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RESEARCH

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TMPRSS6 rs855791 polymorphism is associated with iron deficiency in a cohort of Sri Lankan pregnant women

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Abstract

Background: Hepcidin is the key regulator of systemic iron homeostasis and is downregulated by matriptase 2 (MT2), a protease encoded by *TMPRSS6* gene. In the presence of low iron levels, MT2 cleaves membrane-bound hemojuvelin (HJV), causing a negative regulation of hepcidin at the gene level, and restores iron balance. rs855791T > C, a missense variant in the catalytic domain of MT2, causes valine to alanine change at 736 position. The current study aimed to investigate the association of *TMPRSS6* rs855791 on iron status among a cohort of pregnant women in Sri Lanka and to predict the possible molecular mechanisms.

Methods: The study was conducted among 73 pregnant women at ≤ 12 weeks of gestation. Iron deficiency was defined as serum ferritin < 30 µg/L after adjusting for inflammation. rs855791 was genotyped with a PCR–RFLP, and its association with iron deficiency was analyzed using binary logistic regression. Docking of HJV with MT2 protein encoded by the two rs855791 alleles was undertaken in silico to predict the molecular mechanism of the observed associations.

Results: The majority of the study population (70%) were iron deficient. Among the subjects, T allele was prevalent in the iron deficient group with a frequency of 61.8%, with a nearly twofold enhanced risk for iron deficiency (OR = 2.566, 95%CI; *P* = 0.011). For TT genotype, the risk of iron deficiency was nearly sixfold (OR = 5.867; 95%CI; *P* = 0.023). According to the *in silico* analysis, MT2 736A and HJV complex is more stable with an interface energy of − 7.934 kJ/mol compared to the MT2 736 V and HJV complex which generates an interface energy of − 4.689 kJ/mol.

Conclusion: The current study suggests that the iron regulatory effect of rs855791 of *TMPRSS6* is brought about by the differences in thermodynamic stability of the two protein complexes made by MT2 and HJV proteins. The prevalence of iron deficiency observed among Sri Lankan pregnant women may be an interplay between the prevalence of rs855791 T allele and the low dietary iron intake.

Keywords: Genetic association studies, Hepcidin, Homology models, Iron deficiency anemia, Iron homeostasis, Membrane bound hemojuvelin, Serum ferritin

Introduction

Iron deficiency (ID) and iron deficiency anemia (IDA) [1] are a major public health problem, accounting for 75% of global mortality [2]. Pregnant women and women of childbearing age are at high risk of developing ID due to their high iron requirements [3]. According to the

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latest national survey in Sri Lanka, the prevalence of ID among pregnant women was 36.9%. [4]. ID is a multifactorial disease where numerous factors such as acute and chronic infections [5], socioeconomic conditions [6], diet [7] and genetics [8–10] are at interplay.

Hepcidin is the key regulator of systemic iron homeostasis [11] and is primarily produced by the liver. Hepcidin binds to ferroportin (Fpn1) which is the only iron transporter, on plasma membrane of enterocytes, hepatocytes and macrophages and induces internalization and degradation. Hepcidin is encoded by the human antimicrobial peptide (*HAMP*) gene [12]. The normal production of hepcidin from *HAMP* is influenced by many factors. For example, mutations such as C282Y and H63D in the human hemochromatosis gene (*HFE*) has shown to produce hepcidin deficiency in the liver [13–15]. In addition, various systemic factors like changes in body iron stores, inflammation, hypoxia, rate of erythropoiesis [16] as well as the availability regulatory messenger molecules like matriptase 2 (MT2) can affect the hepcidin levels in the liver [17].

Although genetic factors contributing to ID are still not fully elucidated, it is possible for the polymorphisms present within the genes encoding for iron regulatory proteins such as *HJV, TMPRSS6, TFR1, TFR2, HFE, IL6, BMPR1 and BMPR2* to play a significant role in causing ID. Accordingly, many genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) that affect body iron levels [8, 10, 18]. Among these SNPs, rs855791, a missense variation located at the exon 17, functional (catalytic) domain (2321 T > C) in the *TMPRSS6* gene [19] has shown significant associations with the iron parameters in GWAS [20, 21] as well as in candidate gene studies in Taiwan and Chinese Han population [22, 23]. *TMPRSS6* gene encodes for MT2 which is a type II transmembrane serine proteinase. In the presence of ID, MT2 cleaves membrane-bound hemojuvelin (HJV), blocking the *bone morphogenetic protein—sons of mothers against decapentaplegic* (BMP-SMAD) signaling pathway, which is one of the regulatory pathways for hepcidin gene transcription. This negatively regulates the *HAMP* gene expression causing serum hepcidin to go down allowing an increase in iron absorption [17]. TMPRSS6 rs855791 causes a valine (V) to alanine (A) change at 736 position in the MT2 protein (V736A variant) [19, 24] altering its ability to inhibit hepcidin transcription [25].

Given this context, the current study was conducted to investigate the association of the SNP rs855791 with iron deficiency among a cohort of Sri Lankan pregnant women. To the best of our knowledge, there were no studies conducted on the genetic basis of ID among Sri Lankan women, pregnant or otherwise. To elucidate the molecular mechanism of observed association, an *in silico* analysis consisting of protein–protein docking model was also conducted.

Methods

Study participants

The current study used serum parameters and food data of 73 participants selected randomly from a clinic-based follow-up study conducted among a cohort of Sri Lankan pregnant women to assess anemia, iron and folate status following the iron supplementation programme of the National Food Authority. A detailed discussion on the recruitment of this cohort has already been published [26].

In brief, healthy pregnant women aged 18–36 years at ≤ 12 weeks of gestation not diagnosed with long standing illnesses such as chronic liver diseases which can affect the micronutrient metabolism or on medication that are known to interfere with micronutrient metabolism (e.g., antiepileptic drugs such as acetazolamide, carbamazepine and clobazam, antibiotics such as trimethoprim) were recruited prior to taking any nutritional supplements. Pregnant women with a past or family history of thalassemia and other hematological disorders, with a history of pregnancy-associated complications (e.g., pre-eclampsia or gestational diabetes mellitus), with multiple pregnancy, suspected of having congenital malformations in the fetus and with a current infection (identified by C-reactive protein (CRP) measurement > 10 mg/L) were excluded from the study. Concentration of serum ferritin was measured by enzyme-linked immunosorbent assays (ELISA), while the sensitive immunoturbidimetric assay by Dimension® was used to measure high-sensitivity C-reactive protein (hs-CRP). Serum ferritin values were adjusted for inflammation using a correction factor calculated for the samples (1.34). Iron deficiency was defined as serum ferritin < 30 µg/L. The study was approved by the Ethics Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka.

Molecular genetics investigation

DNA was extracted from peripheral leukocytes using the Wizard® Genomic DNA purification Kit (Promega, USA) and was quantified using Nanodrop 2000c (Thermo Fisher Scientific). rs855791 T > C polymorphism of *TMPRSS6* was genotyped by a PCR-RFLP assay (polymerase chain reaction followed by restriction fragment length polymorphism) designed in-house. Primers, F:5'-GTCACCATGAACCCCACA-3' and R:5'-TCCTTTCTC CCTCCT CTCTC-3' were designed with Primer3Plus 0.4.0 to amplify a 380 bp DNA fragment flanking the SNP locus. PCR reaction was carried out in a total volume of

25 µl, with 1.5 mM $MgCl_2$, 200 µM dNTP, 0.4 µM of each primer, 1.25 U *Taq* polymerase, 0.6 ng / µl DNA and 1X GoTaq® Flexi buffer. Amplification conditions were initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 40 s, primer annealing at 60 °C for 40 s, chain extension at 72 °C for 40 s and a final extension at 72 °C for 10 min. The PCR products were checked on a 2% agarose gel for successful amplification.

Digestion of PCR products was carried out with the restriction enzyme *Hae*III (*Bsu*RI) following manufacture's recommendations (Thermo Fisher Scientific, USA), and the bands were separated on a 2% agarose gel (Fig. 1).

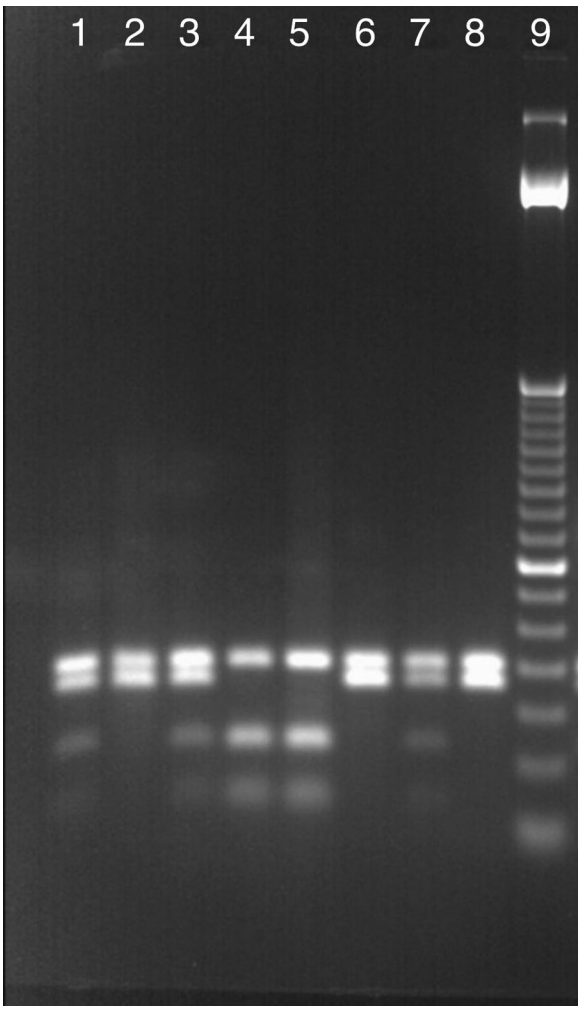

Fig. 1 Agarose gel electrophoresis image showing RFLP banding pattern of rs855791 (cropped gel picture); lane 9: 50 bp ladder; lanes 1,3,7: TC genotype (fragment sizes: 201 bp, 179 bp, 114 bp and 65 bp); lanes 2,6,8: TT genotype (fragment sizes: 201 bp and 179 bp); lanes 4, 5: CC genotype (fragment sizes: 201 bp, 114 bp and 65 bp). (Full length gels are presented in Additional file 1: Fig. S1)

Statistical analysis

Samples with serum ferritin < 30 µg/L were categorized as iron deficient, while those with serum ferritin above 30 µg/L were categorized as iron sufficient. The conformity of genotyping results to Hardy–Weinberg equilibrium (HWE) expectation, and the distribution of the three genotypes among iron sufficient and deficient individuals were analyzed using the exact test for chi-square. Association between genotypes and ferritin levels was determined with binary logistic regression via computing the odds ratio (OR). In addition, the distribution of the heme, non-heme and total iron consumption among the iron sufficient and deficient groups was checked separately by the chi-square test.

All statistical analysis was conducted in SPSS 21 (SPSS Inc, Texas, USA) software, and $P < 0.05$ is considered statistically significant.

Assessment of dietary intake

A report of three months food intake prior to the recruitment to the study was obtained via interview based, pre-tested food frequency questionnaire [27]. The iron content (heme, non-heme and total iron) of the consumed food per day was assessed using Asian and Indian food tables [28, 29].

In silico studies

Protein–protein docking

Protein–protein docking models were utilized to postulate residues involved in the binding interaction between MT2 protein encoded by the gene sequences carrying T and C allele of rs855791 and HJV proteins. The homology model of HJV protein and MT2 protein encoded with T allele (MT2 736 V variant) was built using YASARA [30]. The SNP was introduced to the MT2 736 V variant by using Schrödinger Maestro [31] to obtain the MT2 736 A variant protein (encoded by C allele of TMPRSS6 rs855791). The predicted models were validated using Ramachandran plots [32] generated through RAMPAGE online server at the online site http://mordred.bioc.cam.ac.uk/~rapper/rampage.php [33]. Protein complexes were predicted by docking both MT2 736 V variant and MT2 736 A variant TMPRSS6 proteins with HJV separately in two protein–protein docking web servers: PatchDock [34] and FireDock [35]. Top ten results from each method were analyzed and compared. Thereafter, local docking was carried out by using Rosetta web server [36].

Results

Genotype frequencies of the entire study cohort as well as the iron sufficient and deficient cohorts were confirmed to HWE expectations, excluding any possible genotyping

errors. Among the study population, a majority (69.8%; 51/73) was iron deficient, while only 30.1% (22/73) was iron sufficient as assessed by the serum ferritin levels corrected for inflammation.

The allele and genotype distribution observed for TMPRSS6 rs855791 T > C polymorphism is given in Table 1. As shown, the heterozygous genotype (TC) predominated (0.425) among the study population compared to the two homozygote genotypes (TT: 0.342 and CC: 0.247). Further, both the allele and genotype distribution between iron sufficient and deficient groups was significantly different ($P < 0.05$). T allele was found at a higher frequency (0.618) among the iron deficient compared to the iron sufficient (0.386) women, while C allele was more common among the iron sufficient group (0.614) compared to those who were iron deficient (0.382) (Table 1).

When genotype distribution was considered, TT genotype predominates with a relatively high frequency (0.431) among the iron deficient individuals, suggesting a possible risk of iron deficiency associated with TT genotype. On the other hand, most of the iron sufficient individuals (0.5) were found to be heterozygous (TC). The frequency of TT genotype was very low among the iron sufficient group (0.136) (Table 1).

When tested with binary logistic regression (Table 2), a nearly twofold increased risk for iron deficiency was detected to be associated with the T allele compared to the C allele (OR = 2.566; 95%CI 1.241–5.305; $P = 0.011$). Further, there was a nearly sixfold risk of developing iron deficiency with TT genotype relative to the CC genotype (OR = 5.867; 95%CI 1.279–26.903; $P = 0.023$).

Since results obtained indicated that the risk is associated only with TT homozygosity and none of the other two genotypes, TC and CC genotypes, were grouped together, and the data were reanalyzed. This resulted in a nearly fivefold increased risk of TT genotype to be associated with iron deficiency compared to having either TC or CC genotypes (OR = 4.805; 95%CI 1.261–18.309 $P = 0.021$) (Table 2).

Dietary iron assessment

The mean total dietary iron intake of the study population was 11.25 (± 6.72) mg per day, while the mean heme iron intake was 1.32 (± 0.99) mg per day. None of the pregnant women in the population studied were able to meet the Recommended Dietary Allowance (RDA) for iron for pregnant women in Sri Lanka, which is 33 mg per day [37].

When the heme, non-heme and total dietary iron intakes were compared among the iron sufficient and deficient individuals, there were no significant differences observed indicating a lack of association of iron consumption with iron status within the study cohort (respective *P* values; 0.41, 0.20 and 0.34).

Table 1 Distribution of TMPRSS6 rs855791 allele and genotype frequencies among iron sufficient and deficient women

Alleles / Genotypes	Iron deficient group (serum ferritin < 30 µg/L)	Iron sufficient group (serum ferritin ≥ 30 µg/L)	Total study population
C	38.2%	61.4%	45.2%
T	61.8%	38.6%	54.8%
CC	10 (19.6%)	8 (36.3%)	18 (24.7%)
TC	19 (37.2%)	11 (50%)	31 (42.5%)
TT	22 (43.1%)	3 (13.7%)	25 (34.2%)

Table 2 Association of rs855791 genotypes with iron deficiency

Allele/Genotype	Iron deficient group	Iron sufficient group	Odds ratio	95% CI	*P* value
T/C	63	17	2.566	1.241–5.305	0.011*
C/C	39	27	1		0.142
CC/CC	10	8	1		0.638
TT /CC	22	3	5.867	1.279–26.903	0.023*
TC/CC	19	11	1.381	0.420–4.541	0.594
TT/CC&TC	22	3	4.805	1.261–18.309	0.021*
CC/TT&TC	10	8	2.343	0.772–7.110	0.133

*$P < 0.05$; odds ratio was calculated using binary logistic regression

Homology modeling

The homology models of MT2 736 V variant and MT2 736 A variant of MT2 protein generated with YASARA software are shown in Fig. 2. Each protein consists of three domains, i.e., cytoplasmic topological domain (aa 1–55), transmembrane domain (aa 56–76) and extracellular topological domain (aa 77–814) which carry the amino acid residue change due to rs855791 polymorphism. The BLAST coverage for MT2 736 V variant model was 66%, while it was 54% for HJV homologous model. Since the cover ID of proteins to the template was greater than 30%, the homologous models can be considered particularly valid.

The generated structures for MT2 736 V variant, MT2 736 A variant and HJV molecules were visualized with Schrödinger's BioLuminate software [31] (Fig. 2).

The results of protein model validation with Ramachandran plot [32] are shown in Table 3.

Interface energy was calculated for these complexes through ROSIE [36]. MT2 736 V and HJV complex (Fig. 3, A) generated an interface energy of − 4.689 kJ/mol, while the MT2 736 A and HJV complex (Fig. 3, B) generated an interface value of − 7.934 kJ/mol. These results show that the complex formed by HJV, and MT2 736 A is more thermodynamically stable than the complex made from MT2 736 V and the HJV protein. The most thermodynamically stable complex may inhibit the BMP-SMAD pathway leading to negative regulation of the *HAMP*. This will reduce the hepcidin production and result in sufficient plasma iron levels.

Discussion

The present study was set up to analyze the association of TMPRSS6 rs855791 polymorphism with iron deficiency among a Sri Lankan cohort of pregnant women. As of present, there were no studies conducted

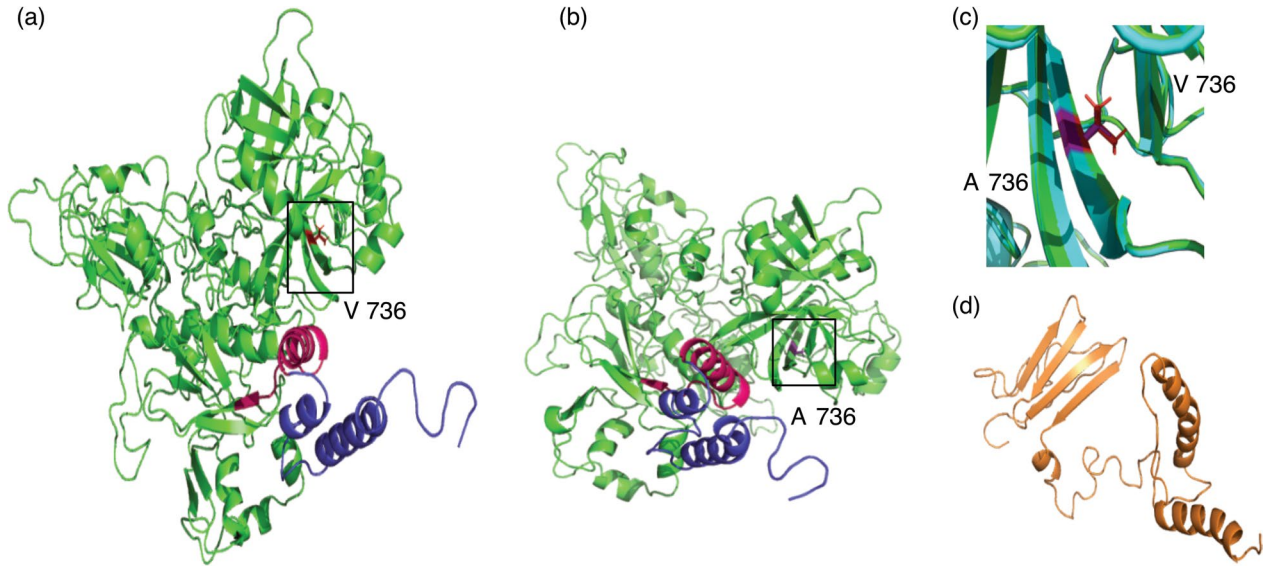

Fig. 2 Homology models of the **A** MT2 736 V variant protein **B** MT2 736 A variant protein (where valine at 736 positions is replaced by alanine) **C** Superimposed MT2 736 V and MT2 736 A **D** HJV protein. The cytoplasmic domain is colored in blue, transmembrane domain is colored in pink, extracellular domain is colored in green, and HJV molecule is colored in orange. All images were generated from YASARA [30]

Table 3 Ramachandran plot analysis results generated by RAMPAGE online server

Protein model	Template used	Number of residues in favored region	Number of residues in allowed region	Number of residues in outlier region
HJV	XRAY RGM domain family member B [*Homo sapiens*]	152 (93.8%)	10 (6.2%)	0 (0%)
MT2 736 V variant	XRAY Plasma kallikrein [*Homo sapiens*]	728 (89.2%)	81 (9.9%)	7 (0.9%)
MT2 736 A variant	XRAY Plasma kallikrein [*Homo sapiens*]	725 (88.8%)	81 (9.9%)	10 (1.2%)

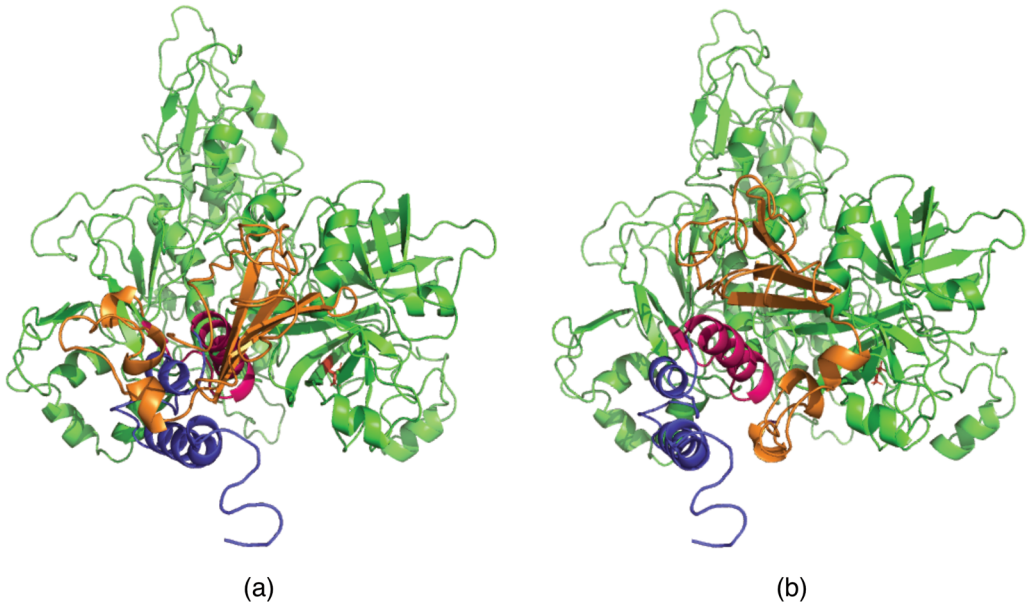

Fig. 3 HJV molecule docked in to the **A** MT2 736 V and **B** the MT2 736 A. The cytoplasmic domain is colored in green, transmembrane domain is colored in pink, extracellular domain is colored in blue, and the HJV molecule is colored in orange. All images were rendered from YASARA [30]

in Sri Lanka to identify the genetic predisposition of iron deficiency (ID) among any risk group, despite the observed high prevalence of ID within the population. In line with several previous research [20–23] conducted on populations in other parts of the world, our study indicated an increased risk of development of ID associated with the rs855791 polymorphism. In addition, the present study suggests for the first time, a possible mechanism through which the observed effect of iron regulation is being exerted by rs855791 polymorphism using an *in silico* protein binding model.

According to the results obtained, the T allele of rs855791 of TMPRSS6 gene predominates among the study cohort. This is comparable to what had been reported previously for other South Asian populations in which T allele predominates with a frequency around of 0.54 (ref: dbsnp https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?do_not_redirect&rs=rs855791 and [38]). On the other hand, in African countries, the C allele predominates with a frequency ranging from 0.82 to 0.93 [8]. This heterogeneity in allele frequency distribution observed for rs855791 TMPRSS6 gene among different ethnicities is suggestive of its significant functional role in different populations. For example, the high prevalence of the C allele in African countries in contrast to Asians and Europeans may be an adaptation to counteract severe famine. Given its higher potency to inhibit hepcidin transcription, the C allele might be advantageous in increasing iron absorption when dietary iron availability is severely limited [25].

The current study population represents a cross section of apparently healthy pregnant women who visited the antenatal clinics within Colombo municipal council area. Among them, a significant percentage (69.8%, 51/73) was iron deficient according to their corrected (for hs-CRP) serum ferritin levels as reported earlier [39]. This highlights the need for immediate attention of the public health authorities in mitigating iron deficiency among pregnant women with a similar socioeconomic background. According to our study results, iron deficiency could have stemmed from two sources: a genetic propensity of the study population toward iron deficiency which is mediated via the rs855791 SNP and the observed low dietary iron intake.

Among our study population, iron deficient subjects showed a high propensity to carry T allele (61.8%) with more than twofold enhanced risk for iron deficiency. Having TT genotype increased the risk further up to sixfold. The polymorphism (T-C) leading to V736A change in the primary structure of MT2 may cause fine alterations in the tertiary conformation of the catalytic domain, leading to changes in the thermodynamic stability of the complex it forms with HJV as observed with the *in silico* study. Accordingly, the MT2 736 V variant which is encoded by the rs855791 T allele may not be able to catalyze the cleavage of HJV efficiently enough due to relatively low thermodynamic stability causing the BMP

pathway to continue, despite low serum iron levels. This might disrupt the negative regulation of *HAMP* gene and could result in relatively high hepcidin levels. Consequently, dietary iron absorption and release of iron from macrophages may be hampered leading to low iron levels in blood circulation [40]. Continuation of this cycle for prolonged periods may lead to iron deficiency [41]. The presence of TT genotype could augment this effect as MT2 enzymes encoded by both copies of the TMPRSS6 gene are altered.

On the other hand, TC genotype has not made a significant effect on iron levels within the study population. This suggests that having a single C allele could cover up for the altered MT2 enzymes encoded by the rs855791T allele among heterozygotes, establishing the normal iron homeostasis. As such, those with TC would carry sufficient amount of iron in their serum similar to those who carry CC genotype, while TT homozygotes are prone to become iron deficient.

Our study is in agreement with two previous studies conducted among Italian [25] and Taiwanese [22] populations, which reported a similar distribution of serum iron levels with the two rs855791 variants. In the latter study, the CC genotype was reported to be associated with a protective role for IDA with a OR of 0.4 (95% CI, 0.17–0.95, $p=0.04$) supporting the observations of the present investigation. Interestingly, in all these populations (Sri Lankans, Italians, Taiwanese) the rs855791 T allele that encodes MT2 736 V was present at a higher frequency than its counterpart, despite its negative impact on iron homeostasis. This along with its evolutionary conserved nature when analyzed in comparison with other species who are closely related to humans [31] further suggests that MT2 736 V variant, which leads to increased hepcidin production and inhibition of iron absorption leading to low iron levels in the circulation, is a recent evolutionary change. Such switching of SNP alleles could have arisen due to systemic inflammation conditions which might have been prevalent among the populations over generations due to numerous environmental stresses. For example, high hepcidin levels which leads to low serum iron in turn have shown to be beneficial in counteracting malaria [41–43].

On the other hand, the low dietary iron intake among the current study population may have contributed to the high prevalence of iron deficiency. The lack of nutritional awareness as well as financial constrains might have played a role in causing this situation. It is also possible that the inadequate iron intake in these pregnant women has inflated the intensity of the genetic association observed between rs855791 and serum iron levels. Recruiting subjects with adequate dietary iron intake would help to remove this confounding factor and to reveal a more accurate picture of serum iron modulation by rs855791. Limited sample size prevented us from exploring this possibility during the analysis. However, it should also be noted that the dietary iron consumption data of this study suffers from recall bias which is a well-known drawback in recall food intake data. In addition, causes such as parasitic infestations and viral infections leading to underlying chronic inflammation, which are known to prevail in tropical countries, may have contributed to aggravate the effect of genetic modulation on iron metabolism. Notwithstanding these limitations, the current study highlights the requirement of stringent public health interventions to prevent iron deficiency among pregnant women in Sri Lanka.

Despite this significant association shown by rs855791 with serum iron levels, the genotype frequencies of the SNP locus were consistent with the Hardy–Weinberg equilibrium (HWE) expectations indicating the absence of significant evolutionary forces acting upon it. This observation agrees with the hypothesis put forward by Nai et al. [25] suggesting that the association of this SNP with iron deficiency is only a recent evolutionary change as discussed above. Otherwise, it is unlikely for a functionally significant polymorphism to remain neutral over an evolutionary time scale.

Bioinformatic analysis of rs855791 was conducted to find out the molecular mechanisms of iron regulation via BMP-SMAD signaling pathway. As indicated by the validation criteria, all the generated models were reliable. These predicted models would be useful in the *in silico* screening of potential drug targets for ID. The natural behavior of amino acids in the secondary structure and the complete tertiary structure has the ability to change the protein stability [44]. Nevertheless, which of these would affect more on the protein stability in most cases is unclear. Though the change between valine and alanine is only a CH_2CH_3 group, with its location they can influence the protein stability. A well-known example that demonstrates the effect of subtle changes in the primary structure of a protein on its conformation and function is found in the case of sickle cell anemia (the amino acid change in glutamic acid to valine causes the disease) [45]. The secondary structural elements of a protein may evolve at different rates. According to Siltberg-Liberies et al. [46], the evolution rate of beta sheets is slower than helical regions and random coils. Some of these could be evolutionarily neutral, while some others can have strong positive or negative selection. In the present scenario, the variant region is in a beta pleated sheet surrounded by several random coils. CH_2CH_3 group of valine acquires more three-dimensional space when compared with that of alanine. Thus, with alanine it would be easier to coil

since the inter-spatial space is low, which makes the protein more stable.

When the obtained interface energy values of the complexes were compared, MT2 736 A variant—HJV, is more thermodynamically stable than the MT2 736 V variant—HJV complex. With a higher stability, MT2 736 A variant is likely to cleave HJV molecule more efficiently with a better protease activity compared to its counterpart, the MT2 736 V variant. This might result in a more efficient inhibition of BMP-HJV pathway, negatively regulating the *HAMP* gene transcription. This will lead to lower production of hepcidin levels resulting in high iron levels in blood circulation.

In this post genomic era, identifying those markers that confer genetic predisposition to complex diseases is of much interest. However, due to the differences in genetic makeup among different ethnicities and the complex interactions they have with their environment, not all such markers could be equally effective to all populations [46]. Our results, though only preliminary considering the number of samples analyzed, indicate that SNP rs855791 can be successfully used as a molecular marker to screen iron deficiency risk among Sri Lankan women especially of the susceptible populations such as women of childbearing age, pregnant women, adolescent girls and the young child. Such early detection and correction of iron deficiency could be one of the first steps of ensuring a healthy future generation in those developing nations including Sri Lanka. In addition, since biochemical markers such as ferritin levels can be masked by inflammation, use of molecular makers would be advantageous [38].

Furthermore, this knowledge on the molecular mechanism of iron deficiency may also be helpful for therapeutic interventions. Although as pointed out by Galesloot et al. [9], rs855791 may also employ a hepcidin-independent mechanism to regulate iron metabolism, the iron deficiency associated with rs855791T allele is at least partly caused by hepcidin deregulation as suggested by our results and many previous studies [19, 20, 47]. Hence, in addition to treating with elemental iron supplements, alternative treatment methods such as hepcidin blockers (small interfering RNA/ antibodies) or alternative supplements using heme iron may be used to ensure iron absorption [48]. This is especially valid for women with inflammation as previous results from the same cohort highlight the ineffectiveness of elemental iron supplements in increasing serum ferritin in pregnant women who had inflammation [49].

However, it is important to confirm the observed risk of iron deficiency modulated by rs855791 through a larger case control study excluding the confounding factors such as low dietary iron intake, parity and diverse ethnicity that are commonly associated with iron deficiency [6]. Measuring the hepcidin levels in the subjects would also aid in determining the molecular mechanism of iron regulation via rs855791 polymorphism.

Conclusions

The present study is indicative of a twofold increased risk of iron deficiency associated with the T allele of the re855791 polymorphism compared to the C allele among the cohort of pregnant women under study. This effect may be due to the relatively low thermodynamic stability of the MT2 736 V variant and HJV complex, as illustrated by our in silico protein–protein docking model. The effect is exerted in a recessive fashion via the TT homozygous genotype, conferring a nearly sixfold increased risk. This observation suggests the possibility of using the TT genotype as a potential molecular marker to screen for iron deficiency in risk groups. The screening can be conducted conveniently using the in-house PCR-RFLP assay established by us, which is technically less demanding for a developing country like Sri Lanka. Furthering the predicted protein models would be useful in in silico screening of potential drug targets for ID.

It was noted that the total dietary iron consumption per day in this study population was only one-third the recommended dietary allowance. This may have interacted with the studied polymorphism, explaining the high prevalence of iron deficiency in the study population. With this respect, it is important that health authorities pay adequate attention to identifying novel strategies to combat iron deficiency and associated disorders in Sri Lanka.

Abbreviations

ID: Iron deficiency; IDA: Iron deficiency anemia; DMT1: Brush border divalent metal transporter 1; IMT: Integrin–mobilferrin pathway; HCP1: Heme carrier protein 1; Fpn1: Ferroportin; HEPH: Ferroxidase hephaestin; *HAMP*: Human antimicrobial peptide gene; MT2: Matriptase 2; TMPRSS6: Transmembrane serine proteinase 6; HJV: Hemojuvelin; BMP-SMAD: Bone morphogenetic protein—sons of mothers against decapentaplegic; JAK-STAT: Janus kinase-signal transducer and activator of transcription; IL-6: Interleukin 6; GWAS: Genome wide association studies; SNPs: Single nucleotide polymorphisms; CRP: C-reactive protein; ELISA: Enzyme-linked immunosorbent assays; PCR-RFLP: Polymerase chain reaction followed by restriction fragment length polymorphism; HWE: Hardy–Weinberg equilibrium; OR: Odds ratio; RDA: Recommended dietary allowance.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-022-00377-8.

Additional file 1. Agarose gel electrophoresis image showing RFLP banding pattern of rs855791 (Uncropped gel picture); lane 9: 50 bp ladder; lanes 1,3,7, 10,11: TC genotype (fragment sizes: 201bp, 179bp, 114bp and 65bp); lanes 2,6,8,12,13,15,18,19,14,17: TT genotype (fragment sizes: 201bp and 179bp); lanes 4, 5: CC genotype (fragment sizes: 201bp, 114bp and

65bp); lane 20: PCR product. (This gel picture was cropped from 9th well to obtain the cropped gel image)

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Author contributions
HLTCA conducted the molecular genetics analysis, food frequency data analysis *and in silico* protein model validation and prepared the first draft of the manuscript. MSKR collected samples and food frequency data and measured the serum parameters. PKLS performed the *in silico* protein modeling and protein–protein docking analysis and drafted the relevant section in the manuscript. TT contributed to study designing, acquisition of samples and interpretation of biochemical and food frequency data and critically reviewed and revised the manuscript. GHG contributed to the study conception, design, molecular genetic analysis and interpretation and critically reviewed and revised the first draft. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was reviewed and approved by Ethics Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka (EC-15–006), and conducted in accordance with the Helsinki Declaration of 1964 and its later amendments. Informed written consent was obtained from all participants prior to their recruitment.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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