British Journal of Nutrition (2024), 131, 193–201 doi:10.1017/S0007114523001848

Anaemia and iron deficiency associate with polymorphism *TMPRSS6* rs855791 in Brazilian children attending day care centres

Natalia Menezes Silva[1], Mirella de Paiva Lopes[2], Raquel Machado Schincaglia[3], Alexandre Siqueira Guedes Coelho[4], Cristiane Cominetti[5] and Maria Claret Costa Monteiro Hadler[1,2]*†

[1]*Graduate Program in Health Sciences, School of Medicine, Federal University of Goiás, Goiânia, GO, Brazil*
[2]*Graduate Program in Nutrition and Health, School of Nutrition, Federal University of Goiás, Goiânia, GO 74605-080, Brazil*
[3]*School of Nutrition, Federal University of Goiás, Goiânia, GO, Brazil*
[4]*School of Agronomy, Federal University of Goiás, Goiânia, GO, Brazil*
[5]*Nutritional Genomics Research Group, Nutrition and Health Graduation Program, School of Nutrition, Federal University of Goiás, Goiânia, GO, Brazil*

(Submitted 22 April 2023 – Final revision received 17 July 2023 – Accepted 11 August 2023 – First published online 22 August 2023)

Abstract

Fe-deficiency anaemia is a major public health concern in children under 5 years of age. *TMPRSS6* gene, encoding matriptase-2 protein, is implicated in Fe homoeostasis and has been associated with anaemia and Fe status in various populations. The aim of this cross-sectional study was to investigate the associations between the single nucleotide polymorphism (SNP) *TMPRSS6* rs855791 and biomarkers of anaemia and Fe deficiency in Brazilian children attending day care centres. A total of 163 children aged 6–42 months were evaluated. Socio-economic, demographic, biochemical, haematological, immunological and genotype data were collected. Multiple logistic and linear regressions with hierarchical selection were used to assess the effects of independent variables on categorised outcomes and blood marker concentrations. Minor allele (T) frequency of rs855791 was 0·399. Each copy of the T allele was associated with a 4·49-fold increased risk of developing anaemia ($P = 0·005$) and a 4·23-fold increased risk of Fe deficiency assessed by serum soluble transferrin receptor (sTfR) ($P < 0·001$). The dose of the T allele was associated with an increase of 0·18 mg/l in sTfR concentrations and reductions of 1·41 fl and 0·52 pg in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), respectively. In conclusion, the T allele of SNP *TMPRSS6* rs855791 was significantly associated with anaemia and Fe deficiency assessed by sTfR in Brazilian children attending day care centres. The effect was dose dependent, with each copy of the T allele being associated with lower MCV and MCH and higher concentrations of sTfR.

Keywords: **Blood cell counts: Genetic polymorphism: Infant: Matriptase-2: Transferrin receptor**

Anaemia is a significant global public health issue, characterised by low blood Hb concentrations and associated with long-term cognitive and motor impairments, particularly in children[1,2]. In Brazil, the prevalence of anaemia and Fe deficiency anaemia among children aged 6 to 59 months is reported to be 10·0 % and 3·6 %, respectively[3]. Fe deficiency is the leading cause of anaemia, and a substantial proportion of the world's population has a diet that is poor in this essential mineral[4]. Apart from dietary intake, Fe is also obtained through recycling of senescent red blood cells within the body[5].

Fe homoeostasis is tightly regulated by hepcidin, a key peptide that plays a crucial role in communication between various sites involved in Fe absorption, utilisation and storage[5–7]. Hepcidin acts by downregulating ferroportin, the protein responsible for releasing Fe from cells. As a result, increased levels of hepcidin are associated with impaired duodenal absorption and recycling of Fe from senescent erythrocytes. Hepcidin expression is influenced by multiple factors, including Fe overload and inflammation, as well as anaemia and hypoxemia, which can lead to decreased hepcidin levels[5]. Understanding the intricate mechanisms involved in Fe homoeostasis is crucial for addressing the public health concern of Fe-deficiency anaemia, particularly in children under 5 years of age.

The serine transmembrane protease 6 (TMPRSS6) gene encodes the matriptase-2 protein (MT-2), which plays a crucial role in the regulation of Fe metabolism. MT-2 has been shown to

Abbreviations: AGP, α-1-acid glycoprotein; CRP, C-reactive protein; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width; SNP, single nucleotide polymorphisms; sTfR, serum soluble transferrin receptor; Fe, iron; Hb, hemoglobin; RT-PCR, real-time polymerase chain reaction; GWAS, genome-wide association studies.

* **Corresponding author:** Maria Claret Costa Monteiro Hadler, email clarethadler@ufg.br

† Coordinator of FORNUTRI Working Group.

suppress hepcidin, a key regulator of Fe homoeostasis, through cleavage of hemojuvelin, a co-receptor for bone morphogenetic proteins involved in hepcidin synthesis[7–9]. By inhibiting hepcidin production, MT-2 enhances the availability of Fe for erythropoiesis and other essential cellular functions[8].

Emerging evidence suggests that genetic factors, including single nucleotide polymorphisms (SNP), contribute significantly to the regulation of Fe stores and the development of Fe deficiency[10]. Of particular interest is the *TMPRSS6* rs855791 SNP)], which has been extensively studied due to its association with various Fe-related parameters[11]. This SNP involves a thymine (T) to cytosine (C) substitution at position 2321 of the gene-coding region, resulting in an alanine to valine substitution at codon 736 (A736V) of the MT-2 protein[12,13]. One in vitro study has demonstrated that the presence of valine at codon 736 is associated with increased concentrations of hepcidin[14]. This suggests that the genetic variation introduced by SNP *TMPRSS6* rs855791 can influence hepcidin regulation and, consequently, impact Fe metabolism and risk for anaemia and Fe deficiency.

A study by Benyamin and cols. (2009) has shown that Caucasian and Asian individuals carrying the T allele of SNP *TMPRSS6* rs855791 exhibit lower serum Fe concentrations and transferrin saturation compared with those who carry the C allele[11]. Furthermore, genome-wide association studies (GWAS) have consistently demonstrated a negative association between genetic variants in *TMPRSS6* (such as rs855791 and rs4820268) and reduced Fe concentrations, transferrin saturation, as well as decreased Hb concentrations, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH)[10,11,15–17].

Based on these findings, we hypothesise that the T allele of SNP *TMPRSS6* rs855791 may be associated with Fe deficiency and anaemia in Brazilian children. Therefore, our aim was to evaluate the associations between SNP *TMPRSS6* rs855791 and biomarkers of anaemia and Fe deficiency in Brazilian children attending day care centres. By examining the associations of this genetic variation with Fe-related parameters, our study will contribute to the understanding of Fe metabolism regulation and provide insights into potential avenues for personalised interventions.

Materials and methods

Study design, participants and ethics

This cross-sectional study utilised baseline data from a randomised clustered clinical trial[18] involving children aged 6 to 42 months who attended Early Childhood Education Centres in a Brazilian capital (Fig. 1). Early Childhood Education Centres with fewer than seven children in the age groups of interest and those that were not operating full time were excluded from the clinical trial. Children with and without anaemia, between 6 and 42 months of age, were included in the study, while those undergoing treatment for anaemia, malaria, HIV, haemoglobinopathies or haemochromatosis; with low birth weight, premature delivery (< 37 weeks), twins or allergy to any components of the fortification sachet and/or ferrous sulphate/folic acid were excluded. Children whose parents/guardians did not authorise DNA extraction, did not have enough blood for

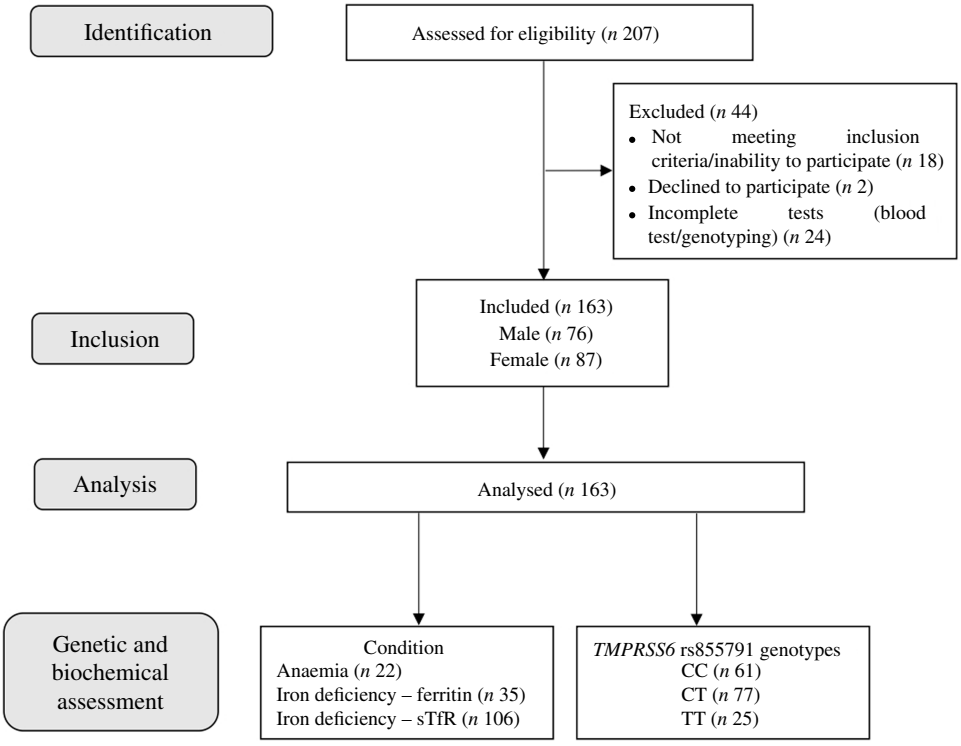

Fig. 1. STROBE flow chart of participants' recruitment.

DNA extraction or had missing data from haematological and biochemical tests were also excluded.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Research Ethics Committee of the Federal University of Goiás (protocol 2·641·285, 8 May 2018, CAAE: 80541717·3·0000·5083). Written informed consent was obtained from all children's parents/guardians. The study 'Effect of fortification with powder nutrients in prevention and treatment of nutrient deficiency' was registered at the Brazilian Registry of Clinical Trials – ReBEC (protocol RBR-4hm7 mz; https://ensaiosclinicos.gov.br/rg/RBR-4hm7mz).

Data collection

Data were collected from March 2018 to August 2018 in ten Early Childhood Education Centres through a structured questionnaire administered to the children's parents/guardians by trained interviewers. The questionnaire collected information on socio-economic, demographic and individual child characteristics, including sex, age, race, family income, number of family members, maternal level of education, day care attendance time, birth weight, length at birth, current weight and exclusive breast-feeding up to 6 months.

Body weight was measured in duplicate using a digital scale (SECA 877®, Seca Deutschland) with a capacity of 200 kg and an accuracy of 100 g, following a standardised protocol[19]. For children younger than 2 years, weight was measured with the child on the lap of the mother/guardian or the teacher/supervisor[20].

Fe intake was determined using two methods: direct food weighing and a 24-h food recall method (R24h). Direct food weighing was conducted at the education centres to assess food consumption[21]. On the same day as the direct weighing, the R24h method was applied to parents or individuals responsible for assessing food consumption at home. Home measures and photographic records were used as auxiliary tools for dietary surveys[22]. Data from the R24h and food weighing were calculated using the Diet Win Professional Plus® software (Brubins Food Commerce, Brazil). Food composition data from national tables[23–25] and food labels were incorporated into the software as needed. Breast-feeding was assessed by estimating the volume of milk based on the number of feeds per day[26].

Venous blood collection was performed by a trained technician. The collection was preferably done after an 8-h fast, but at least 3 h of fasting was ensured for children younger than 12 months. The blood samples were appropriately refrigerated and transported to the laboratory for further analysis.

The blood count was analysed using electronic count (Sysmex XE-2100, Sysmex Corporation). Anaemia was defined as Hb levels below 11 g/dl, and low haematocrit values were defined as below 33 %, based on the recommendations of the WHO[1]. Microcytosis was defined as MCV below 70 fl for children aged 6–23 months and below 73 fl for those aged 24–59 months[1,27]. Hypochromia was identified as MCH values below 22 pg for children aged 6–23 months and below 25 pg for those aged 24–59 months. Altered mean corpuscular haemoglobin concentration was defined as below 32 g/dl for children aged 6–59 months[1,27]. Anisocytosis was defined as red cell distribution width (RDW) above 14·5 %[28,29].

Serum concentrations of α-1-acid glycoprotein (AGP) were analysed using immunoturbidimetry (AU480 Chemistry Analyzer, Beckman Coulter, Brea), while serum concentrations of C-reactive protein (CRP) were determined by turbidimetry (Cobas 8000 c502, Roche Diagnostics). Children with serum AGP levels exceeding 100 mg/dl and/or CRP levels exceeding 0·5 mg/dl were considered to have inflammation[30,31]. Serum ferritin concentrations were analysed by chemiluminescence (UniCel DxI 800, Beckman Coulter), and Fe deficiency assessed by ferritin was defined as ferritin levels below 12 ng/dl in the absence of inflammation and below 30 ng/dl in the presence of inflammation[31]. Serum soluble transferrin receptor (sTfR) concentrations were analysed by nephelometry (BN II System, Siemens Healthineers). Fe deficiency was defined as sTfR levels exceeding 1·76 mg/l, which is the reference value from the analysis kit.

Genomic DNA was extracted from leukocytes in whole blood using the PureLink Genomic DNA Mini Kit® (Thermo Fisher Scientific). Quantification was performed using a Qubit fluorimeter® (Invitrogen), and DNA purity was verified using a NanoDrop spectrophotometer® (Thermo Fisher Scientific). Samples were considered of good quality when the A260/280 ratio fell between 1·7 and 2·0.

Genotyping was conducted using real-time polymerase chain reaction (RT-PCR) with an Applied Biosystems TaqMan® SNP Genotyping assay (Thermo Fisher Scientific) for SNP *TMPRSS6* rs855791. Amplification reactions for SNP genotyping were performed in a StepOne® thermocycler (Thermo Fisher Scientific). As a methodological control, 10 % of the samples were double genotyped. The analysis and processing of fluorescence data for allelic discrimination were performed using the StepOne® v. 2·1 software (Thermo Fisher Scientific).

Statistical analyses and justification of sample size

The data were entered twice using Epi Info version 6·04d (CDC) and Microsoft Excel 2010 (Microsoft) to ensure consistency. To assess the goodness of fit to the normal distribution, the Shapiro–Wilk test was used. Normally distributed continuous variables were presented as mean ± SD, while non-normally distributed variables were presented as median with interquartile range. Categorical variables were presented as absolute (*n*) and relative (%) frequencies.

The sample size calculation was based on an absolute error of 5 %, a power of 80 %, an allocation rate of 1:1 and an effect size of 0·38. The effect size was determined based on the prevalence of anaemia found in a previous Brazilian study[32]. The calculated sample size was 164 children, and an additional 22 % was added to account for potential losses during the data collection process, resulting in a total sample size of 200 children.

Multiple logistic regression models with hierarchical selection of variables were utilised to estimate the effects of various independent variables, including children's sex and age, maternal level of education, family income, number of family members, day care time, weight and length at birth, breast-

feeding up to 6 months, dietary Fe intake, CRP, AGP, current weight, as well as additive and dominance effects of the T allele, on categorised outcomes such as anaemia, Fe deficiency and haematological biomarkers. Linear regression models with hierarchical selection of variables were employed to estimate the effects on blood markers including Hb, ferritin, sTfR and other haematological biomarkers.

In both regression models, the variables were organised into blocks and prioritised based on their presumed influence on the outcome. The distal block encompassed variables such as family income, maternal level of education, number of dependents and children's sex and age. The medial block included day care time, while the proximal block included weight and length at birth, current weight, exclusive breast-feeding up to the sixth month, dietary Fe intake, CRP and AGP concentrations, as well as additive and dominance effects of the T allele of SNP *TMPRSS6* rs855791.

Multiple models were created by testing variables from the distal to the proximal block using an automated stepwise approach. Variables that were associated with the presence of the T allele were identified based on a significance level of P value < 0·05 after adjusting for potential confounders within the same block and superior hierarchical block(s). In cases where significant deviations from normality were detected, the models were adjusted using a boxcox transformation. However, as the results obtained with and without transformation were congruent, the results were presented without any transformation.

The adherence of SNP TMPRSS6 rs855791 to the Hardy–Weinberg equilibrium was assessed using the Pearson χ^2 test, conditioned on the allele frequencies estimated from the study children. All statistical analyses were conducted using R version 4.2.2[33]. The significance level for all analyses was set at 5 %.

Results

Sample characteristics

The final sample consisted of 163 children, of whom 53·4 % were female, with a median age of 24·0 (14·5–34·5) months. The children had been attending the Early Childhood Education Centre facility for a median duration of 13·0 (4·0–24·0) months. The frequency of the minor allele was 0·399. The prevalence of anaemia was 14·4 % (n 22), while the prevalence of Fe deficiency, assessed by serum ferritin and sTfR concentration, was 21·6 % (n 35) and 65·4 % (n 106), respectively. Other socio-economic, demographic, genotype and biochemical data are provided in Table 1.

Association of anaemia, iron deficiency and biochemical and haematological markers with SNP TMPRSS6 rs855791

The distribution of genotypes observed in the study did not deviate significantly from the expected frequencies under the Hardy–Weinberg equilibrium ($\chi^2 = 0·0076$; $P = 0·9305$). In the additive model analysis of SNP *TMPRSS6* rs855791 (CC × CT × TT), the T allele was significantly associated with anaemia and Fe deficiency assessed by sTfR concentrations. For each copy of the T allele, there was a 4·5-fold increase in the odds of presenting anaemia (OR = 4·49, 95 % CI = 1·56, 12·91, $P = 0·005$) and a 4·2-fold increase in the odds of presenting Fe deficiency assessed by sTfR concentrations (OR = 4·23, 95 % CI = 2·07, 8·65, $P < 0·001$). No associations between the dominant model and anaemia were observed, and this model was not included in the final model for the other outcomes.

Anaemia and Fe deficiency assessed by sTfR concentrations were significantly associated with maternal level of education, family income, AGP and CRP ($P < 0·05$). In addition, male sex was associated with a 3·8-fold increase in the odds of developing Fe deficiency assessed by sTfR concentrations compared with female sex (OR = 3·80, 95 % CI = 1·57, 9·20, $P = 0·022$, Table 2). Separate models for males and females were analysed, and differences were observed only for Fe deficiency assessed by sTfR concentrations. For each copy of the T allele, female children had a 6·2-fold increase in the odds of developing Fe deficiency (OR = 6·2, 95 % CI = 2·3, 16·6, $P < 0·001$), and only male children showed a 2·6-fold increase in the odds of presenting high RDW (OR = 2·6, 95 % CI = 1·04, 6·3, $P = 0·041$) for each copy of the T allele (data not shown).

The results of the linear regression analyses revealed significant associations between genetic and demographic factors and various haematological parameters. Each copy of the T allele was found to be associated with an increase of 0·18 mg/l in sTfR concentrations ($P < 0·001$) and reductions of 1·41 fl in MCV ($P = 0·006$) and 0·52 pg in MCH ($P = 0·013$), as shown in Table 3. Moreover, an increase of 0·01 mg/l in sTfR concentration ($P < 0·001$) was observed for each unit increase in AGP. Additionally, with each month increase in the child's age, there was a reduction of 0·01 in sTfR concentrations ($P = 0·004$) and 0·06 in RDW ($P < 0·001$), accompanied by increases of 0·13, 0·07 and 0·04 in MCV, MCH and mean corpuscular haemoglobin concentration, respectively ($P < 0·001$). Furthermore, each additional year of maternal education was associated with an increase of 0·34 in MCV ($P = 0·001$). Notably, male sex was found to be positively associated with sTfR concentrations ($P = 0·013$) and RDW ($P = 0·003$), while being negatively associated with MCV ($P < 0·001$) and MCH ($P < 0·001$), as indicated in Table 3.

Discussion

This is the first study to evaluate associations between SNP *TMPRSS6* rs855791 and various biochemical, haematological and immunological markers related to anaemia and Fe deficiency in Brazilian children. The T allele was associated with an increased odds of developing anaemia and Fe deficiency, as assessed by sTfR concentrations. Additionally, each copy of the T allele was associated with an increase of 0·18 mg/l in sTfR concentrations and reductions of 1·41 fl and 0·52 pg in MCV and MCH, respectively.

Comparing our results with previous studies, the frequency of the T allele in Brazilian children was similar to that found in Polish children with coeliac disease[34] and other Caucasian, European and Asian populations[11,12,35], but higher than South African populations[36]. These differences between populations may potentially impact the outcomes of various diseases, including anaemia and Fe deficiency.

Table 1. Baseline characteristics of the sample (*n* 163)

	Total sample		With anaemia		Without anaemia		
Variables	*n*	%	*n*	%	*n*	%	*P* value
Sex							0·733
Female	87·0	53·4	11·0	50·0	76	54·0	
Male	76·0	46·6	11·0	50·0	65	46·1	
Age (months)							
Median	24·0		13·0		26		< 0·001
IQR	14·5–34·5		10·5–21·8		20·0–35·0		
Skin colour							0·5663
White	76·0	46·6	12·0	54·5	64	45·4	
Brown	78·0	47·8	10·0	45·4	68	48·2	
Black	9·0	5·5	0·0	0·0	9	6·4	
	Median	IQR	Median	IQR	Median	IQR	
Family income (R$)	2600·0	1800·0–3500·0	1954·0	1425·0–2875·0	3000·0	1977·0–4000·0	0·0300
Maternal level of education (full years)	12·0	12·0–15·0	12·0	11·2–12·0	12·0	12·0–16·0	0·0199
Family members	4·0	3·0–4·0	4·0	4·0–5·0	4·0	3·0–4·0	0·0335
Day care time (months)	13·0	4·0–24·0	4·0	4·0–3·0	15·0	5·0–24·0	< 0·001
Birth weight (g)	3240·0	3000·0–3495·0	3467·5	3226·2–3630·0	3202·5	2957·5–3450·0	0·0077
Birth length (cm)	49·0	48·0–51·0	50·0	49·0–51·0	49·0	48·0–51·0	0·0237
Current weight (g)	13·1	11·2–15·0	10·8	9·0–12·8	13·3	11·6–15·3	< 0·001
TMPRSS6 rs855791 genotype							0·0162
C/C							
n	61·0		5·0		56·0		
%	37·4		22·7		39·7		
C/T							
n	77·0		9·0		68·0		
%	47·2		40·9		48·2		
T/T							
n	25·0		8·0		17·0		
%	15·4		36·4		12·1		
MAF	0·399						
Hb (g/dl)	12·4	11·6–13·0	10·6	10·4–10·8	12·5	11·9–13·1	< 0·001
Ferritin (µg/l)	25·8	17·8–38·3	22·4	15·9–27·7	26·6	18·0–40·4	0·1266
CRP (mg/dl)	0·1	0·04–0·33	0·4	0·10–0·80	0·09	0·03–0·18	0·0011
AGP (mg/dl)	85·0	71·0–105·0	102·5	84·2–121·5	84·0	71·0–103·0	0·0064
sTfR (mg/l)	1·9	1·7–2·0	2·2	1·9–2·9	1·9	1·6–2·1	0·0063
	n	%	*n*	%	*n*	%	
Fe deficiency – ferritin							< 0·001
Yes	35·0	21·6	12·0	54·6	23·0	16·4	
No	127·0	78·4	10·0	45·4	117·0	83·6	
Fe deficiency – sTfR							0·2091
Yes	106·0	65·4	17·0	77·3	89·0	63·6	
No	56·0	34·6	5·0	22·7	51·0	36·4	
Haematocrit (%)							0·6889
Deficiency	13·0	8·0	2·0	9·1	11·0	7·8	
Normal	150·0	92·0	20·0	90·9	130·0	92·2	
MCV* (fl)							0·0077
Microcytosis	20·0	12·3	7·0	31·8	13·0	9·2	
Normal	143·0	87·7	15·0	68·2	128·0	90·8	
MCH† (pg)							0·00691
Hypochromia	30·0	18·4	9·0	40·9	21·0	14·9	
Normal	133·0	81·6	13·0	59·1	120·0	85·1	
MCHC‡ (g/dl)							< 0·001
Altered	13·0	8·0	8·0	36·4	5·0	3·6	
Normal	150·0	92·0	14·0	63·6	136·0	96·4	
RDW§ (%)							0·0019
Anisocytosis	69·0	42·3	16·0	72·7	53·0	37·6	
Normal	94·0	57·7	6·0	27·3	88·0	62·4	

Continuous variables are presented as median (interquartile range) and categorical variables are presented as absolute number [percentage]. MAF, minor allele frequency; CRP, C-reactive protein; AGP, alpha-1-acid glycoprotein; sTfR, soluble transferrin receptor; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin, MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width.

* Microcytosis (< 70 fl–6 to 23 months; < 73 fl–24 to 59 months).

†Hypochromia (< 22 pg–6 to 23 months; < 25 pg–24 to 59 months).

‡Altered (< 32 g/dl–6 to 59 months).

§Anisocytosis (> 14·5 %). Sample sizes for sex, age, skin colour, maternal education level, family members, day care time, Hb, SNP *TMPRSS6* rs855791, haematocrit, MCV, MCH, MCHC and RDW: *n* 163; family income, current weight, ferritin, CRP, AGP, sTfR and Fe deficiency–ferritin and sTfR: *n* 162; birth weight: *n* 160; birth length: *n* 156.

Table 2. Association of SNP *TMPRSS6* rs855791 genotypes with biochemical and haematological markers of anaemia and iron deficiency (*n* 153)*

Outcome	OR	95 % CI	*P* value
Anemia†			
Maternal level of education	0·64	0·47, 0·88	0·006
Income	0·99	0·99, 1·00	0·028
AGP	1·03	1·01, 1·05	0·043
rs855791_a	4·49	1·56, 12·91	0·005
rs855791_d	0·25	0·06, 1·16	0·077
Iron deficiency – ferritin‡			
Income	0·99	0·99, 1·00	0·040
rs855791_a	1·45	0·81, 2·61	0·211
Iron deficiency – sTfR§			
Age	0·94	0·90, 0·98	0·003
Male sex	3·80	1·57, 9·20	0·003
CRP	0·40	0·21, 0·75	0·004
AGP	1·05	1·02, 1·08	< 0·001
rs855791_a	4·23	2·07, 8·65	< 0·001
MCV			
Age	0·95	0·90, 0·99	0·044
Male sex	9·63	2·56, 36·21	< 0·001
Income	0·99	0·99, 1·00	0·033
rs855791_a	1·09	0·50, 2·39	0·820
MCH‖			
Male sex	2·93	1·20, 7·16	0·020
Income	0·99	0·99, 1·00	0·008
rs855791_a	1·11	0·59, 2·08	0·742
MCHC¶			
Age	0·91	0·83, 0·99	0·030
Birth weight	1·00	1·00, 1·01	0·040
AGP	1·03	1·01, 1·06	0·004
rs855791_a	1·32	0·53, 3·29	0·544
RDW**			
Age	0·91	0·87, 0·94	< 0·001
Male sex	2·15	1·01, 4·59	0·048
rs855791_a	1·35	0·78, 2·33	0·280

(a), Additive model (CC × CT × TT); d, dominant model (CC × CT + TT); AGP, alpha1-acid glycoprotein; sTfR, soluble transferrin receptor; CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width.
* Only children with complete data for all evaluated markers were included.
†Adjusted for age, number of dependents, birth weight, exclusive breast-feeding up to sixth month and mean weight ($0·05 > P \leq 0·20$).
‡Adjusted for age ($0·05 > P \leq 0·20$).
§Adjusted for exclusive breast-feeding up to sixth month ($0·05 > P \leq 0·20$).
‖Adjusted for AGP ($0·05 > P \leq 0·20$).
¶Adjusted for sex ($0·05 > P \leq 0·20$).
** Adjusted for income, number of dependents and CRP ($0·05 > P \leq 0·20$).

Table 3. Association of the additive model of SNP *TMPRSS6* rs855791 with haematological and biochemical markers – final model (*n* 153)*

Outcome	Regression coefficient	SE	*P* value	R†
Hb†				0·3279
Age (months)	0·03	0·01	0·007	
CRP (mg/dl)	–0·22	0·07	0·003	
Current weight (g)	0·08	0·04	0·048	
rs855791_a	–0·19	0·11	0·077	
Ferritin‡				0·1826
Maternal level of education (years)	1·23	0·41	0·003	
CRP (mg/dl)	3·48	1·48	0·020	
rs855791_a	–2·40	1·65	0·147	
sTfR§				0·2259
Age (months)	–0·01	0·003	0·004	
Male sex	0·17	0·070	0·013	
AGP (mg/dl)	0·01	0·002	< 0·001	
rs855791_a	0·18	0·050	< 0·001	
MCV‖				0·2047
Age (months)	0·13	0·03	< 0·001	
Male sex	–2·69	0·70	< 0·001	
Maternal level of education (years)	0·34	0·12	0·001	
rs855791_a	–1·41	0·51	0·006	
MCH¶				0·2752
Age (months)	0·07	0·01	< 0·001	
Male sex	–1·05	0·28	< 0·001	
Maternal level of education (years)	0·13	0·05	0·020	
rs855791_a	–0·52	0·21	0·013	
MCHC**				0·2510
Age (months)	0·04	0·01	< 0·001	
AGP (mg/dl)	–0·01	0·003	0·003	
rs855791_a	–0·09	0·12	0·430	
RDW				0·3180
Age (months)	–0·06	0·01	< 0·001	
Male sex	0·57	0·19	0·003	
Income (R$)	–0·0001	< 0·001	0·030	
AGP (mg/dl)	0·008	0·004	0·025	
rs855791_a	0·22	0·141	0·116	

(a), Additive model (CC × CT × TT); AGP, alpha1-acid glycoprotein; sTfR, soluble transferrin receptor; CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width.
* Only children with complete data for all evaluated markers were included.
†Adjusted for maternal level of education and birth length ($0·05 > P \leq 0·20$).
‡Adjusted for sex, number of family members and AGP ($0·05 > P \leq 0·20$).
§Adjusted for income, exclusive breast-feeding up to sixth month and CPR ($0·05 > P \leq 0·20$).
‖Adjusted for birth weight ($0·05 > P \leq 0·20$).
¶Adjusted for income, birth weight and AGP ($0·05 > P \leq 0·20$).
** Adjusted for income ($0·05 > P \leq 0·20$).

In line with our study, previous research has also explored the role of *TMPRSS6* gene variants in Fe-related haematological parameters in different populations. Our findings are consistent with previous GWAS that identified associations between SNP *TMPRSS6* rs855791 and lower values of Hb, Fe and MCV in European, North American, Asian and Australian populations[11,12,37]. Consistent with our findings, Batar et al. (2018) reported significant associations between SNP *TMPRSS6* rs855791 and several haematological parameters in Turkish patients with Fe-deficiency anaemia[38]. However, our results differ from a study in Indonesian children, where no associations were observed between this SNP and sTfR and Hb concentrations[39].

In healthy individuals, hepcidin binds to ferroportin, forming a complex that prevents Fe outflow and increases cellular Fe stores as ferritin[5,6]. However, in individuals carrying the SNP *TMPRSS6* rs855791, which causes a change in amino acids in MT-2, the Fe flow in enterocytes is altered, leading to reduced absorption[12]. Carriers of the risk allele exhibit inefficient regulation of hepcidin synthesis, resulting in consistently high concentrations of hepcidin, even during Fe deficiency. This sustained elevation of hepcidin levels prevents the restoration of Fe homoeostasis at both intestinal and macrophage levels, leading to recurrent Fe deficiency. As a result, oral supplementation may not be fully effective in these individuals, as its effectiveness depends on intestinal absorption[11,14,34].

The presence of the T allele of SNP TMPRSS6 rs85579 in carriers is typically associated with low Fe concentrations. This genetic variant promotes the formation of a complex between

responsive elements and Fe-regulating proteins in the mRNA of cellular transferrin receptor, leading to an increase in transferrin receptor synthesis as a compensatory mechanism for Fe uptake[40]. The concentration of transferrin receptor is directly correlated with sTfR concentrations[37]. A meta-analysis conducted in European populations estimated that the T allele is associated with a 0·20 mg/l increase in sTfR concentrations[35], which supports our findings.

While sTfR concentrations could provide valuable information to complement the diagnosis of anaemia, only Hb concentrations have been prioritised. However, a definitive diagnosis of anaemia also considers MCV and MCH values[41,42], which indicate microcytosis and hypochromia associated with Fe deficiency anaemia[43]. Interestingly, our findings revealed associations between SNP *TMPRSS6* rs85579 and MCV and MCH, showing significant reductions in these markers with each copy of the T allele, suggesting the need for further investigation.

Apart from the significant associations with SNP *TMPRSS6* rs85579, it appears that other genetic variations may also be involved in the regulation of sTfR concentrations. For instance, in African individuals, SNP *TF* rs1799852 was associated with lower transferrin levels and higher sTfR concentrations[36]. In Australian individuals, SNP *HFE* rs1800562 and *TF* rs1799852 and rs3811647 together explained 40 % of the variation in sTfR concentrations[11]. Similarly, in African women, SNP *TF* rs1799852 and rs3811647 explained 13 % of the variations in sTfR concentrations[36].

In addition to the associations between SNP *TMPRSS6* rs855791 and markers of anaemia and Fe deficiency, we identified associations between these markers and other variables, including age, income, maternal education level, current weight, sex and inflammatory status. These findings highlight the importance of considering socio-demographic variables when evaluating anaemia and Fe deficiency, as they are often intrinsic determinants of poor nutrition. Our results are supported by other studies that have reported similar relationships between markers of anaemia and Fe deficiency with maternal education level, family income, child's age, sex and concentrations of CRP and AGP[44–46].

It is noteworthy that respiratory and inflammatory diseases are common in children attending day care centres and educational institutions, likely due to close contact with other children and the immaturity of their immune system[47,48]. Fe deficiency and inflammation are closely related, as elevated concentrations of hepcidin are often found in children with pre-existing inflammatory conditions[49]. Therefore, our findings emphasise the importance of further investigating inflammatory markers, anaemia, Fe deficiency and their associations with SNP *TMPRSS6* rs85579.

Our study has several limitations, including the absence of assessment for hepcidin levels. Additionally, we only investigated one SNP in the *TMPRSS6* gene, although it is well established that other genetic variants may also be associated with the outcomes we investigated. Despite the significance of our findings, we acknowledge the need for further investigations in other regions of Brazil, particularly due to the high level of population miscegenation. Nevertheless, this study is the first to analyse the associations between SNP *TMPRSS6* rs855791 and a wide range of parameters related to anaemia and Fe deficiency in Brazilian children, including inflammatory markers, which expands our understanding of the genetic aspects of Fe metabolism.

In conclusion, our study revealed significant associations between the T-risk allele of SNP *TMPRSS6* rs855791 and anaemia and Fe deficiency in Brazilian children attending day care centres. Children carrying the T-risk allele had an increased risk of developing anaemia and Fe deficiency, as evidenced by higher serum concentrations of sTfR. Moreover, the dose of the T allele was associated with lower MCV and MCH values. These findings have implications for directing intervention studies and developing precision nutrition-based strategies to mitigate the adverse effects of these health problems.

It is important to note that the development of effective precision nutrition strategies requires a multifactorial understanding of Fe homoeostasis, including the influence of other genetic variants, dietary factors and environmental influences. Therefore, it is crucial to conduct additional research to identify other relevant genetic markers and their interactions, as well as to investigate gene–environment interactions to refine the selection process for precision nutrition strategies.

Acknowledgements

*Members of FORNUTRI (Fortification with NutriSUS) Working Group: Paulo Sérgio Sucasas da Costa, Ana Paula Viana de Siqueira, Bárbara Pimenta, Vanessa Farias Franco and Nágila Alencar Afyoni. The authors would like to thank all the professionals of the education centres, parents/guardians and children who trusted our work and to the team that helped in data collection.

Financial support

Funding was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [public call CNPq/MS/SCTIE/DECIT/SAS/DAB/CGAN Nº 13/2017, grant Nº 408786/2017–5] and by Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG) [public call Nº 04/2017, Programa Pesquisa para o SUS, FAPEG/SES-GO/CNPq/MS/DECIT/2017 PPSUS-GO, grant Nº 201710267001247]. The funders had no role in the design, analysis or writing of this article.

Authorship

The authors' contributions are as follows: N.M.S. and M.P.L. participated in data acquisition, analysis, interpretation, discussion and wrote the first version of the manuscript; R.M.S. and A.S.G.C. participated in data analysis, interpretation, discussion and reviewed the manuscript. C.C. was the co-supervisor, contributed to data interpretation and wrote and reviewed the manuscript; M.C.C.M.H. was the supervisor responsible for the study conception, data interpretation and reviewed the manuscript. Paulo Sérgio Sucasas da Costa, Ana Paula Viana de Siqueira, Bárbara Pimenta, Vanessa Farias Franco and Nágila Alencar Afyoni for pediatric care, prescription of medications

and data acquisition. Maria Aderuza Hortz who contributed to conceptualization and methodology. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

1. World Health Organization (2001) *Iron Deficiency Anemia. Assessment, Prevention, and Control. A Guide for Programme Managers*. Geneva: WHO. pp. 47–65.
2. World Health Organization (2017) *Nutritional Anaemias: Tools for Effective Prevention and Control*. Geneva: WHO.
3. Universidade Federal do Rio de Janeiro (2020) *Estudo Nacional de Alimentação e Nutrição Infantil – ENANI-2019: Resultados preliminares – Prevalência de anemia e deficiência de vitamina A entre crianças brasileiras de 6 a 59 meses (National Child Food and Nutrition Study – ENANI-2019: Preliminary results – Prevalence of anemia and vitamin A deficiency among Brazilian children aged 6 to 59 months)*. Rio de Janeiro: UFRJ. https://enani.nutricao.ufrj.br/index.php/relatorios/ (accessed January 2023).
4. Kassebaum NJ & GBD 2013 Anemia Collaborators (2016) The global burden of anemia. *Hematol Oncol Clin North Am* **30**, 247–308.
5. Camaschella C, Nai A & Silvestri L (2020) Iron metabolism and iron disorders revisited in the hepcidin era. *Haematologica* **105**, 260–272.
6. Ginzburg YZ (2019) Hepcidin-ferroportin axis in health and disease. *Vitam Horm* **110**, 17–45.
7. Muckenthaler MU, Rivella S, Hentze MW, *et al.* (2017) A red carpet for iron metabolism. *Cell* **168**, 344–361.
8. Nemeth E, Tuttle MS, Powelson J, *et al.* (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* **306**, Suppl. 5704, 2090–2093.
9. Du X, She E, Gelbart T, *et al.* (2008) The serine protease TMPRSS6 is required to sense iron deficiency. *Science* **320**, Suppl. 5879, 1088–1092.
10. Tanaka T, Roy CN & Yao W (2010) A genome-wide association analysis of serum iron concentrations. *Blood* **115**, 94–96.
11. Benyamin B, Ferreira MA, Willemsen G, *et al.* (2009) Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. *Nat Genet* **41**, 1173–1175.
12. 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.
13. 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.
14. Nai A, Pagani A, Silvestri L, *et al.* (2011) TMPRSS6 rs855791 modulates hepcidin transcription *in* vitro and serum hepcidin levels in normal individuals. *Blood* **118**, 4459–4462.
15. Traglia M, Girelli D, Biino G, *et al.* (2011) Association of HFE and TMPRSS6 genetic variants with iron and erythrocyte parameters is only in part dependent on serum hepcidin concentrations. *J Med Genet* **48**, 629–634.
16. Soranzo N, Sanna S, Wheeler E, *et al.* (2010) Common variants at 10 genomic loci influence hemoglobin A1C levels via glycemic and nonglycemic pathways. *Diabetes* **59**, 3229–3239.
17. Oexle K, Ried JS, Hicks AA, *et al.* (2011) Novel association to the proprotein convertase PCSK7 gene locus revealed by analysing soluble transferrin receptor (sTfR) levels. *Hum Mol Genet* **20**, 1042–1047.
18. Machado MM, Lopes MD, Schincaglia RM, *et al.* (2021) Effect of fortification with Multiple Micronutrient Powder on the prevention and treatment of iron deficiency and anaemia in Brazilian children: a randomized clinical trial. *Nutrients* **13**, 2160.
19. Lohman TG, Roche AF & Martorell R (1988) *Anthropometric Standardization Reference Manual*. Chicago: Human Kinetics Books.
20. Monego ET, Vieira EC, Menezes IHFC, *et al.* (2011) Centro Colaborador em Alimentação e Nutrição (CECAN) da Região Centro-Oeste – Ministério da saúde. Antropometria: manual de técnicas e procedimentos (Anthropometry: Manual of Techniques and Procedures), 4th ed. Goiânia: CECAN.
21. Cruz AT, Souza JM & Philippi ST (2003) Avaliação da concordância dos métodos de pesagem direta de alimentos em creches – São Paulo – Brasil (Evaluation of the concordance of methods of direct weighing of food in day care centers – São Paulo – Brazil). *Rev Bras Epidemiol* **6**, 220–226.
22. Zabotto CB, Viana RP & Gil MD (1996) *Registro fotográfico para inquéritos dietéticos: Utensilios e porções (Photographic Record for Dietary Surveys: Utensils and Portions)*. Campinas, Goiânia: UNICAMP, UFG.
23. Giuntini EB (2005) Tabela Brasileira de Composição de Alimentos (Brazilian Food Composition Table). São Paulo: TBCA-USP: 2001–2004.
24. Brasil. IBGE (2011) Pesquisa de Orçamentos Familiares 2008–2009: análise do consumo alimentar pessoal no Brasil (Household Budget Survey 2008–2009: Analysis of Personal Food Consumption in Brazil). Rio de Janeiro: IBGE.
25. Philippi ST (2013) *Tabela de composição de alimentos: Suporte para decisão nutricional (Food Composition Table: Support for Nutritional Decision)*. Barueri, SP: Editora Manole.
26. National Research Council (U.S.) & Subcommittee on the Tenth Edition of the RDAs (1989) Recommended Dietary Allowances, 9th ed. Washington, DC: National Academy Press.
27. Hadler MC, Juliano Y & Sigulem DM (2002) Anemia in infancy: etiology and prevalence. *J Pediatr (Rio J)* **78**, 321–326.
28. Oski FA (1993) Iron deficiency in infancy and childhood. *N Engl J Med* **329**, 90–93.
29. Walters MC & Abelson HT (1996) Interpretation of the complete blood count. *Pediatr Clin North Am* **43**, 599–622.
30. Lundeen EA, Lind JN, Clarke KE, *et al.* (2019) Four years after implementation of a national micronutrient powder program in Kyrgyzstan, prevalence of iron deficiency and iron deficiency anemia is lower, but prevalence of vitamin A deficiency is higher. *Eur J Clin Nutr* **73**, 416–423.
31. World Health Organization (2020) *WHO Guideline on Use of Ferritin Concentrations to Assess Iron Status in Populations*. Geneva: World Health Organization.
32. Cardoso MA, Augusto RA, Bortolini GA, *et al.* (2016) Effect of providing multiple micronutrients in powder through primary healthcare on anemia in young Brazilian children: a multicentre pragmatic controlled trial. *PLoS One* **11**, e0151097.
33. R Core Team (2022) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/
34. Urbaszek K, Drabińska N, Szaflarska-Popławska A, *et al.* (2021) TMPRSS6 rs855791 polymorphism status in children with celiac disease and anemia. *Nutrients* **13**, 2782.
35. Wanjiku WNG, 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**, 1–5.

36. 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.
37. Read RW, Schlauch KA, Elhanan G, *et al.* (2019) GWAS and PheWAS of red blood cell components in a Northern Nevadan cohort. *PloS One* **14**, e0218078.
38. Batar B, Bavunoglu I, Hacioglu 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.
39. Shinta D, Adhiyanto C, Htet MK, *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.
40. Wang CY & Babitt JL (2019) Liver iron sensing and body iron homeostasis. *Blood* **133**, 18–29.
41. Cappellini MD, Russo R, Andolfo I, *et al.* (2020) Inherited microcytic anemias. *Hematol Am Soc Hematol Educ Program* **2020**, 465–470.
42. Dallman PR & Reeves JD (1984) Laboratory diagnosis of iron deficiency. In *Iron Nutrition in Infancy and Childhood*, pp. 11–44 [Stekel A, editor]. New York: Raven Press.
43. Buttarello M (2016) Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *Int J Lab Hematol* **38**, 123–132.
44. Engle-Stone R, Aaron GJ, Huang J, *et al.* (2017) Predictors of anemia in preschool children: biomarkers reflecting inflammation and nutritional determinants of anemia (BRINDA) project. *Am J Clin Nutr* **106**, S402–S415.
45. André HP, Sperandio N, Siqueira RL, *et al.* (2018) Indicadores de insegurança alimentar e nutricional associados à anemia ferropriva em crianças brasileiras: uma revisão sistemática (Indicators of food and nutrition insecurity associated with iron deficiency anemia in Brazilian children: a systematic review). *Cien Saude Colet* **23**, 1159–1167.
46. Zuffo CR, Osório MM, Taconeli CA, *et al.* (2016) Prevalence and risk factors of anemia in children. *J Pediatr (Rio J)* **92**, 353–360.
47. Lambrecht NJ, Bridges D, Wilson ML, *et al.* (2022) Associations of bacterial enteropathogens with systemic inflammation, iron deficiency, and anemia in preschool-age children in southern Ghana. *PloS One* **17**, e0271099.
48. Pedraza DF, Queiroz DD, Sales MC (2014) Doenças infecciosas em crianças pré-escolares brasileiras assistidas em creches (Infectious diseases in Brazilian preschool children assisted in day care centers). *Cien Saude Colet* **19**, 511–528.
49. Prentice AM, Bah A, Jallow MW, *et al.* (2019) Respiratory infections drive hepcidin-mediated blockade of iron absorption leading to iron deficiency anemia in African children. Sci Adv 5, eaav9020.