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Original article

Role of TMPRSS6 rs855791 (T > C) polymorphism in reproductive age women with iron deficiency anemia from Lahore, Pakistan

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ABSTRACT

Background: Iron deficiency anemia (IDA) is the highest nutritional deficiency worldwide. It is a multifactorial disease, with a higher morbidity rate. TMPRSS6 polymorphisms importantly rs855791 is found to play an essential role in iron homeostasis in the human body. The rs855791 (T > C) polymorphism is highly associated with iron levels, and multiple blood parameters, leading to IDA. The role of TMPRSS6 rs855791 polymorphism and the significance of complete blood count (CBC) parameters in the pathogenesis of IDA is not yet studied in the Pakistani population.

Methods: We enrolled 113 cases and 136 controls to conduct a case control study. Complete blood count (CBC) and iron parameters were analyzed for association studies. PCR-RFLP based genotyping was performed.

Results: The TMPRSS6 rs855791 (T > C) polymorphism is significantly associated with IDA pathogenesis as observed in the codominant model and recessive models ($P < 0.05$, OR: 1.5 and 95% CI: 0.9, 2.6, $P < 0.05$, OR: 0.5 and 95% CI: 0.2, 0.9 respectively). Elderly women among cases (30–49 years) were found to be more susceptible to IDA ($P < 0.05$, AOR: 2.1 and 95% CI: 1.0, 4.2). The most significant parameters associated with IDA were red blood cell count (RBC) and hematocrit (Hct%) ($P < 0.05$, AOR: 16.5, 95% CI: 7.6, 35.9 and $P < 0.05$, AOR: 10.1, 95% CI: 2.5, 41.6, respectively).

Conclusion: TMPRSS6 polymorphism at rs855791 (T > C) is significantly associated with IDA susceptibility in reproductive age women in Pakistan. Age, RBC count and Hct% are found to play an important role in IDA pathogenesis in our study population.

1. Introduction

Iron deficiency (ID) is among the most common type of nutritional deficiencies, both in developed and developing countries and reported as the primary cause of anemia in women and children under 5 years (Hershko, 2018). More than 50% of reproductive age women (15–49 years) have been reported as anemic (Ritchie and Roser, 2017). ID occurs due to disturbance of balance among iron uptake, utilisation, and storage in the body. Iron is important for a large number of different processes in body, most important of which is erythropoiesis as iron is component of hemoglobin, found in red blood cells (Abbaspour et al., 2014). Reduced iron level due to decreased absorption from food or excessive blood loss lead to insufficient red blood cells formation subsequently leading to IDA (Burz et al., 2019).

Abbreviations: ID, Iron deficiency; IDA, Iron deficiency anemia; IRIDA, Iron refractory iron deficiency anemia; WHO, World health organization; GWAS, gGenome wide association studies; SNP, Single nucleotide polymorphisms; CBC, Complete blood count; Hct%, Hematocrit%; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; Hb, Hemoglobin; TIBC, Total iron binding capacity; Fe, Iron; PCR-RFLP, Polymerase chain reaction- restriction length polymorphism.

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IDA occurs due to multiple factors including micronutrient deficiency, genetics, infectious diseases and blood loss caused by internal or external injuries (Cappellini et al., 2020). IDA is generally prevalent among women due to menstrual blood loss and nutritional deficiency during pregnancy. IDA is associated with multiple health conditions including heart problems, poor pregnancy outcome, poor motor and mental performance and loss of productivity in adults.

For the year 2011, world health organization (WHO) estimates that nearly 38% of pregnant women, 29% of non-pregnant women and 29% of all women of reproductive age are anemic, while 50% among them are IDA patients (WHO, 2015). In Pakistan, 30 to 60% of women are anemic, and 90.5% of pregnant women are reported to be anemic (Habib et al., 2018). It is proved that IDA causes serious effects to maternal health even the iron supplementation during pregnancy is not much successful, so sufficient attention should be paid to adolescent girls and women of reproductive age long before pregnancy (Akhtar et al., 2013).

Mostly IDA can be treated by oral iron therapy. Iron refractory iron deficiency anemia (IRIDA) patients (1 out of 1 M) are unable to absorb intestinal iron due to elevated hepcidin levels (Pei et al., 2014). Hepcidin is the major hormone regulating the iron homeostasis, controlling the Fe absorption as well as release from macrophages. *TMPRSS6* gene's function is essential in iron deficiency to allow the compensatory mechanism of increased iron absorption (Camaschella et al., 2019). A germline mutation of *TMPRSS6* gene, which is located on chromosome 22q12-q13 and encodes matriptase 2, inhibits hepcidin transcription leading to IRIDA (Dopsaj et al., 2019). In *TMPRSS6* gene rs855791 (T > C) mutation at 2207 position, results in missense shift of valine to Alanine at position 736, by the catalytic and binding sites of matriptase 2 (Dopsaj et al., 2019). This mutation has essential impacts on iron status leading to lower hemoglobin levels, elevated serum hepcidin levels, inhibited iron absorption subsequently resulting in IDA. Characteristic features of IRIDA include normal or high hepcidin levels, low transferrin saturation, and microcytic hypochromic anemia.

Genome wide association studies (GWAS) have reported that *TMPRSS6* SNP's at rs855791, rs4820268 and rs3345321 positions are in strong association with IDA (Benyamin et al., 2009; Chambers et al., 2009). The effects of these SNPs on iron absorption and hepcidin levels in the Pakistani population have not been explored yet. This study is designed to determine the association of *TMPRSS6* rs855791 polymorphism in the pathogenicity of IDA and the significance of complete blood count (CBC) parameters in IDA diagnosis in the reproductive age female of Lahore, Punjab, Pakistan.

2. Methods

2.1. Study population

We conducted a case-control study on females of reproductive age group (15–49 years) including 113 cases and 136 controls. Study subjects were recruited from multiple camps in the University of the Punjab, technical vocational college for women and Citi lab research centre in Lahore. Study subjects were screened for hematological and biochemical parameters. For hematological parameters, complete blood count was performed, and biochemical parameters were determined to explore the iron status of the individuals. Study subjects with hemoglobin (Hb) and ferritin levels lower than 11 g/dL and 10 ng/ml respectively, were included as cases (Pei et al., 2014). Study subjects with hemoglobin levels above 11 g/dl and ferritin above 10 ng/ml were considered as controls with the recommendation of consultant hematologist and patient history. All the subjects with any of the following complications were excluded: infectious disease, gastrointestinal bleeding, thalassemia, renal insufficiency, menopause, and history of gastrointestinal resection. A consent form with information regarding the study was signed by each participant. Written informed consent for the participants under 16 years was taken from their parent or guardian. Ethical approval for the study was obtained from the Ethical Review Board of CRC lab, Lahore, Pakistan (Ref# 26-17/ERB/27th date: 28-7-16).

2.2. Red blood cell parameters

A blood sample of 5 ml was collected from each subject and equally sectioned into two aliquots for blood and serum analysis. Hemoglobin level, haematocrit %, red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by using modern haematology analyzer (Cell Dyn 4000, Abott Laboratories, Chicago,IL) according to manufacturer instructions.

2.3. Biochemical parameters

Biochemical parameters including serum iron, serum ferritin and total iron-binding capacity (TIBC) were analysed. Serum ferritin was determined using ferritin chemiluminescent immunoassay sandwich method (kit Ref 33020 supplied by Beckman Coulter, Inc). Serum Iron was measured using colorimetric assay (Cat. No. 03183696 122 by Roche diagnostics, Germany). TIBC was analysed using colorimetric method (Human kit, Ref.10670, Germany). Transferrin was calculated using measured iron and TIBC:

$$\text{Transferrin saturation} = (\text{Fe}/\text{TIBC}) \times 100$$

2.4. Genotyping

DNA was extracted from whole blood using salting out DNA extraction method (Iranpur-Mobarakeh and Esmailizadeh, 2010). PCR-RFLP was used to investigate the association of rs855791 with the susceptibility of IDA. The sequence of forward and reverse primers is 5′-TAGAGAACAGGGGCTCCAGG-3′ and 5′-ATGTGGGCAG CATCCTTTC-3′, respectively (Nalado et al., 2019; Pei et al., 2014). The NEB *Stu1* restriction enzyme was used for digestion of PCR product.

2.5. Statistical analysis

StataIC version15.1 was used for statistical analysis. Hardy–Weinberg equilibrium (HWE) of the genotypic frequencies among cases and controls was examined. Baseline and clinical characteristics were compared using *t*-test. Logistic regression was used to show the association of CBC and iron parameters with IDA, and strength of association is shown by odds ratio (OR) and 95% confidence interval (CI). Fischer exact test and chi square tests were used for comparison of genotype frequencies among cases and controls. One-way ANOVA was used for the correlation of SNP genotypes with study variables. The P-values less than 0.05 were considered statistically significant.

3. Results

This study is conducted to find out a potential association between IDA susceptibility and *TMPRSS6* gene polymorphism at rs855791. A total of 249 subjects were enrolled in the study. The

genotyping was performed using PCR–RFLP technique. The PCR product (249 bp) was digested with *stu1* restriction enzyme to detect T > C polymorphism (Fig. 1). The genotype frequencies of patients and control subjects were in Hardy–Weinberg equilibrium for both SNPs (Data not shown).

3.1. Baseline and clinical characteristics of participants

The baseline and clinical characteristics were compared for the study participants (Table 1). All the baseline characteristics were significantly different between cases and controls. The mean age of cases vs controls was 30.1 vs 27.2 years, showing cases were of higher age group than controls. Hb level in cases vs control was 9.3 g /dl vs 13.1 g/dl ($P < 0.001$; MD −3.8). As anticipated ferritin, serum iron (Fe) and transferrin percentage were lower in cases vs controls ($P < 0.001$). In addition, MCV in cases was lower as compared to controls 78.4 vs 82.8 ($P < 0.001$; MD: −4.4). MCH and MCHC levels were also low in controls with a $P < 0.001$, MD: −2.6 and $P < 0.001$, MD: −1.4, respectively.

3.2. Association of CBC with the pathogenesis of IDA

CBC is evaluated to find out the cause of anaemia. IDA severity depends on hemoglobin and hematocrit levels. MCV level is also used for diagnosis of the Iron deficiency anemia (Johnson-Wimbley and Graham, 2011). We compared key variables of CBC in cases and controls to find out their association with IDA in our study population (Table 2). Multivariate analysis was done only for the variables that were found significant with univariate analysis ($P < 0.05$). Cases with the upper age range (30 to 49 years) were found to be at high risk of developing IDA, ($P < 0.05$, AOR: 2.1 and 95% CI: 1.0, 4.2). Red blood cell count (RBC) and hematocrit (Hct%) were directly associated with IDA ($P < 0.05$, AOR: 16.5, 95% CI: 7.6, 35.9 and $P < 0.05$, AOR: 10.1, 95% CI: 2.5, 41.6, respectively). Furthermore, MCV, MCH, MCHC and TLC were not significantly different between cases and controls on multivariate analysis ($P > 0.05$).

3.3. Association of TMPRSS6 rs855791 (V736A) with IDA

The rs855791 (V736A) genotype frequencies were in HWE for both cases and controls. We assessed the association of rs855791 with the pathogenicity of IDA in co-dominant, additive, dominant, and recessive models (Table 3). We found that rs855791 (V736A) is significantly associated with IDA as observed in codominant model ($P < 0.05$, OR: 1.5 and 95% CI: 0.9, 2.6). Similarly, significant association was found in recessive model ($P < 0.05$, OR: 0.5 and 95% CI: 0.2, 0.9). However, non-significant P-values were obtained in additive and dominant models ($P > 0.05$). Among the four studied genetic model's, significant P-values (0.0394, and 0.0208 respectively) were observed in co-dominance and recessive models, confirming that rs855791 is significantly associated with IDA. We did not find significant correlation between blood and Fe parameters with TMPRSS6 rs855791 genotypes (Supplementary Table 1).

4. Discussion

Reproductive age women and children under 5 years are at high risk of developing IDA in Pakistan (Akhtar et al., 2013). The GWAS studies in European and Indian Asian populations and in Chianti (Italy) and Baltimore (Washington DC) populations proved that *TMPRSS6* SNPs are strongly associated with low hemoglobin and lower serum iron concentrations (Chambers et al., 2009; Jallow et al., 2019; Tanaka et al., 2010). This study was designed to investigate role of TMPRSS6 gene rs855791 mutation in reproductive-age women with IDA in Pakistan. We found that TMPRSS6 rs855791 is significantly associated with IDA in our study subjects. Similar results were observed from the association studies conducted on Taiwani reproductive age women diagnosed with IDA, elderly Chinese women who were anemia patients, persistent IDA patients of (4-47yrs) from Italy and Indonesian children of 12–17 months (An et al., 2012; Pei et al., 2014; Poggiali et al., 2015).

In our study, the codominant and recessive models show that CC genotype frequency is significantly increased in patient's and may have protective role in susceptibility to IDA. Similar results were observed in a case-control study conducted on 67 women with IDA and 107 healthy volunteers from Taiwan (Pei et al., 2014). However, another study conducted on 260 non-dialysis chronic kidney disease patients did not find CC genotype association with IDA (Nalado et al., 2019). The results of our study are further supported by an *in vitro* study on cells infected either with either MT2736V or MT2736A plasmid found consistent decreases in hepcidin levels in the cells that were infected with plasmid containing 736^{A} variant than in 736^{V} variant. This study indicates that CC genotype inhibits hepcidin more effectively then TT genotype, presuming that C homozygous genotype subjects have lower hepcidin levels in the general population (Nai et al., 2011).

Akhtar *et al.,* reported that majority of the pregnant women and feeding mothers have IDA. Furthermore, another study reported IDA prevalence in 50% of reproductive age women (Akhtar et al., 2013; Habib et al., 2018). We selected the reproductive age women as study population since they are highly diagnosed with general anemia as well as IDA globally (De Benoist et al., 2008). In the present study we showed TMPPRSS6 rs855791 polymorphism significantly associated with IDA pathogenesis in menstruating women.

This study also showed that RBC count and Hct% are important factors in the diagnosis of IDA as reported globally (Nagababu et al., 2008; Pei et al., 2014). Interestingly, we observed that majority of the RBC parameters were significantly reduced in IDA cases vs controls. Similarly, reduced values of RBC's parameters were observed in the Iranian IDA patients (Nikzad et al., 2018).

Women of higher reproductive age are mostly anemic due to increasing gynecological issues related to pregnancy, postpartum bleeding, and menorrhagia. IDA cases were of higher age group

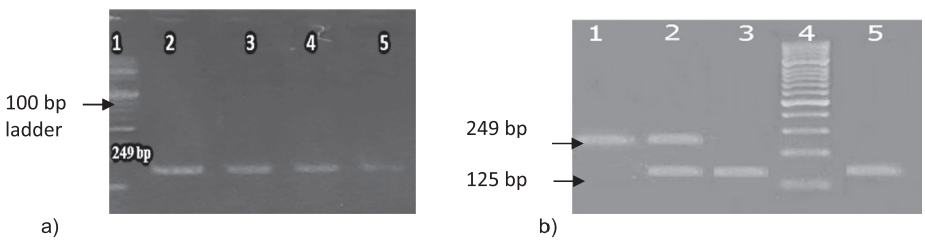

Fig. 1. (a) Genotyping of *TMPRSS6* by PCR (PCR product = 249 bp). (b) Genotyping of TMPRSS6 by RFLP. PCR products were digested by stu1. Single band 249 bp represented homozygous C and single band at 125 bp represented homozygous T. Two bands represented heterozygous TC.

Table 1
Baseline and clinical characteristics of study participants.

Variable	Case (n = 113)	Control (n = 136)	Mean difference (95% CI)	P-value
Age, years	30.1 (6.9)	27.2 (6.7)	2.9 (1.2, 4.6)	0.0007
Hemoglobin (Hb), g/dL	9.3 (1.5)	13.1 (0.8)	−3.8 (−4.1, 3.5)	<0.0001
Red blood cell count (RBC), Count × 10^{12}/L	4.1 (0.5)	4.6 (0.5)	−0.5 (−0.6, −0.4)	<0.0001
Hematocrit (Hct), %	31.9 (3.2)	38.2 (4.7)	−6.3 (−7.3, −5.2)	<0.0001
Mean corpuscular volume (MCV), fL	78.4 (8.6)	82.8 (7.7)	−4.4 (−6.5, −2.4)	<0.0001
Mean corpuscular hemoglobin (MCH), pg/cell	24.0 (4.2)	26.6 (3.7)	−2.6 (−3.6, −1.6)	<0.0001
Mean corpuscular hemoglobin concentration (MCHC), %	29.9 (2.8)	31.4 (2.2)	−1.4 (−2.0, −0.8)	<0.0001
Total leucocyte count (TLC), ×10^9/L	7.1 (2.3)	8.3 (1.9)	−1.2 (−1.7, 0.7)	<0.0001
Ferritin, ng/mL	4.7 (1.7)	53.2 (14.4)	−48.5 (−51.2, −45.9)	<0.0001
Serum Iron (Fe), IU/L	26.7 (7.8)	96.6 (26.7)	−69.9 (−74.9, −64.7)	<0.0001
Total iron binding capacity (TIBC), ug/dL	451.6 (27.3)	297.7 (32.9)	153.9 (146.3, 161.6)	<0.0001
Transferrin, %	5.9 (1.8)	32.9 (10.2)	−26.9 (−28.9, −25.0)	<0.0001

Table 2
Association of complete blood count with IDA.

Variable		Cases (n = 113)	Control (n = 136)	Univariate Analysis		Multivariate Analysis	
				OR (95% CI)	P-value	OR (95% CI)	P-value
Age, years	14–29	61	93	–	–	–	–
	30–49	52	43	1.8 (1.1, 3.1)	0.020	2.1 (1.0, 4.2)	0.040
RBC, Count × 10^{12}/L	≥3.8 & ≤5.8	84	133	–	–	–	–
	< 3.8	29	3	15.3 (4.5, 51.8)	0.000	16.5 (7.6, 35.9)	0.000
Hct %	≥36 & ≤46	15	102	–	–	–	–
	<36	98	34	19.6 (10.0, 38.2)	0.000	10.1 (2.5, 41.6)	0.001
MCV, fl	≥76 & ≤96	78	113	–	–	–	–
	<76 & >96	35	23	2.2 (1.2, 4.0)	0.010	1.2 (0.5, 2.9)	0.680
MCH, pg/cell	≥27 & ≤32	40	80	–	–	–	–
	<27 & >32	73	56	2.6 (1.6, 4.4)	0.000	1.1 (0.5, 2.3)	0.895
MCHC %	≥30 & ≤37	58	109	–	–	–	–
	<30	55	27	3.8 (2.2, 6.7)	0.000	0.9 (0.4, 2.3)	0.997
TLC × 10^9/L	≥4 & ≤11.0	100	124	–	–	–	–
	<4	2	4	0.6 (0.1, 3.5)	0.585	0.2 (0.0, 1.1)	0.066
	>11.0	11	8	1.7 (0.7, 4.4)	0.270	1.9 (0.5, 7.2)	0.336

RBC: red blood cell count, Hct%: Hematocrit%, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, TLC: Total leucocyte count.

Table 3
Different genetic models for TMPRSS6 rs855791 (T > C) association with IDA.

rs855791 (T > C)	Patients (%)	Controls (%)	OR (95% CI)	P-value
Codominant				
CC	17 (15.0)	38 (27.9)	1.5 (0.9, 2.6)	0.0394*
TC	58 (51.3)	64 (47.1)		
TT	38 (33.6)	34 (25)		
Additive				
C	37.5 (43.9)	51 (37.5)	0.7 (0.4, 1.3)	0.3757
T	48 (56.1)	49 (36)		
Dominant				
TT	38 (33.6)	34 (25)	1.5 (0.9, 2.6)	0.1606
TC + CC	75 (66.4)	102 (75)		
Recessive				
CC	17 (15.0)	38 (27.9)	0.5 (0.2, 0.9)	0.0208*
TC + TT	96 (84.9)	98 (72.1)		

P-value obtained by Fischer exact test and chi square test.
Abbreviations: OR Odds Ratio; CI; Confidence Interval.
P-values less than 0.05 were considered statistically significant.

than controls in our population and results remained significant in regression analysis as well. Pei et al. reported that menstruating women of elder age are more susceptible to IDA due to heavy menstrual blood loss (Marret et al., 2010; Pei et al., 2014). Furthermore, Marret *et al.,* also reported that elderly women are more vulnerable to IDA (Marret et al., 2010).

The limitation of our study is that we could not measure hepcidin levels in our study population. Hepcidin analysis is not available in any testing laboratory of Pakistan. In addition, the cost of hepcidin measuring Kit limits its use for research in developing countries.

TMPRSS6 rs855791 SNP is crucial in the pathogenicity of IDA as shown in our study as well as globally inferring that genetics is an important factor in IDA pathogenesis. Age is also found as an important factor for IDA onset in women. CBC factors as RBC and Hct% are also found significantly associated with IDA. Our study is an effort to fill the research gap in the study of the genetic basis of IDA in the Pakistani IDA diagnosed population.

5. Conclusion

Our research provides a way forward to broaden the study throughout the country and implement policies to reduce IDA leading to a healthy lifestyle. TMPRSS6 and its polymorphism can also lead to IRIDA which still is unexplored in Pakistani population. This study will help the health care workers, and researchers to further work on the extrinsic and intrinsic factors of IDA in Pakistani reproductive age women.

6. Ethical approval and consent to participate

The 27th meeting of the ethical committee of Citi lab and Research Centre approved the project with project number CLRC-17-277. Committee had evaluated all project material including consent to participate in Performa. All participants gave written or thumbprint consent to participate.

7. Consent for publication

All the authors consented for the paper publication.

8. Availability of data and material

The data used and analyzed during the current study available from the corresponding author.

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Authors contribution

Nasira M. Lone designed the study, designed the questionnaire, collected data from participants, applied statistics and drafted this manuscript. Saba Riaz contributed to study design and helped in finalizing the manuscript. Syed Hasnain Sajjad Shah and Mariya Farooq participated in sample collection and molecular characterization. Mizna Arif helped in sample collection and performing biochemical and iron parameters. Sidra Younis helped in statistical analysis, writing, and finalizing the manuscript. All authors critically reviewed the manuscript and approved the final version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.11.004.

References

Abbaspour, N., Hurrell, R., Kelishadi, R., 2014. Review on iron and its importance for human health. J. Res. Med. Sci.: Off. J. Isfahan Univ. Med. Sci. 19 (2), 164–174. 24778671.

Akhtar, S., Ahmed, A., Ahmad, A., Ali, Z., Riaz, M., Ismail, T., 2013. Iron status of the Pakistani population-current issues and strategies. Asia Pacific J. Clin. Nutrit. 22 (3), 340–347. https://doi.org/10.6133/apjcn.2013.22.3.17.

An, P., Wu, Q., Wang, H., Guan, Y.u., Mu, M., Liao, Y., Zhou, D., Song, P., Wang, C., Meng, L., Man, Q., Li, L., Zhang, J., Wang, F., 2012. TMPRSS6, but not TF, TFR2 or BMP2 variants are associated with increased risk of iron-deficiency anemia. Hum. Mol. Genet. 21 (9), 2124–2131. https://doi.org/10.1093/hmg/dds028.

Benyamin, B., Ferreira, M.A.R., Willemsen, G., Gordon, S., Middelberg, R.P.S., McEvoy, B.P., Hottenga, J.-J., Henders, A.K., Campbell, M.J., Wallace, L., Frazer, I.H., Heath, A.C., de Geus, E.J.C., Nyholt, D.R., Visscher, P.M., Penninx, B.W., Boomsma, D.I., Martin, N.G., Montgomery, G.W., Whitfield, J.B., 2009. Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. Nat. Genet. 41 (11), 1173–1175. https://doi.org/10.1038/ng.456.

Burz, C., Cismaru, A., Pop, V., Bojan, A., 2018. Iron-Deficiency Anemia Iron Deficiency Anemia: IntechOpen.

Camaschella, C., Pagani, A., Nai, A., Silvestri, L., 2019. Hepcidin and anemia: a tight relationship. Front. Physiol. 10, 1–7. https://doi.org/10.3389/fphys.2019.01294.

Cappellini, M.D., Musallam, K.M., Taher, A.T., 2020. Iron deficiency anaemia revisited. J. Intern. Med. 287 (2), 153–170. https://doi.org/10.1111/joim.13004.

Chambers, J.C., Zhang, W., Li, Y., Sehmi, J., Wass, M.N., Zabaneh, D., Hoggart, C., Bayele, H., McCarthy, M.I., Peltonen, L., Freimer, N.B., Srai, S.K., Maxwell, P.H., Sternberg, M.J.E., Ruokonen, A., Abecasis, G., Jarvelin, M.-R., Scott, J., Elliott, P., Kooner, J.S., 2009. Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. Nat. Genet. 41 (11), 1170–1172. https://doi.org/10.1038/ng.462.

De Benoist, B., Cogswell, M., Egli, I., McLean, E., 2008. Worldwide prevalence of anaemia 1993–2005; WHO Global Database of anaemia.

Dopsaj, V., Topić, A., Savković, M., Milinković, N., Novaković, I., Ćujić, D., Simić-Ogrizović, S., 2019. Associations of common variants in HFE and TMPRSS6 genes with hepcidin-25 and iron status parameters in patients with end-stage renal disease. Disease Markers 2019, 1–10. https://doi.org/10.1155/2019/4864370. 864370.

Habib, M.A., Raynes-Greenow, C., Soofi, S.B., Ali, N., Nausheen, S., Ahmed, I., Black, K. I., 2018. Prevalence and determinants of iron deficiency anemia among non-pregnant women of reproductive age in Pakistan. Asia Pacific J. Clin. Nutrit. 27 (1), 195–203. https://doi.org/10.6133/apjcn.042017.14. 29222899.

Hershko, C., 2018. Assessment of iron deficiency. Haematologica 103 (12), 1939–1942. https://doi.org/10.3324/haematol.2018.205575.

Iranpur-Mobarakeh, V., Esmailizadeh, A., 2010. Rapid-extraction-of-high-quality-DNA-from-whole-blood-storedat-4-C-for-long-period-protocol Online PID: 4175.

Jallow, M.W., Campino, S., Prentice, A.M., Cerami, C., 2019. A recall-by-genotype study on polymorphisms in the TMPRSS6 gene and oral iron absorption: a study protocol. F1000Res 8, 701. https://doi.org/10.12688/f1000research.19080.1.

Johnson-Wimbley, T.D., Graham, D.Y., 2011. Diagnosis and management of iron deficiency anemia in the 21st century. Therap. Adv. Gastroenterol. 4 (3), 177–184. https://doi.org/10.1177/1756283X11398736.

Marret, H., Fauconnier, A., Chabbert-Buffet, N., Cravello, L., Golfier, F., Gondry, J., Agostini, A., Bazot, M., Brailly-Tabard, S., Brun, J.-L., De Raucourt, E., Gervaise, A., Gompel, A., Graesslin, O., Huchon, C., Lucot, J.-P., Plu-Bureau, G., Roman, H., Fernandez, H., 2010. Clinical practice guidelines on menorrhagia: management of abnormal uterine bleeding before menopause. Eur. J. Obstetr. Gynecol. Reprod. Biol. 152 (2), 133–137. https://doi.org/10.1016/j.ejogrb.2010.07.016.

Nagababu, E., Gulyani, S., Earley, C.J., Cutler, R.G., Mattson, M.P., Rifkind, J.M., 2008. Iron-deficiency anaemia enhances red blood cell oxidative stress. Free Radical Res. 42 (9), 824–829. https://doi.org/10.1080/10715760802459879.

Nai, A., Pagani, A., Silvestri, L., Campostrini, N., Corbella, M., Girelli, D., Traglia, M., Toniolo, D., Camaschella, C., 2011. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. Blood 118 (16), 4459–4462. https://doi.org/10.1182/blood-2011-06-364034.

Nalado, A.M., Dickens, C., Dix-Peek, T., Mahlangu, J.N., Olorunfemi, G., Paget, G., Naicker, S., 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), 1–9. 30911357.

Nikzad, Z., Iravani, M., Abedi, P., Shahbazian, N., Saki, A., Shander, A., 2018. The relationship between iron deficiency anemia and sexual function and satisfaction among reproductive-aged Iranian women. PLoS ONE 13 (12), 1–10. https://doi.org/10.1371/journal.pone.0208485.t004.

Pei, S.-N., Ma, M.-C., You, H.-L., Fu, H.-C., Kuo, C.-Y., Rau, K.-M., Wang, M.-C., Lee, C.-T., 2014. TMPRSS6 rs855791 polymorphism influences the susceptibility to iron deficiency anemia in women at reproductive age. Int. J. Med. Sci. 11 (6), 614–619. https://doi.org/10.7150/ijms.8582.

Poggiali, E., Andreozzi, F., Nava, I., Consonni, D., Graziadei, G., Cappellini, M.D., 2015. The role of TMPRSS6 polymorphisms in iron deficiency anemia partially responsive to oral iron treatment: TMPRSS6 Polymorphisms in Iron Deficiency Anemia. Am. J. Hematol. 90 (4), 306–309. https://doi.org/10.1002/ajh.23929.

Ritchie, H., Roser, M., 2017. Micronutrient deficiency. Our World in data.

Tanaka, T., Roy, C.N., Yao, W., Matteini, A., Semba, R.D., Arking, D., Walston, J.D., Fried, L.P., Singleton, A., Guralnik, J., Abecasis, G.R., Bandinelli, S., Longo, D.L., Ferrucci, L., 2010. A genome-wide association analysis of serum iron concentrations. Blood 115 (1), 94–96. https://doi.org/10.1182/blood-2009-07-232496.

WHO, 2015. The Global Prevalence of Anaemia in 2011. World Health Organization, Geneva.