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To the editor:

Plasma hepcidin of Ethiopian highlanders with steady-state hypoxia

Hepcidin impedes iron absorption and is suppressed when erythropoietic iron requirements are increased. Recent studies show that during acute exposure to high-altitude hypoxia, plasma hepcidin concentrations drop when iron demands for erythropoiesis and hemoglobin synthesis are sharply increased.[1,2] However, the effects of chronic exposure to high-altitude hypoxia with stable erythropoietic iron requirements have not been examined. We hypothesized that plasma hepcidin would not be suppressed in iron-replete individuals chronically adapted to high altitude.

People of Amhara and Oromo ethnicity have been living at high altitude in Ethiopia for more than 5000 years and about 500 years, respectively, and have been shown to differ from one another in hemoglobin and oxyhemoglobin percentage.[3] Healthy volunteers from 3700 to 4000 m (high altitude) and 1200 to 1500 m (low altitude) were recruited, and they provided blood samples for analyses (see the supplemental Video(s)/Data Set(s) link at the top of the online article for genetic analysis methodology). The sample reported here had normal calculated body iron stores[4] (Figure 1D) and did not have infection or inflammation, assessed with C-reactive protein levels and malarial plasmodium DNA.

In contrast to acutely exposed Europeans[2] (see also the online supplement), high-altitude Amhara had higher plasma hepcidin, and high-altitude Oromo had similar hepcidin, compared with their respective lowland counterparts (Figure 1E). Furthermore, Amhara had higher plasma hepcidin and oxyhemoglobin percentage as well as lower hemoglobin and erythropoietin than Oromo at high altitudes (Figure 1A-C,E). Within Ethiopian subsamples, age, sex, BMI, erythropoietin, hemoglobin, oxyhemoglobin percentage, and transferrin receptor were not correlated with hepcidin (natural-log transformed for normality). Like Europeans at low altitude,[5] serum ferritin ($r = 0.35$ to 0.77) and body iron stores ($r = 0.39$ to 0.85) correlated with ln(hepcidin) (all $P < .05$, except in the small sample of female low-altitude Oromo [$n = 6$]). An intronic SNP in *GRAMD3* was associated with plasma hepcidin among Amhara at genome-wide significance ($P = 4.94*10^{-8}$), accounting for 22% of the variation in covariate-adjusted hepcidin level. Allele A of rs7700582 increased hepcidin levels by 8.1 ng/mL only among Amhara, although allele frequency was similar in all 4 subsamples.

Hepcidin was not suppressed in Amhara or Oromo highland samples under steady-state hypoxia, likely because erythropoietic drive was stable.[6] It is interesting to speculate that the higher plasma hepcidin of highlander Amhara, compared with Oromo, is due to lower iron demand indicated by lower hemoglobin and erythropoietin concentrations and higher body iron stores. Variants in *GRAMD3* are associated with macular degeneration, a retinal disease that has been related to abnormalities in hepcidin and iron accumulation.[7,8] Another variant near *GRAMD3* (rs1366100) has been associated with erythrocyte counts,[9] consistent with the idea that this region plays a role in iron metabolism. Thus, the genetic results also support the idea that iron stores are primary regulators of hepcidin levels in hypoxic populations without increased erythropoietic drive. Previous work has shown that various highlander populations demonstrate different responses to hypoxia,[10] which may also be the case with iron regulation.

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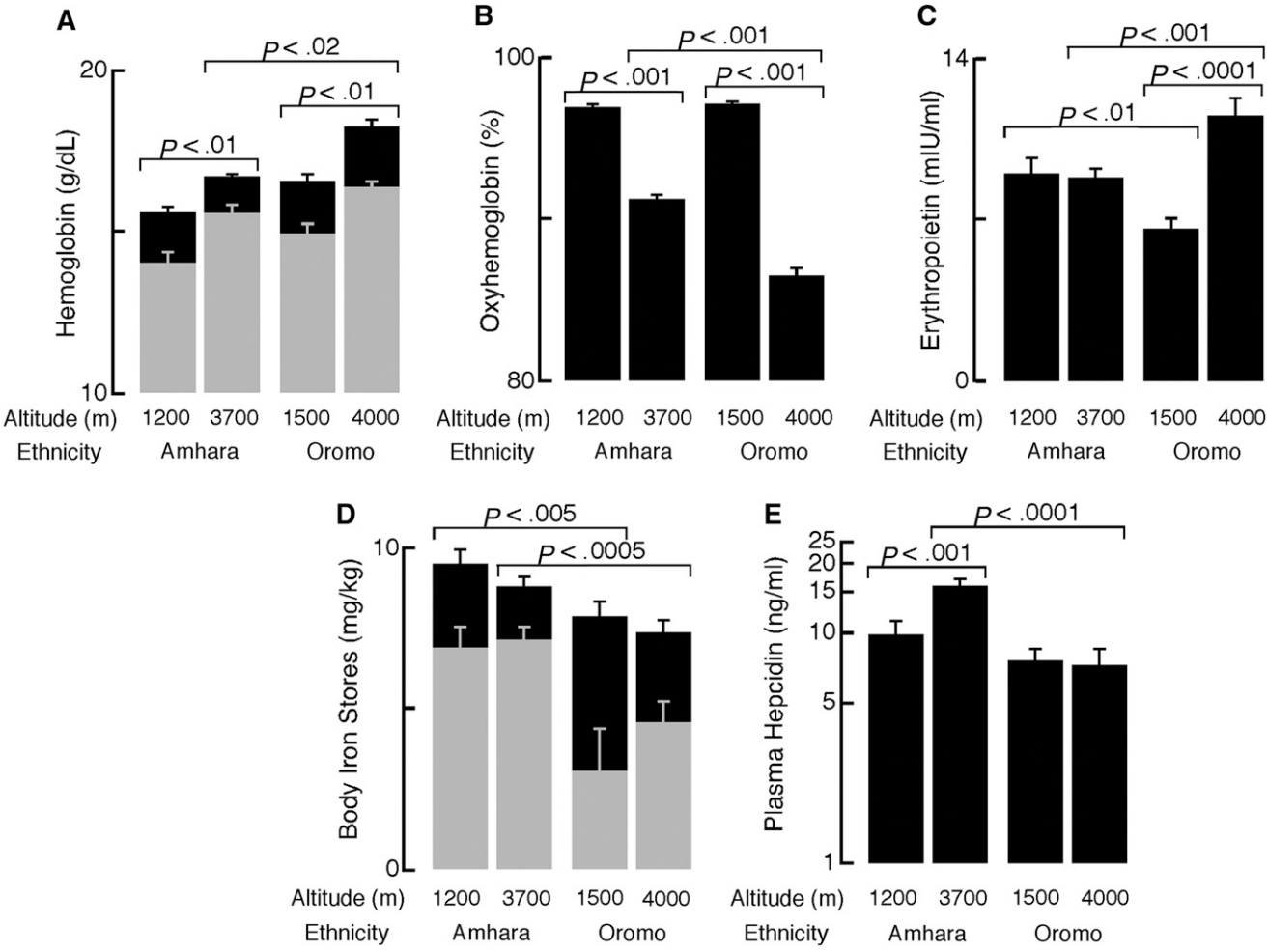

Figure 1. Oxyhemoglobin percentage, hemoglobin, ferritin, hepcidin, and erythropoeitin of Ethiopians differ by ethnicity at high and low altitudes. A total of 116 high-altitude Amhara (27 females, age 32 ± 0.8 years), 49 low-altitude Amhara (9 females, 34 ± 1.4 years), 75 high-altitude Oromo (27 females, 28 ± 1.0 years), and 39 low-altitude Oromo (6 females, 25 ± 0.9 years) were included in the analyses. Data were analyzed using the JMP 9 statistical software (SAS Institute, Cary, NC). Data are reported as mean and standard error of the mean. (A) Hemoglobin was higher in men (*black*) compared with women (*gray*) in all populations (all $P < .005$). Hemoglobin was increased at high altitudes in both ethnic groups, but Oromo had higher hemoglobin than Amhara at high altitudes. Hemoglobin concentration was measured using the cyanmethemoglobin technique (Hemocue, Sweden) immediately after drawing venipuncture samples. (B) Oxyhemoglobin percentage was significantly lower among Ethiopians at high altitude compared with low altitudes. At high altitude (3700-4000 m), Oromo had significantly lower saturations than Amhara. (C) Amhara had no altitude differences in erythropoietin, whereas Oromo had significantly higher erythropoietin at high vs low altitude. Compared with the Oromo, Amhara had lower erythropoietin at high altitude and higher erythropoietin at similar low altitudes. Erythropoietin was measured in serum by ELISA (R&D Systems, Minneapolis, MN). Extreme outliers (EPO > 40 mIU/mL) were excluded in advance of statistical testing. (D) Body iron stores were significantly lower among women (*gray bars*) compared with men (*black bars*) except among low-altitude Amhara (all others, $P < .01$). Neither Amhara nor Oromo had altitude differences in body iron stores. At high altitudes, Amhara had higher body iron stores than Oromo. Body iron stores were calculated as previously described[4] from serum transferrin receptor and ferritin concentrations, measured by enzyme immunoassay and radioimmunoassay (Ramco, Houston, TX), respectively. (E) Amhara highlanders had higher hepcidin than Amhara lowlanders and the Oromo at either altitude. Hepcidin was measured using enzyme-linked immunosorbant assay (ELISA, Bachem, UK) in heparinized plasma. Hepcidin was natural log transformed for statistical testing.

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Contribution: E.L.L. performed research and statistical analysis, interpreted data, and wrote the manuscript. A.J.J. and C.D.K. performed research, collected data, and analyzed and interpreted data. A.G. designed the research and interpreted data. A.D., G.A.-A., and G.M.B. analyzed and interpreted data. S.C.E. designed research, analyzed and interpreted data, and wrote the manuscript. C.M.B. designed and performed the research, collected data, analyzed and interpreted data, and wrote the manuscript.

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To the editor:

Fcγ receptor IIB gene polymorphism in adult Japanese patients with primary immune thrombocytopenia

Several studies have indicated that platelet recovery occurs in a subgroup of immune thrombocytopenia (ITP) patients after successful *Helicobacter pylori* (*H pylori)* eradication.[1,2] Interestingly, a higher response rate to *H pylori* eradication therapy has been reported in Japan and Italy than in the United States and European countries other than Italy,[2] suggesting that the efficacy of *H pylori* eradication is influenced by ethnicity, probably through genetic and environmental factors. In addition, Asahi et al observed that monocytes from *H pylori*–infected ITP patients demonstrated low levels of inhibitory FcγRIIB and enhanced platelet phagocytosis, both of which were reversed after successful *H pylori* eradication.[3]

The *FcγRIIB* 232I/T (Ile/Thr) polymorphism (rs1050501) has been identified as a genetic factor associated with susceptibility to various autoimmune diseases.[4,5] The *FcγRIIB* 232T cannot inhibit activating receptors because it is not present in lipid rafts, resulting in decreased FcγRIIB-mediated inhibition of macrophage and B-cell responses.[6,7]

We analyzed the *FcγRIIB* 232I/T polymorphisms by restriction-fragment-length polymorphism polymerase chain reaction in 206 adult Japanese patients with primary ITP and in 193 healthy controls (supplemental Methods, available on the *Blood* website). The *FcγRIIB* 232T carriers were more frequently detected in ITP patients than in healthy controls ($P = .003$; odds ratio [OR] = 1.87; 95% confidence interval [CI], 1.24-2.82) (Table 1). Our results differed from those described by Breunis et al using 44 adult Dutch patients with ITP and Xu et al using 178 adult Chinese patients with ITP.[8,9] This discrepancy might be explained by study design factors including sample size and ethnic differences. Interestingly, the distribution of the *FcγRIIB* 232T carriers is more common in Asians than in Caucasians.[5] This distribution is similar to the regional differences observed for the effect of *H pylori* eradication therapy in ITP patients.[2]

We compared the distribution of *FcγRIIB* 232I/T polymorphisms between *H pylori*–infected ITP patients and healthy controls or *H pylori*–uninfected ITP patients and healthy controls (Table 1). The frequency of the *FcγRIIB* 232T carriers was significantly higher in *H pylori*–infected ITP patients than in healthy controls (49.0% vs 30.6%; $P = .002$; OR = 2.18; 95% CI, 1.33-3.59). *H pylori* infection plays a role in ITP pathogenesis by altering the FcγR balance of monocytes in favor of activating FcγR, through downregulation of inhibitory FcγRIIB.[3] Furthermore, our data suggest that the functionally impaired *FcγRIIB* 232T carriers may contribute to disease pathogenesis in a subgroup of *H pylori*–infected ITP patients.

We further evaluated associations between *FcγRIIB* 232I/T polymorphisms and therapeutic response rates to *H pylori* eradication in

Table 1. Genotype distributions of the *FcγRIIB* 232I/T polymorphism

	No. (%)			No. (%)		No. (%)		No. (%)		
								H pylori eradication therapy†		
***FcγRIIB* 232I/T polymorphism**	**Healthy controls, n = 193**	**Total ITP patients, n = 206**	***P*, vs healthy controls**	***H pylori*–infected ITP patients, n = 100***	***P*, vs healthy controls**	***H pylori*–uninfected ITP patients, n = 82***	***P*, vs healthy controls**	**Responders, n = 21**	**Nonresponders, n = 21**	***P***
I/I genotype	134 (69.4)	113 (54.8)	.01‡	51 (51.0)	.01‡	47 (57.3)	.2	7 (33.3)	18 (85.7)	.01‡
I/T genotype	56 (29.0)	84 (40.8)		44 (44.0)		33 (40.2)		12 (57.2)	3 (14.3)	
T/T genotype	3 (1.6)	9 (4.4)		5 (5.0)		2 (2.4)		2 (9.5)	0	
Non-T carriers§	134 (69.4)	113 (54.8)	.003	51 (51.0)	.002	47 (57.3)	.053	7 (33.3)	18 (85.7)	.001
T carriers§	59 (30.6)	93 (45.2)		49 (49.0)		35 (42.7)		14 (66.7)	3 (14.3)	

Genotype distributions of the *FcγRIIB* 232I/T polymorphism in ITP patients and healthy controls, in *H pylori*–infected and –uninfected ITP patients, and in responders and nonresponders to *H pylori* eradication therapy. Genotype distributions were tested for statistical significance using the χ-square or Fisher exact test when 1 or more variables was <5.

*Of 182 patients with ITP evaluated for *H pylori* infection status, 100 were confirmed positive for *H pylori* infection based on a positive urea breath test and/or serum anti–*H pylori* antibodies measured by an enzyme-linked immunosorbent assay kit.

†Forty-two *H pylori*–infected ITP patients were administered amoxicillin (750 mg twice daily), clarithromycin (400 mg twice daily), and lansoprazole (30 mg twice daily) for 7 days. Twenty-one ITP patients were responders, defined as having a platelet count higher than 50×10^9/L and doubling of the baseline level at 24 weeks after initiation of the eradication regimen.

‡The corrected *P* (P_{corr}) values were calculated by multiplying the observed *P* value by the number of comparisons made. $P_{corr} = .03$.

§FcγRIIB receptors encoded by *FcγRIIB* 232T are unable to interact with activating receptors and exert inhibitory activity.[6,7] In addition, only few subjects were T/T genotype in this study. Therefore, we compared non-T carriers (I/I genotype) to T carriers (I/T + T/T genotype).