring normal hair development. Critically, the pathophysiological changes in *TMPRSS6*^{-/-} mice are caused by a lack of MT-2 proteolytic activity, as demonstrated by the discovery of similar phenotypic abnormalities in mask mice, a murine model that expresses a proteolytically inactive version of MT-2 via chemical manipulation [42]. Wahedi et al. [41] also discovered that the membrane receptor hemojuvelin is proteolyzed by MT-2, which regulates hepcidin. These shreds of evidence showed that hepcidin is the key regulator of iron metabolism. Finally, by characterising human MT-2 gene mutations in IRIDA patients, Finberg et al., [21] presented strong evidence for the relevance of physiological observations made in *TMPRSS6*^{-/-} and mask mice for connections with human iron disorders. The study of these iron-related illnesses and the corresponding mice models has substantially improved our understanding of how iron homeostasis and hepcidin are regulated, as well as the signalling pathways involved.

Identification of SNPs in the *TMPRSS6* gene

Chronic illness anaemia is an acquired disease seen in people with inflammatory disorders such as infections,



malignancies, and rheumatoid arthritis [9, 27]. However, genetic factors play a significant role as well. Up to date, there is still a lack of understanding of iron-deficient anaemia individuals who are not genetically predisposed to abnormal iron metabolisms. Patients with iron deficiency anaemia who are insensitive to oral iron therapy and show inadequate hematologic recovery with parenteral iron infusions have been documented to have elevated hepcidin levels, a syndrome known as iron refractory iron deficiency anaemia (IRIDA) [13, 21, 44]. Variations in hepcidin regulatory pathway genes have been discovered as potential variables affecting iron status, with the consequences of their presence including aberrant iron status and, most likely, the development of IRIDA. Iron refractory iron deficiency anaemia (IRIDA) was an uncommon iron metabolism condition. Buchanan and Sheehan first characterised this condition in three siblings with IDA who were resistive to oral iron and only partially responsive to paternal iron dextran, implying a hereditary aetiology

[45]. Only 27 years later, Finberg et al. [21] revealed that this syndrome was caused by mutations in the *TMPRSS6* gene, which was located on chromosome 22q12-q13 and encodes MT-2.

Most studies on *TMPRSS6* SNPs that influence biochemical parameters were conducted in Caucasians, and fewer studies were run among the Asian population [46–52]. However, multiple *TMPRSS6* SNPs have been identified across all studies, profoundly associated with IRIDA, IDA, low iron, and blood indices. SNPs that have been identified were classified as synonymous, missense, intron, 5'-UTR and intergenic variants. The most frequently reported *TMPRSS6* SNP were rs855791 and rs4820268, linked to biomarkers of poor iron status and low blood indices. Other *TMPRSS6* SNPs were also linked to iron deficiency biomarkers such as rs2235321, rs2235324, rs5756504, rs5756506, and rs1421312. These SNPs were commonly found among the African, European, Caucasian, and Asian populations.

In 2009, genome-wide association studies (GWAS) on Indian Asian and European ancestry revealed that the most closely associated SNP with low haemoglobin (Hb) is rs855791, located on exon 17. Moreover, among both populations, the proportion of population variance for low Hb attributed by rs855791 is higher in Indian Asians (0.31%) than Europeans (0.25%) [53]. A study on Australian individuals discovered a significant association between the SNP rs855791, low transferrin saturation (TS) and serum iron (SI) among adolescents and adults. Furthermore, a strong association of rs855791 with low Hb levels and mean corpuscular volume (MCV) were observed. In addition, less than 3% of the proportion variance on low iron and blood parameters was attributed by rs855791 [16]. Based on the discovery that *TMPRSS6* polymorphisms may be risk factors for IDA, Beutler et al. [54] investigated the role of *TMPRSS6* gene polymorphisms in iron-deficient adults. As reported previously, Poggiali et al. [55] also revealed that the heterozygous and homozygous genotype of rs855791 was related to low SI, TS, Hb, and MCV in the IDA patients.

Among the European populations, rs855791 showed the strongest association with low SI, confirming previous studies' results. SNP of rs4820268 on exon 13 also showed a significant association and revealed a strong signal as rs855791, resulting in low SI, TS, Hb, MCV, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) [18, 48, 53, 56, 57]. High linkage disequilibrium (LD) between rs855791 and rs4820268 caused a further effect on iron imbalances [15, 16]. The two most prevalent SNPs in *TMPRSS6* are synonymous SNPs in the LDLRA domain (rs4820268) and a non-synonymous SNP (rs855791), causing the change in the MT-2 serine protease domain. Most likely, the synonymous SNP is in LD with a functional SNP within or around *TMPRSS6* [15] and had caused an iron imbalance due to the loss of the inhibitory effect of MT-2. Two other SNPs (rs2235320 and rs5756504) were moderately associated with low SI concentration in the IDA group among the European population due to these SNPs showed moderate LD with rs855791 and rs4820268 [15]. Other European populations also reported an association of SNPs, rs855791, rs2235320, rs4820268, rs11704654, and rs2543519, as the most recent significantly associated with iron refractory anaemia [58]. However, they did not find any association of the identified SNPs with clinical parameters such as Hb, MCV, SI, serum ferritin and TS, probably due to their small sample size ($n = 66$). Despite that, a possible association of *TMPRSS6* genetic variants with IRIDA caused the abnormal regulations of hepcidin expression as some of the SNPs act as a non-synonymous variant that altered the amino acid sequence, resulting in different MT-2 catalytic activity.

A meta-analysis study of African cohorts found that another SNP, rs2413450 in the *TMPRSS6* gene, is also associated with iron deficiency with a significant association with Hb concentrations [18]. However, in their meta-analysis, the most consistent SNP association with lower Hb concentrations were rs855791 [59] and rs4820268, as reported in the European population [15, 53]. However, the minor allele frequency (MAF) of rs855791 was higher in Caucasians and Asians than in Africans, similarly reported in a recent study [60]. Meanwhile, among the female black South African, rs228918 and rs228921 were identified and associated with increased soluble transferrin receptor (sTfR) concentration with homozygous (GG) allele combination compared to the combination of allele AA, reflecting the low iron status [61]. In contrast, healthy African-Gambian adults reported no significant association of genotype SNPs (rs855791, rs4820268 and rs2235321) with SI and haematological traits before and after the iron supplementation [62]. However, Jallow et al. [60] studied the effect of genotyped SNPs on the hepcidin concentration during pre- and post-iron supplementation. The analysis found that the concentration of hepcidin level is higher in the homozygous (GG) rs855791 carriers than in AG carriers. Findings on the African population contradicted the previous GWAS result, where the rs855791 AA has been associated with increased hepcidin concentrations accompanied by a low level of TS and SI in Europeans and Asians [63]. In addition, Nalado et al. [23] discovered that the rs855791 allele was not the risk allele in IDA in a study of chronic renal disease patients in South Africa, which exhibited similar results with the healthy African-Gambian diet population. Hence, *TMPRSS6* SNPs may exhibit different effects on the level of hepcidin, iron and blood biomarkers due to the difference in the population genetic background.

Transmembrane protease serine 6 (TMPRSS6) polymorphisms commonly described to be linked to IRIDA with relatively increased hepcidin levels; Kloss-Brandstätter et al. [64] sequenced the *TMPRSS6* exons and exon-intron boundaries and discovered another SNP (rs11704654) in a Serbian family with asymptomatic non-consanguineous parents and three out of four children suffering from IRIDA. Other previous commonly reported *TMPRSS6* SNPs were very frequent in the Dutch population in which adults from the population carrying the risk variant also exhibit low iron levels but not the severe iron insufficiency reported in the children [64]. These disparities could be attributed to the fact that the iron parameters of very young children were compared with those of adults. The phenotype could only be seen in cases of high iron demand, which occurs in early childhood and adolescence when high amounts of the iron are required for haemoglobin synthesis and growth.

This is consistent with findings in *TMPRSS6*-variant affected people, who have been demonstrated to have a more severe phenotype in early childhood and a milder phenotype with ageing [44, 58]. The Danish population study among the healthy adults blood donors reported that rs855791 has an additive effect of a decrease in iron stores or mainly known as ferritin in males, whereas no significant effect was observed among the female donors [65].

In an association study of SNPs in iron-related genes of multi-ethnic populations including whites, African Americans, Hispanic, and Asians, rs2111833 and rs1421312 in the *TMPRSS6* gene showed a significant association with iron biomarkers in which the strongest association was found for SNP rs2111833 in whites, where it showed an inverse correlation with SI and TS. In contrast, rs2111833 showed a weaker association with serum iron among Asians. Meanwhile, rs2111833 showed the strongest direct association with an increased level of unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) in Asians. In the African American group, an inverse relationship of SI and TS with rs1421312 was observed [66]. The SNP of rs2111833 was also identified in a recent study among Saudi Arabia female students between the age of 18 and 25. The common functional SNP (rs855791) were also found in the study subjects [19]. Al-Amer et al. [19] reported that rs855791 was significantly associated with low serum iron and serum ferritin but showed no association with low Hb, red blood cell (RBC), platelets and white blood cell (WBC). Contrary to the Caucasian adults, rs2111833 identified among the female Saudi Arabia showed no significant association with iron and blood parameters. Furthermore, Al-Amer and colleagues also reported that rs855791 showed a significant association with the risk of IDA, whereas for rs2111833 showed no significant association with IDA. Variability in environmental factors such as nutrient intake and lifestyle may explain the discrepancy of the results presented in different ethnicities, which could affect the phenotype outcome.

Another study conducted by Elmahdy et al. [24] reported SNP of rs4820268 showed the highest frequency of allele in the IDA group compared to the lean group, and this allele exhibits a highly significant association in the IRIDA group. There was a significant increase in the frequency of mutations in the IDA group and a highly significant increase in the frequency of mutations in the IRIDA group relating to the SNP of rs855791. Both SNPs of rs855791 and rs4820268 were highly associated with a significant decrease in Hb, MCV and MCH among the IDA patients that exhibit both or single mutant variants. A similar result was also observed among the IRIDA patients. Both SNPs are also significantly associated

with the low serum ferritin, serum iron, and high TIBC among the IDA and IRIDA groups. Their findings were consistent with those of Delbini et al. [58], who discovered a highly significant increase in the frequency of SNP rs855791 in people with IRIDA compared to healthy controls. According to the literature search, this is the most recent association study that found both SNPs to be significantly associated with increased hepcidin levels in IRIDA compared to the IDA group. Elmahdy et al. [21] findings agreed with previous findings in which IRIDA patients exhibit abnormally high levels of hepcidin due to variants found in the *TMPRSS6* gene, as reported earlier. Meanwhile, a study of Turkish IDA patients found that mutations in *TMPRSS6* rs855791, rs4820268, and rs2413450 are associated with increased RBC and TIBC in IDA patients [67].

Menstruating women have been identified as the most vulnerable to iron deficiency anaemia, and it is unlikely that heavy menstruating women have normal Hb and sufficient body iron levels. It is unclear whether the *TMPRSS6* SNP (rs855791) is associated with IDA in menstruating women because some do not develop IDA while others do. Among the Taiwanese IDA women, heterozygous (C > T) rs855791 increases the risk for IDA [50, 68]. Despite that, the homozygous (CC) of rs855791 has a protective effect against IDA, particularly in heavy menstruating women. The studied group with non-CC genotypes revealed an inverse correlation with Hb levels and menorrhagic condition compared to the homozygous (CC) genotype [50]. In vitro, the rs855791 C genotype suppresses hepcidin more effectively, and in the general population, C homozygotes have lower serum hepcidin levels and higher transferrin saturation [63]. A population-based study also showed that the *TMPRSS6* genetic variants (rs855791 and rs4820268) were strongly associated with the susceptibility of IDA in elderly Chinese women, in which the common SNPs were significantly associated with low iron biomarkers (SI and TS) and low Hb [57].

Shinta and colleagues discovered a link between the *TMPRSS6* gene polymorphism and iron intake/iron status in under-two-year-old toddlers in Lombok, Indonesia. All of these findings support the direct link between *TMPRSS6* gene mutations and IDA, as well as several clinical characteristics [46]. As previously identified that rs855791 and rs4820268 as the common variants associated with low iron and blood parameters among other populations. Both SNPs were also frequently identified among the children with IDA rs855791 and rs4820268, exhibiting significant association with low serum ferritin. However, no other associations were found between sTfR and Hb [46]. Gichohi-Wainaina et al. [59] reported that the minor allele frequency (MAF) of rs855791 is higher

in the Asian population than in the Caucasian. Across Caucasian and Asian populations, associations between *TMPRSS6* SNPs and anaemia are consistent [59]. Among the adult Japanese population, rs5756504 was associated with low Hb, MCV, and MCH in both genders [52]. Furthermore, among the Chinese adolescents, rs4820268 was associated with a low level of SF. However, there was no association with SF level with rs855791, a missense mutation in *TMPRSS6* with previously documented effect on iron status among the Chinese adults [57]. Meanwhile, a systemic evaluation of a paediatric cohort with IRIDA in India revealed several variants on the *TMPRSS6* gene, with most of the cases exhibiting 9 potentially deleterious intronic regions and 60% of the IRIDA cases revealing two benign exonic variations [47]. Bhatia et al. [47] also reported that four of the deleterious intronic region and both exonic variations were frequently found in most of the cases and most likely to act synergistically, contributing to an IRIDA phenotype.

In summary, SNPs identified in the *TMPRSS6* gene showed association across the multiple populations with different iron and blood parameters such as SI, TS, TIBC, unsaturated iron-binding capacity (UIBC), Hb, RBC, MCV and others. Findings from previous studies suggest a role for the identified SNPs in increasing the risk of iron deficiency in affected persons. Although GWAS has successfully identified risk alleles for complex genetic characteristics, the discovered risk alleles for a given trait do not fully explain the trait heritability. Furthermore, the allele frequencies and biological adaptations differ by ethnicity due to numerous environmental influences. Population-specific genetic investigations are therefore necessary. In addition, functional investigations are required to delve deeper into the mechanisms involved in the regulation of iron metabolism. All the identified SNPs of the *TMPRSS6* gene associated with various iron and blood indices are summarised in Table 1.

Risk allele of *TMPRSS6* gene polymorphism

In human GWAS, common genetic variations of *TMPRSS6* have been linked to erythrocyte and iron parameters [15, 18, 59, 61, 69]. The SNP rs855791 has recently been identified as the top-hit in GWAS linked to changes in SI, TS, erythrocyte MCV, Hb levels, and glycated Hb levels [62, 64]. The population frequency of the single nucleotide polymorphism (SNP) rs855791 of *TMPRSS6* is approximately 0.5 in Caucasians [16, 53], ≈ 0.6 in Japanese [51], and ≈ 0.2 – 0.1 in African Americans. SNP of rs855791 results in a non-synonymous substitution at the protease's catalytic and active site, which is strongly associated with iron status and erythrocyte characteristics, thus leading to IDA and/or IRIDA [19, 47, 53, 61, 65]. T allele variations of the rs855791 in

the *TMPRSS6* gene have been linked to an increased risk of ID and iron-deficient anaemia (IDA) [57]. In a Taiwanese case-control study, homozygotes for the SNP rs855791 CC had a lower prevalence of IDA than people with the CT or TT variant [50]. Meanwhile, variants (TT) in rs855791 are also related to lower TS and serum ferritin (SF), higher hepcidin, and higher ratios of hepcidin to iron indices in European and Saudi Arabia populations [19, 24, 63]. Surprisingly, the TT variation was also related to higher declines in SF and haemoglobin (Hb) after several donations in first-time blood donors, indicating a decreased capacity to replenish stockpiles following donation [65]. Among the African study population, two alleles, A and G of rs855791, resulted in the decrease in the different blood and iron parameters such as SI, Hb, and increased soluble transferrin receptors (sTfR) [18, 59, 61].

Changed in the alleles of rs855791 (G>A) revealed the strongest association with Hb concentration in the combined analysis study of Europeans and Indian Asian ancestry [53]. Among Europeans, rs855791 represents 0.25% of the population variance in haemoglobin, but in Indian Asians, it explained 0.31%. In the replication sample, Indian Asians were more common than Europeans to have the A allele of rs855791 associated with lower haemoglobin levels [53]. In contrast, a study of reproductive-age Pakistani women found that the rs855791 T allele is linked to the incidence of IDA [70]. A similar finding of the risk allele among Pakistani women was reported in a recent study by Buerkli et al. [68]. In a study of iron absorption in Taiwanese women utilising stable iron isotopes, the T allele was linked to a higher risk of developing iron deficiency, with serum iron and transferrin saturation being lower in the TT version of rs855791 [68]. This revealed that women with the *TMPRSS6* rs855791 (2321 C>T) polymorphism have impaired iron homeostasis, which affects oral iron absorption and may increase the risk of IDA. The reason why rs855791 has a big impact on contributing to the risk of IDA and exhibits in most of the IRIDA cases [14, 21] is that rs855791 is a non-synonymous variant in which it causes an alanine to valine amino acid change at a position of 736 nearby catalytic and binding sites of MT-2 in the *TMPRSS6* sequence [63, 71]. The non-synonymous variant may alter the amino acid sequence and may result in the malfunction or truncated form of active proteins involved in the iron regulatory pathways [26].

Minor allele frequency (MAF) in SNPs differs by racial and ethnic group. In the case of rs4820268, carriers of the G/G genotype had considerably lower SF levels than carriers of the A allele [48]. The MAF of rs855791 is lower (10%) in African people than that in East Asians (57%), South Asians (54%) and Europeans (39%). Similarly, the

Table 1 List of identified SNPs in the *TMPRSS6* gene across global population. (Location: Line 390)

SNP	Population/Ethnicity	Associated trait	Body Mass Index (BMI)	Age	Age group	Reference
rs9610643	India	Associated with IRIDA	Unspecified	6 months to 11 years old	Children	Bhatia et al. [47]
rs855791	European and Indian ancestry	Associated with Hb, MCV, MCH, and MCHC	Unspecified	Unspecified	Unspecified	Chambers et al. [53]
rs855791	Australians	Associated with reduced SI, TS, Hb, and MCV Associated with reduced SI, TS, Hb, and MCV	Unspecified Unspecified	10–17 years old 18–84 years old	Children and young adults Adults	Benyamin et al. [16]
rs855791	Caucasians	Associated with ID	More than 18 kg/m ²	35–75 years old	Adults	Beutler et al. [54]
rs855791	Italy and USA	Associated with low SI	Unspecified	50–80 years old	Adults	Tanaka et al. [15]
rs855791	Women Gambian African	Associated with low Hb and serum ferritin	18 kg/m ² > BMI > 30 kg/m ²	32–86 years old	Adults	Gichohi-Wainaina et al. [59, 61]
rs855791	Dutch ethnicity	Associated with low Hb, SI, and ferritin Associated with low SI and ferritin	Unspecified	1–17 years old 18–41 years old	Children Adults	Kloss-Brandstätter et al. [64]
rs855791	Danish blood donors	T allele associated with low serum ferritin in male donors	Unspecified	18–67 years old	Adults	Sørensen et al. [65]
rs855791	Female Saudi Arabia	T allele exhibit significant association with low SI and SF in IDA group	Unspecified	18–26 years old	Adults	Al Amer et al. [19]
rs855791	Egyptian	Associated with IDA and IRIDA	More than 18 kg/m ²	22–45 years old	Adults	Elmahdy et al. [24]
rs855791	Turkish	Associated with iron and blood parameters among IDA patients	Unspecified	18–50 years old	Adults	Batar et al. [67]
rs855791	Taiwanese	Associated with IDA, C allele showed as protective variant	Unspecified	18–40 years old	Adults	Pei et al. [50]
rs855791	Han Chinese	Associated in the risk of IDA, low SI, Hb and TS	Unspecified	50–70 years old	Adults	An et al. [57]
rs855791	India	Associated with IRIDA	Unspecified	6 months to 11 years old	Children	Bhatia et al. [47]
rs855791	Indonesia	Associated with low SI and ferritin	Unspecified	Below 2 years old	Children	Shinta et al. [46]
rs78174698	India	Associated with IRIDA	Unspecified	6 months to 11 years old	Children	Bhatia et al. [47]
rs4820268	European and Indian ancestry	Associated with Hb MCV, MCH and MCHC	Unspecified	Unspecified		Chambers et al. [53]
rs4820268	Dutch ethnicity	Associated with low Hb, SI and ferritin Associated with low SI and ferritin	Unspecified	1–17 years old 18–41 years old	Children Adults	Kloss-Brandstätter et al. [64]
rs4820268	African population	Associated with low Hb	Unspecified	5–13 years old		Gichohi-Wainaina et al. [18]
rs4820268	Italy and USA	Associated with low SI	Unspecified	50–80 years old	Adults	Tanaka et al. [15]
rs4820268	Egyptian	Associated in IRIDA	More than 18 kg/m ²	22–45 years old	Adults	Elmahdy et al. [24]
rs4820268	Chinese Adolescents	Associated with low SF	Unspecified	12–17 years old	Young adults	Piao et al. [48]
rs4820268	Han Chinese	Associated in the risk of IDA, low SI, Hb and TS	Unspecified	50–70 years old	Adults	An et al. [57]

Table 1 (continued)

SNP	Population/Ethnicity	Associated trait	Body Mass Index (BMI)	Age	Age group	Reference
rs4820268	Indonesia	Associated with low SI and ferritin	Unspecified	Below 2 years old	Children	Shinta et al. [46]
rs2413450	African population	Associated with low Hb	Unspecified	5–13 years old	Children	Gichohi-Wainaina et al. [18]
rs2160906	Italy and USA	Associated with low SI	Unspecified	50–80 years old	Adults	Tanaka et al. [15]
rs2111833	Whites, African Americans, Hispanics and Asians	Associated with SI, TS, UIBC and TIBC	Unspecified	25–50 years old	Adults	McLaren et al. [66]
rs2235324	Caucasians	Associated with ID	More than 18 kg/m ²	35–75 years old	Adults	Beutler et al. [54]
rs2235320	Italy and USA	Moderately associated with low SI	Unspecified	50–80 years old	Adults	Tanaka et al. [15]
rs228918	Women Gambian African	Associated with high sTfR level	18 kg/m ² > BMI > 30 kg/m ²	32–86 years old	Adults	Gichohi-Wainaina et al. [59, 61]
rs228921	Women Gambian African	Associated with high sTfR level	18 kg/m ² > BMI > 30 kg/m ²	32–86 years old	Adults	Gichohi-Wainaina et al. [59, 61]
rs2543519	India	Associated with IRIDA	Unspecified	6 months to 11 years old	Children	Bhatia et al. [47]
rs2072860	India	Associated with IRIDA	Unspecified	6 months to 11 years old	Children	Bhatia et al. [47]
rs5756504	Italy and USA	Moderately associated with low SI	Unspecified	50–80 years old	Adults	Tanaka et al. [15]
rs5756504	Japanese	Associated with Hb, MCV and MCH	Unspecified	35–69 years old	Adults	Seiki et al. [52]
rs5756516	India	Associated with IRIDA	Unspecified	6 months to 11 years old	Children	Bhatia et al. [47]
rs1421312	African American	Associated with SI and TS	Unspecified	25–50 years old	Adults	McLaren et al. [66]
rs1421312	Italy and USA	Associated with SI	Unspecified	50–80 years old	Adults	Tanaka et al. [15]

MAF of rs4820268 is lower in Africans (28%) than in Europeans (42%), although the MAF of rs2235321 in Africans (41%) is comparable to the European population (42%) [59]. SNPs of rs855791 and rs4820268 in *TMPRSS6* have been linked to lower iron and transferrin saturation. Lower SI concentrations, lower Hb levels, smaller red cells, and higher variability in red cell size have all been associated with the exon 13 variant, rs4820268 [15]. This variant has also been linked to a reduction in hepcidin levels in the urine [72].

Animal and in vitro research have revealed that deleting the *TMPRSS6* serine protease domain removes hepcidin inhibition [21]. The amino acid changed by rs855791 is positioned close to both the catalytic and specificity sites of the serine protease, according to comparison with other serine proteases and molecular modelling using PHYRE [53]. SNP rs855791 could be a causative variant, operating through altered protease activity or substrate binding, and rs4820268 was found to be in the linkage disequilibrium with rs855791, causing the rs4820268 to exhibit similar signals [15]. This association could be explained by changes in protease function and hepcidin-mediated iron homeostasis management. South and East

Asians had the highest iron risk alleles, while Africans, Americans, and Europeans had low iron risk alleles for both SNPs of rs855791 and rs4820268 [60]. The risk-associated T allele *TMPRSS6* A736V (rs855791) is common in the general population, with earlier research estimating a prevalence of 45% [63].

In other variants such as rs78174698, MAF is low across most of the global population except for the South Asian population, which the MAF showed to be more than 10% [60]. However, based on the previous studies, the association of other *TMPRSS6* SNPs with iron and blood parameters was reported; data reporting the risk alleles contributing to IDA were found to be in the intronic region of *TMPRSS6* gene that was still inadequate. This could be due to the smaller sample size of the study population, as well as persistent malnutrition and infections that cause IDA. It is possible that the differences in the allele frequency among the studied population occurred through the founder effects as humans migrated out from their original population [60]. According to our knowledge, the IRIDA phenotype is most likely explained by a combined effect of numerous heterozygous or homozygous intronic and/or exonic genetic

alterations in the *TMPRSS6* gene within single instances reported in the previous study.

At present, the significance of non-coding genetic variants in developing various complex features, human disease, and cancer is well understood. The absorption, transport, usage, and storage of iron in the body are all governed by a complex interplay of numerous genes. As a result, it is possible that the numerous phenotypes associated with iron deficiency or IRIDA-like

traits are attributable to an additive effect of changes in the *TMPRSS6* gene's non-coding regions. Variants located on the non-coding *TMPRSS6* gene may influence the expression of MT-2 as the variants could be on the upstream region or near the promoter region of the *TMPRSS6* gene. Findings that support the high frequency of intronic–exonic variants with a synergistic effect in the development of anaemia in human groups predisposed to the IDA/IRIDA-like phenotype could not be ignored [47]. This is due to the fact

Table 2 Risk allele of *TMPRSS6* gene polymorphism across global population associated with iron and blood parameters imbalance. (Location: Line 474)

SNP	Type of variant (Amino acid change)	Minor allele	Major allele	Risk allele	Population
rs9610643	Intron variant	G	A	N/A	India
rs855791	Missense variant (A736V)	A	G	A	European and Indian ancestry
		A	G	A	Australians
		A	G	A	Caucasians
		T	C	T	Italy and USA
		A	G	A	Women Gambian African
		G	A	A	Dutch ethnicity
		A	G	T	Danish blood donors
		T	C	T	Female Saudi Arabia
		T	C	T	Egyptian
		A	G	N/A	Turkish
		A	G	T	Taiwanese
		A	G	A	Han Chinese
rs78174698	Exon variant	A	G	N/A	India
		A	G	N/A	India
rs4820268	Missense variant	A	G	A	European and Indian ancestry
		A	G	A	Dutch ethnicity
		G	A	G	African population
		G	A	G	Italy and USA
		C	T	C	Egyptian
		G	A	G	Chinese adolescents
		G	A	G	Han Chinese
rs2413450	Intron variant	T	C	A	African population
rs2160906	Intron variant	A	G	N/A	Italy and USA
rs2111833	Synonymous variant	T	C	T	Whites, African Americans, Hispanics and Asians
rs2235324	Missense variant (K253E)	G	A	G	Caucasians
rs2235320	Intron variant	T	G	T	Italy and USA
rs228918	Intergenic variant	G	A	G	Women Gambian African
rs228921	Intergenic variant	G	A	G	Women Gambian African
rs2543519	Intron variant	G	A	N/A	India
rs2072860	Intron variant	G	A	N/A	India
rs5756504	Intron variant	T	C	T	Italy and USA
rs5756516	Intron variant	T	C	N/A	India
rs1421312	Intron variant	G	A	N/A	African American
		G	A	N/A	Italy and USA

that synonymous variants located in such regions can alter protein production, conformation, and function, mostly due to protein misfolding [47]. All the identified risk alleles of the *TMPRSS6* gene associated with various iron and blood indices are summarised in Table 2.

TMPRSS6 SNPs associated with other diseases

Polymorphisms of the *TMPRSS6* gene are not only seen to cause iron insufficiency and anaemia but also seen in other diseases such as celiac disease and chronic kidney diseases (CKD). One of the most common extra-intestinal symptoms of celiac disease is iron deficiency anaemia (IDA). Although IDA normally resolves with a gluten-free diet (GFD), some people experience persistent IDA, the mechanisms of which are unknown. However, studies found that rs855791 was significantly increased among celiac disease patients with persistent IDA compared to non-persistent IDA, and their findings suggested that the *TMPRSS6*'s involvement in predicting oral iron response and modifying hepcidin impact on iron absorption. In contrast, an association between rs855791 and persistent IDA among children with celiac disease was not observed [71]. These findings suggested that persistent IDA in children with celiac disease was uncommon. Meanwhile, *TMPRSS6* polymorphism of rs855791 was also reported among CKD patients [23]. Recent research had also linked *TMPRSS6* gene polymorphism to higher hepcidin levels in end-stage renal disease [22].

As the prevalence of overweight and obesity has increased globally, more research on adiposity and its vulnerability to ID in children and adults have been carried out [73, 74]. In the first study conducted in 1962, researchers discovered that obese adolescents had lower serum iron levels than non-obese adolescents [75]. Similar findings in obese adults indicated a strong association between iron insufficiency and obesity [76], validating the adverse relationship between adiposity and iron status. Even though obesity and ID are two different forms of nutritional problems current evidence suggests that overweight and obese people are more likely to develop ID. Various factors have been suggested as contributing to the relation between these health problems, such as adiposity-inflammatory condition, low level of physical activity and increased iron requirement due to the larger blood volume needed in obesity. However, none of these investigations could provide a clear explanation of how ID develops in obese individuals. Previous studies have found associations of *TMPRSS6* variants in various anaemic and IDA settings. However, none have linked the *TMPRSS6* gene polymorphism to increased hepcidin, low iron and haemoglobin in overweight and obese individuals.

Therefore, a study on *TMPRSS6* gene variants is warranted as the number of obese individuals with ID increases globally in both developed and developing countries. Furthermore, future studies on adiposity may help other researchers further understand the underlying iron metabolism due to the presence of SNPs that causes iron imbalance and elevated hepcidin via in vitro and in vivo demonstration. Thus, it may assist in designation of the new drugs and intervention to curb the arising ID problem among obese groups, indirectly helping the government reduce the health burden. In conclusion, previous studies showed that *TMPRSS6* gene polymorphism did not only caused iron imbalance among IRIDA and IDA patients but also exhibited similar signals in other diseases. These indicate the importance of the *TMPRSS6* gene in regulating the body iron metabolism, and genetic testing on *TMPRSS6* variants among IDA and pre-diagnosed IDA individuals might be the alternative treatment in combating this disease.

Conclusion

The *TMPRSS6* gene variants associated with iron levels were shown to be ethnic-specific, with the top two SNPs (rs855791 and rs4820268) being extremely common among Asians. Given the high prevalence of anaemia and iron deficiency in Asian countries such as India, China, Thailand, and Indonesia, as well as an increase in the prevalence of these diseases in Malaysia, little is known about the frequency of other identified SNPs in the *TMPRSS6* gene among Southeast Asians. While environmental and physiological factors contribute to the development of ID and IDA, genetic factors, for example SNPs, may predispose individuals to the condition. Other genes' epistatic effects cannot be ruled out. Further investigation into the functional characterisation of these unique genetic variants found in the *TMPRSS6* gene is required to better understand the underlying mechanisms of iron homeostasis in ID and IDA patients. These include determining the correlation of *TMPRSS6* SNPs with Hb, SI, and ferritin levels in IDA patients following iron supplementation. The association of *TMPRSS6* SNPs with inflammatory markers under inflammatory conditions could also be explored to see if such SNPs affect iron status outcomes. Genetic testing and rigorous mapping of genetic influences on iron status could aid in the development of novel diagnostic and iron intervention strategies in the future.

Abbreviations

DNA: Deoxyribonucleic acid; FPN: Ferroportin; DMT1: Divalent metal transporter 1; ID: Iron deficiency; IDA: Iron deficiency anaemia; IRIDA: Iron refractory iron deficiency anaemia; *TMPRSS6*: Transmembrane protease serine 6; MT-2:

Matriptase-2; HJV: Hemojuvelin; SNP: Single nucleotide polymorphism; BMP: Bone morphogenetic proteins; SMAD: Son of mother against decapentaplegic proteins; HAMP: Hepcidin antimicrobial peptide; GWAS: Genome-wide association studies; Hb: Haemoglobin; RBC: Red blood cells; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; SI: Serum iron; SF: Serum ferritin; TS: Transferrin saturation; TIBC: Total iron-binding capacity; UIBC: Unsaturated iron-binding capacity; sTfR: Soluble transferrin receptor; LD: Linkage disequilibrium; MAF: Minor allele frequency; PHYRE: Protein fold recognition server; GFD: Gluten-free diet; CKD: Chronic kidney disease.

Acknowledgements

Not applicable.

Author contributions

FNEMA and AAR have made a substantial contribution to the draft and are the major contributors to writing the manuscript. WAWMS interpreted regarding the haematological and iron study parameters. YSK and ZK contributed to the interpretation of the findings. All authors read and approved the final manuscript.

Funding

This project is supported by the Ministry of Higher Education's Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers (FRGS-RACER); RACER/1/2019/SKK08/UITM//5 and Universiti Teknologi MARA's Geran Insentif Penyelidikan (GIP); 600-RMC/GIP5/3(157/2021).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Institute For Medical Molecular Biotechnology, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia. ²Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia. ³Department of Physiology, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia. ⁴Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia. ⁵Maternofetal and Embryo (MatE) Research Group, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia.

Received: 15 April 2022 Accepted: 25 October 2022

Published online: 02 November 2022

References

- Dev S, Babitt JL (2017) Overview of iron metabolism in health and disease. *Hemodial Int* 21:S6–S20
- Anderson GJ, Frazer DM (2017) Current understanding of iron homeostasis. *Am J Clin Nutr* 106:1559S–1566S
- Miller JL (2013) Iron deficiency anemia: a common and curable disease. *Cold Spring Harb Perspect Med* 3:1–13
- Kassebaum NJ, Jasrasaria R, Naghavi M et al (2014) A systematic analysis of global anemia burden from 1990 to 2010. *Blood* 123:615–624
- Vos T, Abajobir AA, Abbafati C et al (2017) Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the global burden of disease study 2016. *Lancet* 390:1211–1259
- Chaparro CM, Suchdev PS (2019) Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. *Ann NY Acad Sci* 1450:15–31
- Gardner W, Kassebaum N (2020) Global, regional, and national prevalence of anemia and its causes in 204 countries and territories, 1990–2019. *Curr Dev Nutr* 4(Suppl 2):830. https://doi.org/10.1093/cdn/nzaa053_035
- Kotwal A (2016) Iron deficiency anaemia among children in South East Asia: determinants, importance, prevention and control strategies. *Curr Med Res Pract* 6:117–122
- Camaschella C (2019) Iron deficiency. *Blood* 133:30–39
- Al-Alimi AA, Bashanfer S, Morish MA (2018) Prevalence of iron deficiency anemia among University students in Hodeida province, Yemen. *Anemia* 2018:1–7
- Cappellini MD, Musallam KM, Taher AT (2020) Iron deficiency anaemia revisited. *J Intern Med* 287:153–170
- Cable RG, Glynn SA, Kiss JE et al (2012) Iron deficiency in blood donors: the REDS-II donor iron status evaluation (RISE) study. *Transfusion* 52:702–711
- Brissot P, Bernard DG, Brissot E et al (2018) Rare anemias due to genetic iron metabolism defects. *Mutat Res Rev Mutat Res* 777:52–63
- Capra AP, Ferro E, Cannavò L et al (2017) A child with severe iron-deficiency anemia and a complex TMPRSS6 genotype. *Hematology* 22:559–564
- Tanaka T, Roy CN, Yao W et al (2010) A genome-wide association analysis of serum iron concentrations. *Blood* 115:94–96
- Benyamin B, Ferreira MAR, Willemsen G et al (2009) Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. *Nat Genet* 41:1173–1175
- Pandey S, Pandey SK, Shah V (2018) Role of HAMP genetic variants on pathophysiology of iron deficiency anemia. *Indian J Clin Biochem* 33:479–482
- Gichohi-Wainaina WN, Tanaka T, Towers GW et al (2016) Associations between common variants in iron-related genes with haematological traits in populations of african ancestry. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0157996>
- Al-amer O, Hawasawi Y, Abdulwahab A et al (2020) Study the association of transmembrane serine protease 6 gene polymorphisms with iron deficiency status in Saudi Arabia. *Gene* 751:144767
- Al-Jamea LH, Woodman A, Heiba NM et al (2021) Genetic analysis of TMPRSS6 gene in Saudi female patients with iron deficiency anemia. *Hematol Oncol Stem Cell Ther* 14:41–50
- Finberg KE, Heeney MM, Campagna DR et al (2008) Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nat Genet* 40:569–571
- Dopsaj V, Topić A, Savković M et al (2019) Associations of common variants in HFE and TMPRSS6 genes with hepcidin-25 and iron status parameters in patients with end-stage renal disease. *Dis Mark* 2019:1–10
- Nalado AM, Dickens C, Dix-Peek T et al (2019) TMPRSS6 rs855791 polymorphism and susceptibility to iron deficiency anaemia in non-dialysis chronic kidney disease patients in South Africa. *Int J Mol Epidemiol Genet* 10:1–9
- Elmahdy M, Elhakeem H, Gaber F et al (2018) TMPRSS6 gene polymorphism and serum hepcidin in iron deficiency anemia. *Egypt J Hosp Med* 73:7090–7103
- Yoshida N, Sakaguchi H, Matsumoto K et al (2012) Two novel mutations in the tmprss6 gene associated with iron-refractory iron-deficiency anaemia (irida) and partial expression in the heterozygous form Iron-refractory. *Br J Haematol* 158:666–668
- Ismail S, Essawi M (2012) Genetic polymorphism studies in humans. *Middle East J Med Genet* 1:57–63
- Ramsay AJ, Hooper JD, Folgueras AR et al (2009) Matriptase-2 (TMPRSS6): A proteolytic regulator of iron homeostasis. *Haematologica* 94:840–849
- Hooper JD, Campagnolo L, Goodarzi G et al (2003) Mouse matriptase-2: Identification, characterization and comparative mRNA expression analysis with mouse hepsin in adult and embryonic tissues. *Biochem J* 373:689–702

29. Qiao B, Sugianto P, Fung E et al (2012) Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. *Cell Metab* 15:918–924
30. Aschemeyer S, Qiao B, Stefanova D et al (2018) Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. *Blood* 131:899–910
31. Kautz L, Meynard D, Monnier A et al (2008) Iron regulates phosphorylation of Smad1/5/8 and gene expression of Bmp6, Smad 7, Id1, and Atoh8 in the mouse liver. *Blood* 112:1503–1509
32. Wang RH, Li C, Xu X et al (2005) A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. *Cell Metab* 2:399–409
33. Babitt JL, Huang FW, Wrighting DM et al (2006) Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. *Nat Genet* 38:531–539
34. Silvestri L, Pagani A, Nai A et al (2008) The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. *Cell Metab* 8:502–511
35. Finberg KE, Whittlesey RL, Fleming MD et al (2010) Down-regulation of Bmp/Smad signaling by Tmprss6 is required for maintenance of systemic iron homeostasis. *Blood* 115:3817–3826
36. Enns CA, Jue S, Zhang AS (2020) The ectodomain of matriptase-2 plays an important nonproteolytic role in suppressing hepcidin expression in mice. *Blood* 136:989–1001
37. Nicolas G, Bennoun M, Devaux I et al (2001) Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proc Natl Acad Sci U S A* 98:8780–8785
38. Roettol A, Papanikolaou G, Politou M et al (2003) Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nat Genet* 33:21–22
39. Zarghamian P, Azarkeivan A, Arabkhazaeli A et al (2020) Hepcidin gene polymorphisms and iron overload in β -thalassemia major patients refractory to iron chelating therapy. *BMC Med Genet* 21:1–5
40. Nicolas G, Bennoun M, Porteu A et al (2002) Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *Proc Natl Acad Sci U S A* 99:4596–4601
41. Wahedi M, Wortham AM, Kleven MD et al (2017) Matriptase-2 suppresses hepcidin expression by cleaving multiple components of the hepcidin induction pathway. *J Biol Chem* 292:18354–18371
42. Du X, She E, Gelbart T et al (2008) The serine protease TMPRSS6 is required to sense iron deficiency. *Science* 320:1088–1092
43. Folgueras AR, De Lara FM, Pendás AM et al (2008) Membrane-bound serine protease matriptase-2 (Tmprss6) is an essential regulator of iron homeostasis. *Blood* 112:2539–2545
44. De Falco L, Totaro F, Nai A et al (2010) Novel TMPRSS6 mutations associated with iron-refractory iron deficiency anemia (IRIDA). *Hum Mutat.* <https://doi.org/10.1002/humu.21243>
45. Buchanan GR, Sheehan RG (1981) Malabsorption and defective utilization of iron in three siblings. *J Pediatr* 98:723–728
46. Shinta D, Asmarinah C et al (2019) The association of TMPRSS6 gene polymorphism and iron intake with iron status among under-two-year-old children in Lombok, Indonesia. *Nutrients* 11:878
47. Bhatia P, Singh A, Hegde A et al (2017) Systematic evaluation of paediatric cohort with iron refractory iron deficiency anaemia (IRIDA) phenotype reveals multiple TMPRSS6 gene variations. *Br J Haematol* 177:311–318
48. Piao W, Wang L, Zhang T et al (2017) A single-nucleotide polymorphism in transferrin is associated with soluble transferrin receptor in Chinese adolescents. *Asia Pac J Clin Nutr* 26:1170–1178
49. Athiyarath R, Shaktivel K, Abraham V et al (2015) Association of genetic variants with response to iron supplements in pregnancy. *Genes Nutr* 10:1–8
50. Pei SN, Ma MC, You HL et al (2014) TMPRSS6 rs855791 polymorphism influences the susceptibility to iron deficiency anemia in women at reproductive age. *Int J Med Sci* 11:614–619
51. Kamatani Y, Matsuda K, Okada Y et al (2010) Genome-wide association study of hematological and biochemical traits in a Japanese population. *Nat Genet* 42:210–215
52. Seiki T, Naito M, Hishida A et al (2018) Association of genetic polymorphisms with erythrocyte traits: verification of SNPs reported in a previous GWAS in a Japanese population. *Gene* 642:172–177
53. Chambers JC, Zhang W, Li Y et al (2009) Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. *Nat Genet* 41:1170–1172
54. Beutler E, Van Geet C, te Loo DMW et al (2010) Polymorphisms and mutations of human TMPRSS6 in iron deficiency anemia. *Blood Cells Mol Dis* 44:1–7
55. Poggiali E, Andreozzi F, Nava I et al (2015) The role of TMPRSS6 polymorphisms in iron deficiency anemia partially responsive to oral iron treatment. *Am J Hematol* 90:306–309
56. Hsueh W, Bennett PH, Esparza-romero J et al (2018) variants in mexican pima Indians: marked allelic. *Ann Hum Genet* 82:287–299
57. An P, Wu Q, Wang H et al (2012) TMPRSS6, but not TF, TFR2 or BMP2 variants are associated with increased risk of iron-deficiency anemia. *Hum Mol Genet* 21:2124–2131
58. Delbini P, Vaja V, Graziadei G et al (2010) Genetic variability of TMPRSS6 and its association with iron deficiency anaemia. *Br J Haematol* 151:281–284
59. Gichohi-Wainaina WN, Towers GW, Swinkels DW et al (2015) Inter-ethnic differences in genetic variants within the transmembrane protease, serine 6 (TMPRSS6) gene associated with iron status indicators: a systematic review with meta-analyses. *Genes Nutr* 10:1–15
60. Jallow MW, Cerami C, Clark TG et al (2020) Differences in the frequency of genetic variants associated with iron imbalance among global populations. *PLoS ONE* 15:e0235141
61. Gichohi-Wainaina WN, Melse-Boonstra A, Swinkels DW et al (2015) Common variants and haplotypes in the TF, TNF- α , and TMPRSS6 genes are associated with iron status in a female black South African population. *J Nutr* 145:945–953
62. Jallow MW, Campino S, Saidykhan A et al (2021) Common variants in the TMPRSS6 gene alter hepcidin but not plasma iron in response to oral iron in healthy gambian adults: a recall-by-genotype study. *Curr Dev Nutr* 5:1–10
63. Nai A, Pagani A, Silvestri L et al (2011) TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum. *Blood J* 118:4459–4463
64. Kloss-Brandstätter A, Erhart G, Lamina C et al (2012) Candidate gene sequencing of SLC11A2 and TMPRSS6 in a family with severe anaemia: common SNPs, rare haplotypes, no causative mutation. *PLoS ONE* 7:1–8
65. Sørensen E, Rigas AS, Thørmøer LW et al (2016) Genetic factors influencing ferritin levels in 14,126 blood donors: results from the danish blood donor study. *Transfusion* 56:622–627
66. McLaren CE, McLachlan S, Garner CP et al (2012) Associations between single nucleotide polymorphisms in iron-related genes and iron status in multiethnic populations. *PLoS ONE* 7:e38339
67. Batar B, Bavunoglu I, Hacıoglu Y et al (2018) The role of TMPRSS6 gene variants in iron-related hematological parameters in Turkish patients with iron deficiency anemia. *Gene* 673:201–205
68. Buerkli S, Pei SN, Hsiao SC et al (2021) The TMPRSS6 variant (SNP rs855791) affects iron metabolism and oral iron absorption - a stable iron isotope study in Taiwanese women. *Haematologica* 106:2897–2905
69. Jallow MW, Campino S, Prentice AM et al (2019) A recall-by-genotype study on polymorphisms in the TMPRSS6 gene and oral iron absorption: a study protocol [version 1; peer review: 2 approved with reservations]. *F1000Research* 8:1–12
70. Lone NM, Shah SHS, Farooq M et al (2020) Role of TMPRSS6 rs855791 (T > C) polymorphism in reproductive age women with iron deficiency anemia from Lahore, Pakistan. *Saudi J Biol Sci* 28:748–753
71. Urbaszek K, Drabińska N, Szaflarska-Popławska A et al (2021) Tmprss6 rs855791 polymorphism status in children with celiac disease and anemia. *Nutrients.* <https://doi.org/10.3390/nu13082782>
72. Pichler I, Minelli C, Sanna S et al (2011) Identification of a common variant in the TFR2 gene implicated in the physiological regulation of serum iron levels. *Hum Mol Genet* 20:1232–1240
73. Shekariz R, Mehdi VM (2017) Iron profile and inflammatory status of overweight and obese women in Sari, north of Iran. *Int J Hematol Stem Cell Res* 11:108–113
74. Siyaram D, Bhatia P, Dayal D et al (2018) Hypoferremic state in overweight and obese children. *Indian Pediatr* 55:72–73
75. Wenzel BJ, Stults HB, Mayer J (1962) Hypoferræmia in obese adolescents. *Lancet* 280:327–328

76. Jordaan EM, Van den Berg VL, Van Rooyen FC et al (2020) Obesity is associated with anaemia and iron deficiency indicators among women in the rural Free State, South Africa. *S Afr J Clin Nutr* 33:1–7

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)
