Original Article

A single-nucleotide polymorphism in transferrin is associated with soluble transferrin receptor in Chinese adolescents

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Background and Objectives: Associations between genetic variants in the hepcidin regulation pathway and iron status have been reported in previous studies. Most of these studies were conducted in populations of European descent and relatively few studies have been conducted in Chinese populations. In this study, we evaluated associations between single-nucleotide polymorphisms (SNPs) in the hepcidin regulation pathway, serum ferritin (SF) and soluble transferrin receptor (sTfR) in Chinese adolescents. **Methods and Study Design:** In total, 692 students from rural boarding schools were selected from six cities in China. The participants were divided into case and control groups according to criteria for SF and sTfR. Furthermore, 33 SNPs in *TMPRSS6*, *TF*, *TFR2*, *BMP2*, *BMP4*, *HJV*, *CYBRD1*, *HFE*, *IL6*, *PCSK7*, *HAMP*, *KIAA1468*, and *SRPRB* were selected. Associations between the genetic variants and SF or sTfR were detected. **Results:** For SF, rs4820268 in *TMPRSS6* was associated with an SF <25 ng/mL status. Carriers of the G/G genotype of rs4820268 exhibited significantly lower SF levels than A allele carriers did (p=0.047). For sTfR, rs1880669 in *TF*, rs4901474 in *BMP4*, and rs7536827 in *HJV* were significantly associated with an sTfR ≥4.4 mg/L status. However, in general linear model analysis, after adjustment for age, sex, and location, only rs1880669 exhibited a stable association with higher sTfR levels (p=0.032). **Conclusions:** We found rs4820268, in *TMPRSS6* that was associated with a low SF level, as previously reported, and a new association between 1880669 in *TF* and sTfR.

Key Words: single-nucleotide polymorphism, hepcidin, serum ferritin, soluble transferrin receptor, Chinese adolescents

INTRODUCTION

Iron is an essential element involved in energy metabolism and other biochemical processes, including oxygen transport in blood, oxidative phosphorylation in cellular respiration, erythropoiesis, and DNA synthesis.[1-3] Anemia is a blood disorder affecting approximately one quarter of the global population, particularly pregnant women and young children, because of their high iron requirements.[4] Iron deficiency (ID), a major cause of anemia, can result in intellectual reduction, motor function damage,[5] and iron deficiency anemia (IDA). In addition to nutritional factors and infectious diseases, a possible relationship between single-nucleotide polymorphisms (SNPs) and IDs has been reported in recent studies conducted using genome-wide association technology.[3,6-8] This evidence strongly suggests that iron status might depend on genetics and nutrition. SNPs in *BMP2*,[9] *BMP4*,[9] *HAMP*,[9] *CYBRD1*,[10] *HFE*,[11-13] *HJV*,[9,14] *IL6*,[15-17] *KIAA1468*,[8] *PCSK7*,[3] *SRPRB*,[11,18] *TF*,[8,11,19,20] *TFR2*,[21] *TMPRSS6*,[6,20,22,23] and *LOC105375147*[19] have been reported to have a positive association with the status of serum ferritin (SF), iron store (IS), soluble transferrin receptor (sTfR), hemoglobin (Hb), total iron-binding capacity, unsaturated iron-binding capacity, hemochromatosis (HFE), and ferroportin. Iron metabolism is regulated by the concerted action of several genes and proteins. In previous studies, the most positive genetic variants influencing iron status were observed in the hepcidin regulation pathway. Hepcidin, a circulating peptide hormone produced mainly in the liver, is coded by *HAMP*, and its expression level has been identified as a key factor for iron homeostasis.[20] Variations in the genes involved in the hepcidin regulation pathway have been considered as potential factors affecting iron status, and the consequences of the presence of these variants include

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Manuscript received 22 February 2016. Initial review completed 25 April 2016. Revision accepted 26 July 2016.
doi: 10.6133/apjcn.112016.04

abnormal iron status and probably the development of iron-refractory iron deficiency anemia (IRIDA).[24] In similar studies, significant associations have been reported among fluctuations in iron status, the gene polymorphisms of iron sensors on the membranes of hepatocytes, and the trigger proteins in the hepcidin regulation pathway.[9,25,26]

Each iron status biomarker has advantages and limitations. For instance, Hb is a widely used screening biomarker for ID. However, Hb is unsuitable for assessing the iron status of an individual because of its instability, low sensitivity, and low specificity.[27] On the basis of previous reports, SF remains the optimal indicator of IS in the absence of infection or inflammation.[28] Another indicator, sTfR, a measure of cellular iron deficiency, is directed by iron-deficient erythropoiesis and is not strongly affected by concurrent infection or inflammation.[29] After comparing the features of each of the aforementioned indicators, SF and sTfR were selected for this study. Although many related studies are available, most of those studies focused on populations of European descent. To provide new data in this field, a case-control study was designed to observe the associations between SNPs in the genes involved the hepcidin regulation pathway and SF and sTfR in a Chinese adolescent population.

METHODS

Study population

A case-control approach was employed to investigate the relationship between polymorphisms in genes involved in the hepcidin regulation pathway and SF and sTfR. Both male and female students who studied in the first grade of junior high school at rural boarding schools were enrolled with the fully informed consent of guardians and participants from the following six cities in China: Zhaoqing, Guangdong Province; Chengdu, Sichuan Province; Wenshang, Shandong Province; Wuzhong, Ningxia Hui Autonomous Region; and Tianjin and Songyang, Zhejiang Province. Questionnaires were designed to collect the personal and family data of the participants through a person-to-person interview. The information included demographics and the quantity of food consumed from the school canteens during week days and at home during weekends. Students with inflammation or infection were excluded, and those who did not consume meals from the school canteens, on the basis of the information obtained from the interview questionnaires, were also excluded from our study.

Measurement of SF, sTfR, and CRP

Overnight fasting venous blood samples were collected using blood collection tubes (SST^{TM} II Advance, Becton, Dickinson and Company, USA) by local CDC staff and local clinicians. The collected blood samples were allowed to stand undisturbed for 30 min. The sera were separated from the red blood cells through centrifugation at 3000 rpm at the worksite, frozen, and then sent to the laboratory of the Capital Institute of Pediatrics. All sera and red blood cells were stored at −80 °C until use in analysis. SF, sTfR, and C-reactive protein (CRP) were measured through a Roche Tina-quant immunoturbidimetric assay by using a Hitachi 7080 clinical analyzer (Roche Diagnostics) with the same batch of reagents. All assays and quality control were strictly conducted according to the manufacturer's instructions and reference samples. According to the detection results for SF and sTfR, the participants were divided into two groups: controls and cases. Cases were defined according to SF <25 ng/mL or sTfR ≥4.4 mg/L (kit reference). CRP was regarded as an indicator of infection or inflammation.[30] Samples were excluded from our analysis if CRP ≥5 mg/L[31] to eliminate the effects on SF and sTfR.

DNA extraction and genotyping

Genomic DNA was extracted from the frozen samples of red blood cells by using a magnetic bead DNA extraction kit (Bioteke Corporation), and the procedure for automatic extraction systems was performed according to the manufacturer's instructions. The concentration and purity of extracted DNA samples were measured by reading their absorbance at 260 and 280 nm. All the samples were stored at −20 °C for further use. The iPLEX platform (Sequenom, Inc., San Diego, CA, USA) was used for genotyping. The PCR and extension primers were designed using MassARRAY Assay Design 3.1 software (Sequenom, Inc.). All genotyping reactions were performed according to the manufacturer's iPLEX Application Guide (Sequenom, Inc.).

Statistical analysis

SPSS version 17.0 (SPSS, Inc., Chicago, IL), SNP Stats (http://bioinfo.iconcologia.net/snpstats/start.htm), and Haploview v4.2[32] were used for all calculations with a significance level of 0.05. The thresholds of qualified SNPs for association testing and linkage disequilibrium (LD) analysis were (1) call rate >0.8, (2) Hardy–Weinberg equilibrium test p>0.05 (for controls), (3) minor allele frequency >0.05. Results were expressed as the mean±SD. Tests of the distribution of participants and Hardy–Weinberg equilibrium were performed using chi-square analysis. Differences in continuous variables among the genotype-based groups were analyzed through ANOVA followed by a Bonferroni post hoc test. A general linear model analysis adjusted for sex, age, and location was performed to investigate whether the positive SNPs were correlated with SF and sTfR independent of the concentration. The pairwise linkage disequilibrium for diallelic sequence variants located in one gene was computed using Haploview v4.2.

Ethics statements

Written informed consent was obtained from all participants and their guardians. The protocol of this study was evaluated and approved by the ethical committee of the Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention (2013-002).

RESULTS

Characteristics of study population

In total, 681 rural boarding school students from six cities in China, aged 12 to 17 years, were recruited for this study. The demographic information and biomarker levels, namely sex, age, SF, sTfR, and CRP, are presented in

Table 1. The average age of the participants was 13.8±1.12 years and no differences were observed between males and females; therefore, the effect of age on IS was eliminated. The average serum levels of SF and sTfR were 62.6±42.7 ng/mL and 3.53±1.37 mg/mL, respectively, and no discrepancies were observed between males and females.

Characteristics of the candidate SNPs

Through the genotyping analysis of 10 genes, 33 single nucleotide variant sites were identified, among which 27 SNPs satisfied our criteria and were used for further analysis (Table 2).

Associations of genetic variants and SF

One SNP, rs4820268, in *TMPRSS6* was associated with the SF <25 ng/mL status. Carriers of G/G in rs4820268 were 3.48 (95% CI: 1.55–7.82, p=0.003) times more likely to develop the SF <25 ng/mL status than the carriers of A/A. In the dominant model, the G allele in this SNP was observed as a factor for SF <25 ng/mL; the OR of this model was 2.48 (95% CI: 1.16–5.33, p=0.01). Although the OR (G/G: OR: 2.19, 95% CI: 1.32–3.66, p=0.003) decreased slightly in the recessive model analysis, the effect of the G allele in rs4820268 on SF was also significant. Participants with G/G genotypes had a significantly lower SF level (57.8±36.0 ng/mL) than those of participants with A/A and A/G (65.79±47.70 ng/mL) genotypes (p=0.047) in the recessive model (Table 3). The general linear model analysis revealed that rs4820268 in TMPRSS6 (p=0.002) and sex (p=0.002) were related to SF levels, but age and location were not (Table 4). No other association with the SF level was observed, including rs855791, a missense mutation in *TMPRSS6*, whose effect on iron status was confirmed previously.[6]

Associations of genetic variants with sTfR

Three SNPs were found to be significantly associated

Table 1. Demographic characteristics of the participants

Characteristics	Men†	Women†	p	Total
N	353	328		681
Age, year	13.8±1.30	13.7±1.10	0.098	13.8±1.10
SF, ng/mL	64.5±45.7	60.6±39.3	0.232	62.6±42.7
sTfR, mg/L	3.63±1.00	3.44±1.67	0.076	3.53±1.37
CRP, mg/L	0.62±0.76	0.53±0.75	0.105	0.58±0.76

†Data are presented as the mean±SD.

Table 2. SNPs selected

Gene	Chr.	SNP	MAF (total)	MAF (men)	MAF (women)	Biomarkers associated of genes in previous studies
BMP2	20	rs173107	0.34	0.35	0.32	SF[9]
		rs235756	0.13	0.13	0.13	
		rs6077060	0.18	0.18	0.18	
BMP4	14	rs4901474	0.35	0.35	0.35	SF[9]
CYBRD1	2	rs2356782	0.29	0.30	0.29	SF[10]
HJV	1	rs16827043	0.23	0.21	0.25	SF[9], ferroprotin[14]
		rs7536827	0.47	0.49	0.44	
KIAA1468	18	rs9948708	0.16	0.13	0.17	TIBC[8]
PCSK7	11	rs236918	0.46	0.44	0.49	sTfR [3]
SRPRB	3	rs6794945	0.47	0.46	0.48	SF[11,18]
TF	3	rs1358024	0.39	0.38	0.40	TIBC[8,11,19], SF[11], sTfR[20]
		rs1525892	0.23	0.23	0.23	
		rs1799852	0.24	0.24	0.25	
		rs1880669	0.54	0.52	0.55	
		rs3811647	0.40	0.41	0.40	
		rs3811658	0.42	0.42	0.42	
		rs7638018	0.41	0.43	0.42	
		rs8177248	0.42	0.43	0.41	
TFR2	7	rs4434553	0.09	0.08	0.10	SI[21]
		rs7385804	0.19	0.19	0.19	
TMPRSS6	22	rs11704654	0.14	0.14	0.14	IDA[6,22], sTfR[23], SF [20]
		rs1421312	0.42	0.44	0.40	
		rs2111833	0.32	0.36	0.29	
		rs2235321	0.37	0.39	0.35	
		rs2543519	0.50	0.54	0.47	
		rs4820268	0.46	0.45	0.46	
		rs855791	0.44	0.46	0.42	

MAF: minimum allele frequency; Chr.: chromosome.

Table 3. Results of associations between rs4820268 and SF

SNP	Gene	Model	Genotype	Control	Case†	OR (95% CIs)	*p*-value*	SF, ng/mL	*p*-value*
rs4820268	*TMPRSS6*	Genotypic	A/A	126 (94.0%)	8 (6.0%)	1	0.0033	65.0±44.6	0.135
			A/G	242 (89.3%)	29 (10.7%)	1.89 (0.84-4.25)		66.2±49.2	
			G/G	145 (81.9%)	32 (18.1%)	3.48 (1.55-7.82)		57.8±36.0	
		Dominant	A/A	126 (94.0%)	8 (6.0%)	1	0.01	64.9±44.6	0.643
			A/G+G/G	387 (86.4%)	61 (13.6%)	2.48 (1.16-5.33)		62.9±44.6	
		Recessive	A/A+A/G	368 (90.9%)	37 (9.1%)	1	0.0029	65.8±47.7	0.047
			G/G	145 (81.9%)	32 (18.1%)	2.19 (1.32-3.66)		57.8±36.0	

Data are presented as the mean±SD.
†SF <25 ng/mL.
*$p<0.05$ is considered statistically significant.

Table 4. General linear model with SF level

Variables	Adjusted R^2	*F*	*p*-values*
	0.0032		
Age, years		1.484	0.224
Gender (men: 1, women: 0)		9.547	0.002
Location		2.529	0.112
rs4820268 (1: A/A, 2: A/G, 3: G/G)		6.086	0.002

All *p* values were two-tailed. *$p<0.05$ is considered statistically significant.

with the sTfR ≥4.4 mg/L status, namely rs1880669 in *TF*, rs4901474 in *BMP4*, and rs7536827 in *HJV*. Eight SNPs in *TF* were tested, but only rs1880669 showed an association with the sTfR ≥4.4 mg/L status. The genotypes A/G and A/A in rs1880669 were significantly associated with the sTfR ≥4.4 mg/L status; the ORs were 2.38 (95% CI: 1.09–5.21, $p<0.05$) and 2.47 (95% CI: 1.08–5.65, $p<0.05$), respectively. The effect of the A allele was also confirmed in the dominant model (OR: 2.41, 95% CI: 1.13–5.16, $p<0.05$). Among the SNPs in *BMP4*, rs4901474 was found to be associated with the sTfR ≥4.4 mg/L status in the dominant model. Therefore, the T allele was revealed as a risk factor for the case group. The SNP rs7536827 in *HJV* was detected and was also associated with the sTfR ≥4.4 mg/L status. Homozygosity for A in rs7536827 was suggested to be a risk factor for the sTfR ≥4.4 mg/L status in the recessive model. For rs1880669 in *TF*, participants with the A allele had significantly higher sTfR levels (3.59±1.47 mg/L) than those with G/G (3.35±0.83 mg/L) genotypes ($p=0.014$) did. For rs7536827 in *HJV*, participants with A/A genotypes had significantly higher sTfR levels (3.71±1.92 mg/L) than those with A/T (3.37±0.96 mg/L) genotypes ($p=0.033$) did. In addition, the participants with A/A genotypes had significantly higher sTfR levels (3.71±1.92 mg/L) than those with T alleles (3.43±1.02 mg/L) ($p=0.021$) did. No other SNPs exhibited any associations with sTfR (Table 5). The general linear model analysis revealed that the variant rs1880669 in *TF* was significantly associated with sTfR levels ($p=0.032$), but the effects of rs4901474 and rs7536827 on sTfR levels were nullified (Table 6). LD analyses showed that rs1880669 in *TF* and rs7536827 in HJV were independent ($r^2<0.33$). The LD plots among SNPs that were selected on *TF* and *HJV* are depicted in Figure 1. No haplotype was associated with sTfR.

DISCUSSION

The variants genotyped in our study exhibited associations with iron metabolism, particularly in the hepcidin-mediated iron metabolism pathway. The gene *TMPRSS6*, which encodes the protein matriptase-2, has an inhibitory effect on the production of hepcidin through the cleavage of hemojuvelin, a BMP coreceptor.[33] Mutations in *TMPRSS6* have been reported to be strongly associated with the indicators of iron and iron-related diseases. In our study, we observed that rs4820268 in *TMPRSS6* was associated with low SF levels, and homozygosity for G was a risk factor. This result was consistent with previous reports[34,35] and confirmed the association between *TMPRSS6* and iron status. Another crucial SNP, rs855791 in *TMPRSS6*, has been reported to be associated with many indicators of iron status and iron-related diseases.[6,24] However, we failed to replicate these results in our study. It is possible that rs4820268 is not in linkage disequilibrium ($r^2=0.044$) with rs855791 in the Chinese population; thus, its association with iron traits are different. The protein sTfR comprises two identical subunits connected by a pair of disulfide bridges to form a 190 kDa molecule.[36,37] According to previous studies,[36,38-40] the sTfR level should be negatively correlated with the body iron level. The proteins encoded by *TF*, *BMP4*, and *HJV* are the triggers of *HAMP*.[26] Although the mechanisms underlying mutations are not yet well explained, the mutations in the coding region binding terminal domains of *TF* probably alter the combining power to ferric iron and thereby affect the efficiency of iron transport. The variants rs1880669 in *TF*, rs4901474 in *BMP4*, and rs7536827 in *HJV* were found to be associated with sTfR. In the general linear analysis adjusted for age, sex, and location, the effects of rs4901474 and 7536827 were not detected. The effects of *BMP4* and *HJV* polymorphisms on sTfR were weaker than those of TF polymorphism. Homozygosity for A in rs1880669 was associated with a 2.54-fold higher likelihood of a high sTfR status than that of homozygosity for G. A similar result was observed in the dominant model. We observed for the first time that the A allele in rs1880669 increased the risk of high sTfR in this Chinese adolescent population.

Variants at other loci that were found to affect the phenotypes of iron through a signaling or transfer pathway were not detected in our study.[3,44] In addition, related studies[3,7,22,24] have revealed that different mutations in the same or different genes may play different roles and affect phenotypes to varying degrees. These findings may explain why the mutation rs4820268 in *TMPRSS6* was associated with SF and the variant rs1880669 in *TF* was associated with sTfR. These phenomena were also reported in related studies that have focused on the associations between genetic factors and IDA,[6] IRIDA,[24] or related indices of iron status.[3,10] Menstruation, a risk factor for ID and even IDA in women, was considered in several studies.[41] However, some studies have shown that menstruation did not affect the iron level or iron disorders.[23] Our results of the analysis on sTfR were consistent with reports[7,21,23,42] indicating that menstruation need not be considered a risk factor in investigating the associations between genetic variants and iron status. However, in the analysis on SF, sex manifested its effect on SF levels, which might mainly be caused by instability and sensitivity of SF.[43] These results suggested that the effects of genetic polymorphisms on iron status were more severe than those of physiologic metabolism. Although the

Table 5. Results of associations between SNPs and sTfR

SNP	Gene	model	Genotype	Control	Case[†]	OR (95% CIs)	*p*-value[*]	sTfR, mg/L	*p*-value[*]
rs1880669	*TF*	Genotypic	G/G	117 (93.6%)	8 (6.4%)	1	0.043	3.35±0.83	0.108
			A/G	276 (86.0%)	45 (14.0%)	2.38 (1.09-5.21)		3.54±1.10	
			A/A	154 (85.6%)	26 (14.4%)	2.47 (1.08-5.65)		3.68±1.97	
		Dominant	G/G	117 (93.6%)	8 (6.4%)	1	0.012	3.35±0.83	0.014
			A/G+A/A	430 (85.8%)	71 (14.2%)	2.41 (1.13-5.16)		3.59±1.47	
		Recessive	G/G+A/G	393 (88.1%)	53 (11.9%)	1	0.39	3.48±1.03	0.21
			A/A	154 (85.6%)	26 (14.4%)	1.23 (0.76-2.07)		3.68±1.97	
rs4901474	*BMP4*	Genotypic	C/C	241 (91.6%)	22 (8.4%)	1	0.069	3.43±1.16	0.191
			C/T	225 (85.2%)	39 (14.8%)	1.90 (1.09-3.30)		3.64±1.71	
			T/T	74 (88.1%)	10 (11.9%)	1.49 (0.67-3.27)		3.43±0.86	
		Dominant	C/C	241 (91.6%)	22 (8.4%)	1	0.027	3.43±1.16	0.169
			C/T+T/T	299 (85.9%)	49 (14.1%)	1.80 (1.06-3.05)		3.59±1.55	
		Recessive	C/C+C/T	466 (88.4%)	61 (11.6%)	1	0.93	3.54±1.47	0.524
			T/T	74 (88.1%)	10 (11.9%)	1.03 (0.51-2.10)		3.43±0.86	
rs7536827	*HJV*	Genotypic	T/T	131 (87.3%)	19 (12.7%)	1	0.034	3.54±1.12	0.033
			A/T	250 (91.6%)	23 (8.4%)	0.63 (0.33-1.21)		3.37±0.96	
			A/A	153 (83.6%)	30 (16.4%)	1.35 (0.73-2.51)		3.71±1.92[‡]	
		Dominant	T/T	131 (87.3%)	19 (12.7%)	1	0.73	3.54±1.12	0.795
			A/T+A/A	403 (88.4%)	53 (11.6%)	0.91 (0.52-1.59)		3.51±1.43	
		Recessive	T/T+A/T	381 (90.0%)	42 (10.0%)	1	0.028	3.43±1.02	0.021
			A/A	153 (83.6%)	30 (16.4%)	1.78 (1.07-2.95)		3.71±1.92	

[†]sTfR >4.4 mg/mL.
All *p* values were two sided. [*]$p<0.05$ is considered statistically significant.
[‡]denotes a significant difference between the A/A genotype and A/T genotype.

Table 6. General linear model with sTfR levels

Variables	Adjusted R^2	*F*	*p*-values*
	0.10		
Age, years		0.110	0.740
Gender (men: 1, women: 0)		1.970	0.161
Location		0.020	0.888
rs1880669 (1: G/G, 2: A/G, 3: A/A)		1.118	0.032
rs4901474 (1: C/C, 2: C/T, 3: T/T)		2.125	0.120
rs7536827 (1: T/T, 2: A/T, 3: A/A)		2.701	0.068

All *p* values were two sided. *$p < 0.05$ is considered statistically significant.

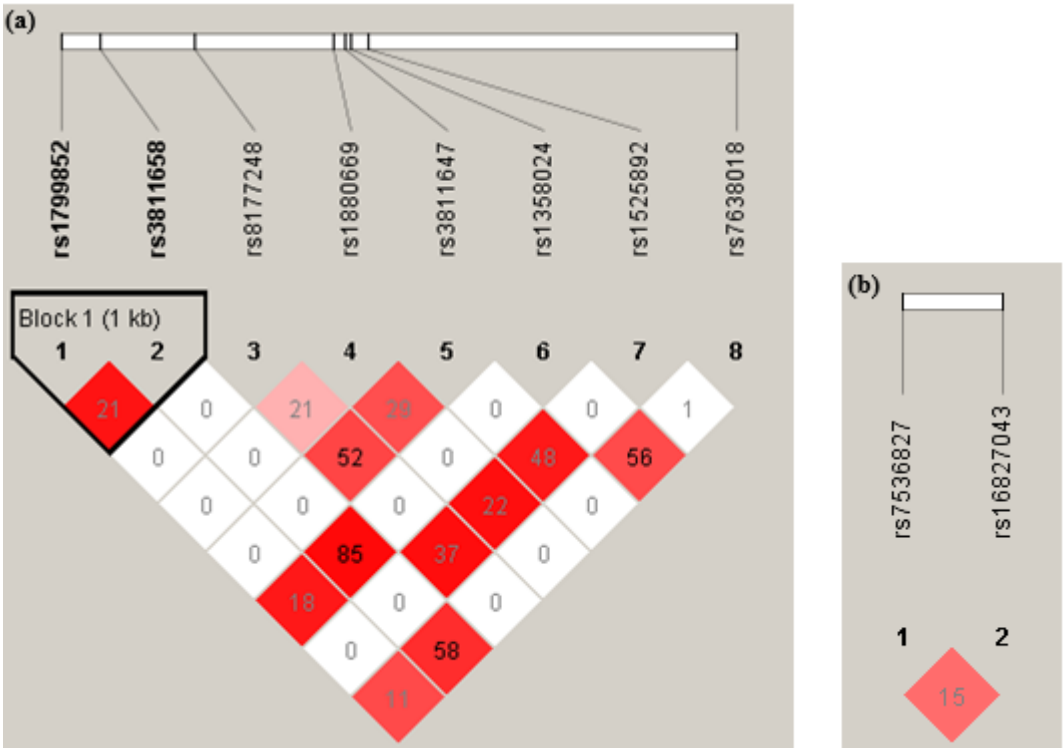

Figure 1. (a) Linkage disequilibrium information of rs1880669 in *TF* based on r^2; (b) Linkage disequilibrium information of rs7536827 in HJV based on r^2.

variants reported in previous studies were inconsistent, the effects of crucial genes on iron status were confirmed in this study.

The protein encoded by *HFE* regulates iron absorption by modulating transferrin receptor and interacts with transferrin. Previous studies have shown that the most common missense mutation, C282Y, along with another variant, H63D, in the *HFE* gene can influence the parameters of iron metabolism and lead to genetic hemochromatosis.[44] Although positive results regarding the roles of the *HFE* gene were identified in populations of European descent, no associations were observed in this study, despite the possibility that the allelic frequencies of these variants were rare in Chinese populations.[6]

Considering the complexity of iron homeostasis, collaborative effects should be further resolved. According to previous studies and the results from our research, we assumed that the strength of products encoded by different genes varies. The spatial configuration of proteins is probably determined by crucial amino acids located at pivotal loci, which might be explained by the fact that some of the detected SNPs are sufficiently potent to affect phenotypes but others are weaker. Although the precise underlying mechanisms have yet to be defined, previous studies and our study have established that *TMPRSS6*, *TF*, *BMP4*, *HJV*, and related genes are associated with iron status. Our study revealed an association between genetic variants and SF and sTfR in a Chinese adolescent population. Moreover, a novel association between rs1880669 in *TF* and sTfR levels was identified in the Chinese adolescent population. The results of this study could strengthen the evidence of the functional role of the genes involved in iron regulation.

Conclusions

In summary, the present study documented associations between genetic variants and indicators of iron status. We observed a previously reported association between an SNP, rs4820268, in *TMPRSS6* and *SF* and identified a new association between 1880669 in *TF* and sTfR.

ACKNOWLEDGEMENTS

We thank our colleagues at the centers for disease control and prevention in Guangdong Province, Ningxia Province, Shandong Province, Sichuan Province, and Zhejiang Province. We

appreciate all members who participated in and supported the study from the Chinese Center for Disease Control and Prevention and the Capital Institute of Pediatrics.

AUTHOR DISCLOSURES

None of the authors have a conflict of interest. This study was supported by the Research Special Fund for the Public Welfare Industry of Health (No. 201202012).

REFERENCES

1. Calarge CA, Ziegler EE. Iron deficiency in pediatric patients in long-term risperidone treatment. J Child Adolesc Psychopharmacol. 2013;23:101-9.
2. Franchini M, Montagnana M, Lippi G. Hepcidin and iron metabolism: from laboratory to clinical implications. Clin Chim Acta. 2010;21:1565-9.
3. Oexle K, Ried JS, Hicks AA, Tanaka T, Hayward C, Bruegel M et al. Novel association to the proprotein convertase PCSK7 gene locus revealed by analysing soluble transferrin receptor (sTfR) levels. Hum Mol Genet. 2011; 411:1042-7.
4. WHO. Assessing the iron status of populations. Geneva: World Health Organization; 2007.
5. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics. 2000;105:e51.
6. An P, Wu Q, Wang H, Guan Y, Mu M, Liao Y et al. TMPRSS6, but not TF, TFR2 or BMP2 variants are associated with increased risk of iron-deficiency anemia. Hum Mol Genet. 2012;21:2124-31.
7. Lee PL, Barton JC, Khaw PL, Bhattacharjee SY, Barton JC. Common TMPRSS6 mutations and iron, erythrocyte, and pica phenotypes in 48 women with iron deficiency or depletion. Blood Cell Mol Dis. 2012;48:124-7.
8. McLaren CE, McLachlan S, Garner CP, Vulpe CD, Gordeuk VR, Eckfeldt JH et al. Associations between single nucleotide polymorphisms in iron-related genes and iron status in multiethnic populations. PLoS One. 2012;7:e38339. doi: 10.1371/journal.pone.0038339.
9. Milet J, Dehais V, Bourgain C, Jouanolle AM, Mosser A, Perrin M et al. Common variants in the BMP2, BMP4, and HJV genes of the hepcidin regulation pathway modulate HFE hemochromatosis penetrance. Am J Hum Gene. 2007; 81:799-807.
10. Constantine CC, Anderson GJ, Vulpe CD, McLaren CE, Bahlo M, Yeap HL et al. A novel association between a SNP in CYBRD1 and serum ferritin levels in a cohort study of HFE hereditary haemochromatosis. Brit J Haematol. 2009; 147:140-9.
11. Benyamin B, McRae AF, Zhu G, Gordon S, Henders AK, Palotie A et al. Variants in TF and HFE explain 40% of genetic variation in serum-transferrin levels. Am J Hum Gene. 2009;84:60-5.
12. Merryweather-Clarke AT, Cadet E, Bomford A, Capron D, Viprakasit V, Miller A et al. Digenic inheritance of mutations in HAMP and HFE results in different types of haemochromatosis. Hum Mol Genet. 2003;12:2241-7.
13. Whitfield JB, Cullen LM, Jazwinska EC, Powell LW, Heath AC, Zhu G, Duffy DL, Martin NG. Effects of HFE C282Y and H63D polymorphisms and polygenic background on iron stores in a large community sample of twins. Am J Hum Gene. 2000;66:1246-58.
14. Lee DH, Zhou LJ, Zhou Z, Xie JX, Jung JU, Liu Y, Xi CX, Mei L, Xiong WC. Neogenin inhibits HJV secretion and regulates BMP-induced hepcidin expression and iron homeostasis. Blood. 2010;115:3136-45.
15. Milet J, Déhais V, Bourgain C, Jouanolle AM, Mosser A, Perrin M et al. Common variants in the BMP2, BMP4, and HJV genes of the hepcidin regulation pathway modulate HFE hemochromatosis penetrance. Am J Hum Gene. 2007; 81:799-807.
16. Jason J, Archibald LK, Nwanyanwu OC, Bell M, Jensen RJ, Gunter E et al. The effects of iron deficiency on lymphocyte cytokine production and activation: preservation of hepatic iron but not at all cost. Clin Exp Immunol. 2001;126:466-73.
17. Muñoz M, Villar I, García-Erce JA. An update on iron physiology. World J Gastroentero. 2009;37:4617. doi: 10. 3748/wjg.15.4617.
18. Benyamin B, Esko T, Ried JS, Radhakrishnan A, Vermeulen SH, Traglia M et al. Novel loci affecting iron homeostasis and their effects in individuals at risk for hemochromatosis. Nat Commun. 2014;5:4926. doi: 10.1038/ncomms5926.
19. McLaren CE, Garner CP, Constantine CC, McLachlan S, Vulpe CD, Snively BM et al. Genome-wide association study identifies genetic loci associated with iron deficiency. PLoS One. 2011;6:e17390. doi: 10.1371/journal.pone.00173 90.
20. Benyamin B, Ferreira MAR, Willemsen G, Gordon S, Middelberg RPS, McEvoy BP et al. Common variants in TMPRSS6 are associated with iron status and erythrocyte volume. Nat Genet. 2009;41:1173-5.
21. Pichler I, Minelli C, Sanna S, Tanaka T, Schwienbacher C, Naitza S et al. Identification of a common variant in the TFR2 gene implicated in the physiological regulation of serum iron levels. Hum Mol Genet. 2011;20:1232-40.
22. Delbini P, Vaja V, Graziadei G, Duca L, Nava I, Refaldi C, Cappellini MD. Genetic variability of TMPRSS6 and its association with iron deficiency anaemia. Br J Haematol. 2010;151:281-4.
23. He M, Workalemahu T, Manson JE, Hu FB, Qi L. Genetic determinants for body iron store and type 2 diabetes risk in US men and women. PLoS One. 2012;7:e40919. doi: 10. 1371/journal.pone.0040919.
24. Finberg KE, Heeney MM, Campagna DR, Aydınok Y, Pearson HA, Hartman KR et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet. 2008;40:569-71.
25. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta. 2012;1823:1434-43.
26. Silva B, Faustino P. An overview of molecular basis of iron metabolism regulation and the associated pathologies. Biochim Biophys Acta. 2015;1852:1347-59.
27. Cook JD. Diagnosis and management of iron-deficiency anaemia. Best Pract Res Cl H. 2005;2:319-32.
28. Zimmermann MB. Methods to assess iron and iodine status. Br J Nutr. 2008;99(Suppl 3):S2-S9. doi: 10.1017/S0007114 50800679X .
29. Archer NM, Brugnara C. Diagnosis of iron-deficient states. Crit Rev Clin Lab Sci. 2015;52:256-72.
30. Aguilar R, Moraleda C, Quintó L, Renom M, Mussacate L, Macete E, Aguilar JL, Alonso PL, Menendez C. Challenges in the diagnosis of iron deficiency in children exposed to high prevalence of infections. PLoS One. 2012;7:e50584. doi: 10.1371/journal.pone.0050584.
31. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem. 1996; 34:517-20.

32. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263-5.
33. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, Camaschella C. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab. 2008;8:502-11.
34. Nai A, Pagani A, Silvestri L, Campostrini N, Corbella M, Girelli D, Traglia M, Toniolo D, Camaschella C. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. Blood. 2011; 118:4459-62.
35. Gan W, Guan Y, Wu Q, An P, Zhu J, Lu L et al. Association of TMPRSS6 polymorphisms with ferritin, hemoglobin, and type 2 diabetes risk in a Chinese Han population. Am J Clin Nutr. 2012;95:626-32.
36. Skikne BS. Serum transferrin receptor. Am J Hematol. 2008; 11:872-75.
37. Kamer B, Dółka E, Pasowska R, Świątkowska E. The usefulness of soluble transferrin receptor (sTfR) in differentiating anemia occurring in young children. Folia Histochem Cytobiol. 2012;50:473-9.
38. Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. Annu Rev Med. 1993;44:63-74.
39. Castel R, Tax MG, Droogendijk J, Leers MP, Beukers R, Levin MD, Sonneveld P, Berendes PB. The transferrin/log (ferritin) ratio: a new tool for the diagnosis of iron deficiency anemia. Clin Chem Lab Med. 2012;50:1343-9.
40. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta. 2003;329:9-22.
41. Blanco-Rojo R, Toxqui L, López-Parra AM, Baeza-Richer C, Pérez-Granados AM, Arroyo-Pardo E, Vaquero MP. Influence of diet, menstruation and genetic factors on iron status: a cross-sectional study in Spanish women of childbearing age. Int J Mol Sci. 2014;15:4077-87.
42. Beutler E, Van Geet C, te Loo DM, Gelbart T, Crain K, Truksa J, Lee PL. Polymorphisms and mutations of human TMPRSS6 in iron deficiency anemia. Blood Cells Mol Dis. 2010;44:16-21.
43. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr. 2010:92:546-55.
44. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ et al. Iron-overload–related disease in HFE hereditary hemochromatosis. New Engl J Med. 2008; 358:221-30.