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Brief report

A genome-wide association analysis of serum iron concentrations

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To investigate genetic variants that affect iron concentrations in persons not affected by overt genetic disorders of iron metabolism, a genome-wide association study was conducted in the InCHIANTI Study (N = 1206) and the Baltimore Longitudinal Study of Aging (N = 713). The top 2 single-nucleotide polymorphisms were examined for replication in the Women's Health and Aging Study (WHAS) I and II (N = 569). The single-nucleotide polymorphism most strongly associated with lower serum iron concentration was rs4820268 ($P = 5.12 \times 10^{-9}$), located in exon 13 of the transmembrane protease serine 6 (*TMPRSS6*) gene, an enzyme that promotes iron absorption and recycling by inhibiting hepcidin antimicrobial peptide transcription. The allele associated with lower iron concentrations was also associated with lower hemoglobin levels, smaller red cells, and more variability in red cell size (high red blood cell distribution width). Our results confirm the association of *TMPRSS6* variants with iron level and provide further evidence of association with other anemia-related phenotypes. (Blood. 2010;115:94-96)

Introduction

Iron is an important cofactor for enzymes performing basic functions in human physiology.[1] Iron deficiency has important pathologic consequences including but not limited to anemia.[2] Iron is also toxic and can react with oxygen species to form chemically active free radicals that damage macromolecules and cellular organelles.[3] To avoid both deficiencies and toxicity, iron homeostasis is tightly regulated.

Iron balance is maintained through regulation of dietary iron uptake and systemic distribution with only very small quantities eliminated through bleeding and shedding of the intestinal mucosa.[4] Studies have suggested that variability in iron concentrations is in part genetically determined with heritability estimates of 20% to 30%.[5,6]

Over the past decade, heritable, overt pathologic iron deficiencies and iron overload have been attributed to mutations in a number of key genes that control iron homeostasis.[1] However, whether iron levels are affected by genetic variants in subjects who are not affected by these Mendelian diseases is unclear. To address this question, we conducted a genome-wide association study (GWAS) in the InCHIANTI and the Baltimore Longitudinal Study of Aging (BLSA) and confirmed our results in the Women's Health and Aging Study (WHAS).

Methods

Study subjects

The InCHIANTI study is a population-based epidemiologic study performed in a sample of the population living in the Chianti region of Tuscany, Italy.[7] The BLSA study is a population-based study conducted in a sample of volunteers predominantly from the Baltimore–Washington, DC, area.[8] The WHAS I and II are companion prospective, observational studies of the causes and consequences of disability in older women.[9,10] The 3 studies were approved by the institutional review boards at their respective institutions.

Iron-related measurements

Serum iron was measured using a colorimetric assay (Roche Diagnostics; InCHIANTI;WHAS) or Fe slide method (VITROS 750, Johnson & Johnson; BLSA). Serum ferritin was measured using Quest Diagnostics Laboratory (formerly Ciba-Corning Laboratories; WHAS), chemiluminescent immunoassay (Abbott Diagnostic; INCHIANTI) or an immunoassay-type 2-stage sandwich method using 2 antiferritin antibodies (Advia Centaur, Bayer; BLSA). Other traits (hemoglobin, hematocrit, red blood cell width, mean corpuscular volume, red blood cell count, and platelets) were assessed using autoanalyzer SYSMEX SE-9000 (Sysmex Corporation; InCHIANTI), coulter hematology analyzer (WHAS), and SYSMEX XE-series (Sysmex Corporation).

Genotyping

Genome-wide genotyping of the InCHIANTI and BLSA was assessed using the Illumina Infinium HumanHap 550K.[11,12] Association analysis was conducted on 475 322 single-nucleotide polymorphisms (SNPs) that passed quality control (minor allele frequency $\geq 1\%$, genotyping completeness $\geq 99\%$, and Hardy Weinberg-equilibrium > 0.0001). Genotyping of rs855791 and rs4820268 in WHAS was performed using AppliedBiosystems TaqMan Assays on Demand.

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Table 1. Characteristics of the 4 participating studies

	BLSA (n = 713)	InCHIANTI (n = 1206)	WHASI (n = 375)	WHASII (n = 194)
Age, y*	65.5 (14.8)	68.3 (15.5)	78.3 (8.0)	74 (2.7)
Male†	54.0 (389)	44.4 (536)	—	—
Iron, μg/dL*	82.9 (32.3)	83.3 (26)	77.6 (26.2)	84.9 (29.5)
Red blood cells, 10^6/μL*	4.6 (0.6)	4.5 (0.4)	4.3 (0.5)	4.4 (0.3)
Hemoglobin, g/dL*	13.9 (1.7)	13.8 (1.4)	13.2 (1.3)	13.5 (1.1)
Mean corpuscular volume, fL*	92.3 (4.5)	90.3 (4.8)	94.4 (6.0)	92.6 (5.1)
Hematocrit, percentage*	42.3 (5.2)	40.7 (3.5)	40.3 (4.1)	40.3 (3.2)
Ferritin, ng/mL*	94.7 (111.9)	140.7 (144.6)	118.7 (191.7)	108.0 (98.8)
Platelets, 1000/μL*	244.7 (157.4)	227.6 (67.1)	267.6 (84.4)	240.8 (56.2)
Red blood cell width, percentage*	13.3 (0.9)	13.7 (1.0)	14.3 (1.6)	13.8 (1.2)

— indicates not applicable.
*Values expressed as mean (SD).
†Value expressed as percentage (N).

Statistical analysis

For association analysis, inverse normal transformation was applied to iron concentrations to avoid inflated type I error resulting from nonnormal data distribution.[13] An additive genetic model was tested by fitting a simple regression adjusted for age[2] and sex using the fastAssoc option in MERLIN as previously described.[13,14] For the BLSA, the analysis was restricted to subjects with European ancestry, and each analysis was further adjusted for the top 2 principal components derived from an EIGENSTRAT analysis using approximately 10 000 randomly selected SNPs from the 550K SNP panel.[15] The genomic control method was used to control for residual effects of population structure and cryptic relatedness.[16] Genome-wide significance was recognized for P values of 5×10^{-7}.[17] The summary results were combined using an inverse variance and sample size weighted meta-analysis using METAL (http://www.sph.umich.edu/csg/abecasis/Metal/). The meta-analysis results from the GWAS studies are available through dbGAP (accession no. phs000215.v1).

Results and discussion

The mean serum iron concentrations were comparable across the study populations (Table 1). Because the population examined in this study represents older persons, the genetic effects on iron resulting from menstrual blood loss were minimized. Polymorphisms associated with serum iron levels with P value less than 1×10^{-5} are listed in supplemental Table 1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article). A common polymorphism on chromosome 22 in the transmembrane serine protease 6 (*TMPRSS6*) or matriptase-2 gene reached genome-wide significance (supplemental Table 1; supplemental Figure 1). Variant rs855791 in exon 17 showed the strongest association ($P = 3.93 \times 10^{-7}$), confirming results from a recent GWAS study of iron levels.[18] The second strongest SNP, rs4820268 on exon 13, was in linkage disequilibrium (LD) with rs855791 ($r^2 = 0.9$), representing the same signal. Both rs855791 ($P = .037$) and rs4820268 ($P = .003$) were significantly associated with iron concentrations in combined analysis of WHAS I and II studies. A meta-analysis of the 2 GWAS and the 2 replication studies resulted in a genome-wide significant P value of 4.16×10^{-8} (rs855791) and 5.12×10^{-9} (rs4820268; Table 2). An additional 14 SNPs within 10 kb of *TMPRSS6* were represented in the GWAS panel (supplemental Table 2). The SNPs in moderate LD with the top SNPs (r2 = 0.2-0.3 in Hapmap CEU population) were modestly associated with serum iron concentrations (rs2235320, rs5756504).

The *TMPRSS6* signal affects a high proportion of the population because the frequency of the iron-lowering allele is high (~ 45%). However, because each of the *TMPRSS6* variant explains only approximately 1% of the variance in iron concentrations, other unknown genetic

Table 2. Meta-analysis of the associations of *TMPRSS6* SNPs with iron and iron-related traits

	InCHIANTI		BLSA		WHASI		WHASII		Meta-analysis	
	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
rs855791										
Iron, μg/dL	3.22 (1.05)	.001	7.21 (1.64)	2.66×10^{-5}	3.33 (1.93)	.092	3.16 (2.73)	.218	4.11 (0.77)	4.16×10^{-8}
Red blood cell width, percentage	−0.09 (0.04)	.005	−0.12 (0.05)	.004	0.03 (0.12)	.662	−0.29 (0.12)	.054	−0.10 (0.03)	1.13×10^{-4}
Mean corpuscular volume, fL	0.57 (0.20)	.007	0.39 (0.23)	.040	0.19 (0.45)	.519	0.11 (0.52)	.927	0.44 (0.14)	.001
Hemoglobin, g/dL	0.12 (0.05)	.006	0.11 (0.08)	.182	−0.15 (0.10)	.120	0.08 (0.11)	.872	0.08 (0.04)	.040
Hematocrit, percentage	0.27 (0.13)	.028	0.24 (0.25)	.351	−0.51 (0.30)	.100	−0.07 (0.32)	.563	0.14 (0.10)	.221
Red blood cells, n, 10^6/μL	−0.004 (0.02)	.858	0.010 (0.03)	.878	−0.068 (0.03)	.062	−0.010 (0.04)	.772	−0.010 (0.01)	.393
Platelets, 1000/μL	−0.64 (2.70)	.522	−7.48 (8.31)	.519	0.61 (6.24)	.830	1.53 (5.70)	.496	−0.64 (2.19)	.495
Ferritin, ng/mL	6.54 (5.68)	.677	7.15 (6.56)	.070	−5.48 (10.88)	.294	−6.10 (10.18)	.459	3.64 (3.72)	.592
rs4820268										
Iron, μg/dL	3.15 (1.03)	.002	7.31 (1.66)	3.09×10^{-5}	4.49 (1.93)	.021	5.02 (2.72)	.058	4.39 (0.76)	5.12×10^{-9}
Red blood cell width, percentage	−0.07 (0.04)	.033	−0.12 (0.05)	.002	0.02 (0.12)	.883	−0.28 (0.12)	.078	−0.10 (0.03)	3.98×10^{-4}
Mean corpuscular volume, fL	0.45 (0.19)	.032	0.56 (0.24)	.004	0.43 (0.45)	.206	0.43 (0.53)	.464	0.48 (0.14)	1.86×10^{-4}
Hemoglobin, g/dL	0.12 (0.05)	.007	0.11 (0.08)	.162	−0.08 (0.10)	.423	0.06 (0.11)	.970	0.08 (0.04)	.020
Hematocrit, percentage	0.27 (0.12)	.019	0.22 (0.26)	.427	−0.25 (0.30)	.425	−0.19 (0.32)	.367	0.16 (0.10)	.136
Red blood cells, 10^6/μL	0.002 (0.02)	.805	−0.001 (0.03)	.834	−0.049 (0.03)	.187	−0.036 (0.03)	.290	−0.010 (0.01)	.453
Platelets, 1000/μL	−0.99 (2.64)	.444	−6.19 (8.39)	.551	−4.53 (6.26)	.405	3.85 (5.76)	.260	−1.08 (2.17)	.390
Ferritin, ng/mL	5.77 (5.56)	.936	11.19 (6.66)	.055	2.64 (10.99)	.725	0.25 (10.20)	.948	6.36 (3.71)	.479

Effect allele rs855791 (C, average frequency = 0.59), rs4820268 (A, average frequency = 0.54).

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loci probably contribute to the variability in iron levels. Interestingly, variants in *HFE*, including C282Y (rs1800562) previously described in association with iron concentrations, were not significant in this meta-analysis.[18] We examined whether variants in other candidate genes (*CYBRD1*, *HAMP*, *SLC11A2*, *SLC40A1*, *TF*, and *TFRC*) of iron absorption and use were associated with iron concentrations in this study (supplemental Table 3). After adjusting for multiple comparisons, none of the candidate SNPs was significantly associated with iron concentrations.

TMPRSS6 regulates iron absorption through suppression of hepcidin antimicrobial peptide (*HAMP*).[19-21] Hepcidin is a key regulator of iron homeostasis that induces degradation of ferroportin (*SLC40A1*), the only transporter known to facilitate elemental iron egress from macrophages and enterocytes.[22,23] During iron deficiency, hepcidin is downregulated to promote ferroportin-mediated iron uptake and correct the deficiency. Several genes are required for appropriate *HAMP* expression, including the hemochromatosis gene, *HFE*[24] and hemojuvelin (*HFE2*).[25] The 2 leading *TMPRSS6* SNPs are synonymous SNPs in the LDL-receptor class A-like (*LDLRA*) domain (rs4820268) and a missense SNP within the trypsin-like serine protease domain (rs855791). The synonymous SNP most probably is in LD with a functional SNP within or near *TMPRSS6*. Functional analysis of common variants within *TMPRSS6*, in particular rs855791, is warranted.

We examined whether the *TMPRSS6* SNPs were associated with other iron-related hematologic values in the 4 studies (Table 2). Although the *TMPRSS6* SNPs were not associated with anemia prevalence (assessed using hemoglobin values), the alleles associated with lower iron concentrations were also associated with lower mean corpuscular volume, lower hemoglobin levels, and higher red blood cell distribution width. Whether this genetic background is associated with higher risk of developing iron-deficiency anemia should be tested in future studies.

In conclusion, we confirm a previously reported *TMPRSS6* locus in association with lower serum iron concentrations. These variants were also significantly associated with smaller red cells, lower hemoglobin levels, and higher red blood cell distribution width. Because this gene is directly involved in the regulation of dietary iron absorption and use, this SNP may be an informative marker to identify a subpopulation at increased risk of iron-restricted erythropoiesis as a consequence of inefficient absorption of iron from dietary sources.

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Authorship

Contribution: A.M., R.D.S., D.A., J.D.W., L.P.F., A.S., J.G., G.R.A., S.B., D.L.L., and L.F. designed the research; A.S., W.Y., and T.T. conducted the data analysis; and T.T. and C.N.R. wrote the paper.

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