Variability and heritability of hemoglobin concentration: an opportunity to improve understanding of anemia in older adults

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Hemoglobin concentration varies substantially according to age, sex, and race/ethnicity as well as by altitude. Over the past decade, the role of hemoglobin concentration in aging has gained prominence as several prospective studies have shown that decreased hemoglobin levels significantly increase the risk of physical disability, hospitalization, and mortality in older adults living in the community.[1-7] Even *low-normal* hemoglobin levels (i.e., 13-14 g/dL in men and 12-13 g/dL in women) are significantly associated with poor survival.[2,6,7] Cross-sectional data from the United States displayed in Figure 1 illustrate that hemoglobin levels, on average, decrease with advancing age in men throughout adulthood, but a more modest decline occurs in women after the age of 50. An average decline of 0.06 g/dL per year was observed in one prospective study of adults who did not have anemia or chronic kidney disease at baseline.[8] In addition to decreasing levels with older age, variation in the distribution of hemoglobin concentration increases with age as well. To illustrate, Figure 2 shows that average hemoglobin level decreases but variance increases with each successive age group in men, although this pattern is less clear in women it did occur after the reproductive age period (>50 years). Undoubtedly, this increased heterogeneity in hemoglobin concentration reflects the cumulative effects of interaction between intrinsic (i.e., biological) and extrinsic (i.e., social, behavioral and environmental) factors over the life course. The challenge then is to identify subpopulations predisposed to decreased hemoglobin level at older age and develop interventions accordingly to prevent adverse outcomes. Naturally, the role of genetics in impaired erythropoiesis at older ages should be investigated. While substantial progress has been made in understanding the genetic basis of hemoglobinopathies that often follow Mendelian inheritance patterns, relatively little attention has been devoted toward understanding variation in hemoglobin concentration in the general population. Given that the marked differences in hemoglobin distribution by sex and race are sustained across the lifespan (Figure 1), it is conceivable that set points in hemoglobin concentration are to some extent under genetic control.

In this issue of the journal, Sala and colleagues report that the heritability of hemoglobin concentration ranges between 0.34 and 0.42 based on 3 genetically isolated Italian populations of apparently healthy adults.[9] Importantly, their analyses comprised of 3,849 adults with a wide age range (52%, 42%, and 6% were aged 18-49, 50-79, and ≥80 years, respectively) and excluded persons with extreme hemoglobin and mean corpuscular volume (MCV) values as well as those with health conditions that might distort hemoglobin level. Early studies on twins suggested a higher genetic contribution to hemoglobin with heritability estimates ranging from 0.65 to 0.84, but these studies were limited to children and young adults under 35 years of age.[10,11] The estimates by Sala *et al.*[9] are more in line with a study by Garner *et al.*[12] of 775 adult twins age 20-80 years that showed heritability of 0.37. In addition, the heritability estimate from the Framingham Offspring Study of 1,444 persons aged 10-64 (mean: 33 years) was 0.45.[13] Other erythrocyte indices also have significant heritability. Overall, these data indicate that variation in hemoglobin concentration is significantly influenced by genetic factors; however, the combinations of genes that regulate hemoglobin level remain to be identified.

Genome-wide association and linkage analyses of hematologic parameters have been performed in the Framingham Offspring Study.[13,14] The initial study by Lin *et al.* identified significant linkage for hematocrit at chromosome 6q23-24 that contains 2 candidate genes, including EBP41L2 that codes for protein 4.1G, which is a member of the erythrocyte cytoskeletal protein 4.1R gene family, and HEBP2 that codes for a putative heme-binding protein.[13] In a follow-up study using the Affymetrix GeneChip 100K single nucleotide polymorphism (SNP) set in Framingham participants, Yang et al. identified 2 SNPs (rs1582055 and rs4897475) in the EBP41L2 gene that were significantly associated with hematocrit and hemoglobin concentration, but no significant association of SNPs in the HRBP2 candidate gene were observed with erythrocyte indices.[14] Significant linkage for hemoglobin and hematocrit to chromosome 6q23 was also recently reported in a twin study of 14 year-old Australians,[15] replicating the findings from the Framingham Offspring Study. Importantly, neither the Framingham Offspring Study nor the recent Australian twin study observed significant linkage for hemoglobin or hematocrit to areas containing genes for the α- or β-globin chains, erythropoietin, or erythropoietin receptor.[13-15] These initial findings from genome-wide association and linkage studies suggest that variation in hemoglobin concentration in the general population is influenced by gene variants that might differ from polymorphisms that produce the major hemoglobinopathies, although further research, including fine mapping and more association studies with candidate genes in larger samples, is needed.

In addition to providing heritability estimates for hemoglobin concentration, Sala *et al.* report an intriguing finding that hemoglobin concentration varied geographically, with the northern isolate population (Val Borbera) having significantly higher levels than the southern ones (Cilento and Carlantino), even after screening out study participants with MCV values suggestive of thalassemia traits.[9] A difference of approximately 0.4-0.5 g/dL was generally observed across age and sex strata, which is larger than but consistent with

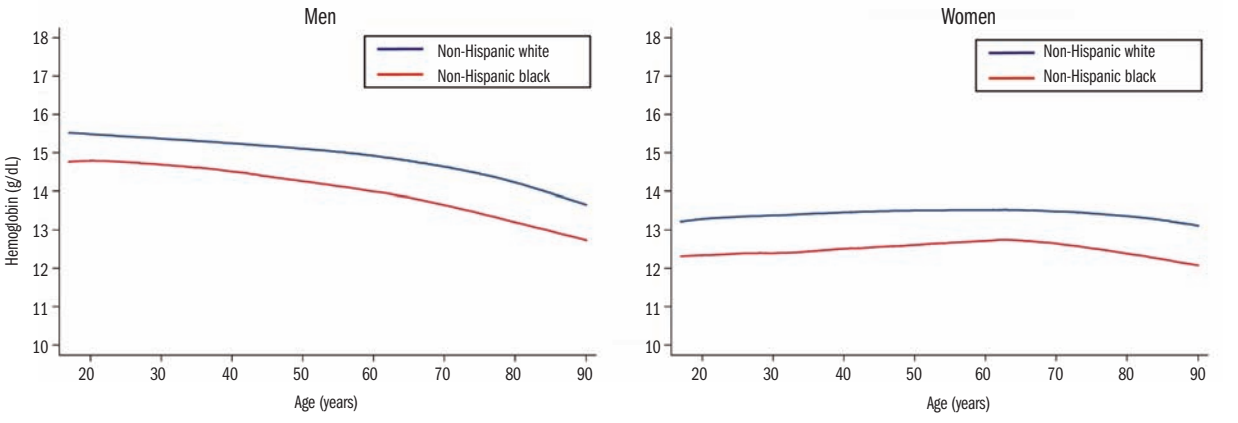

Figure 1. Mean hemoglobin concentration by age, sex, and race in the United States: 1988-1994 Third National Health and Nutrition Examination Survey (N=11,780). The locally weighted mean decreased with advancing age in men, while a more modest decline occurred in women after age 50. In both men and women, non-Hispanic blacks had lower means than non-Hispanic whites (analyses completed by the author).

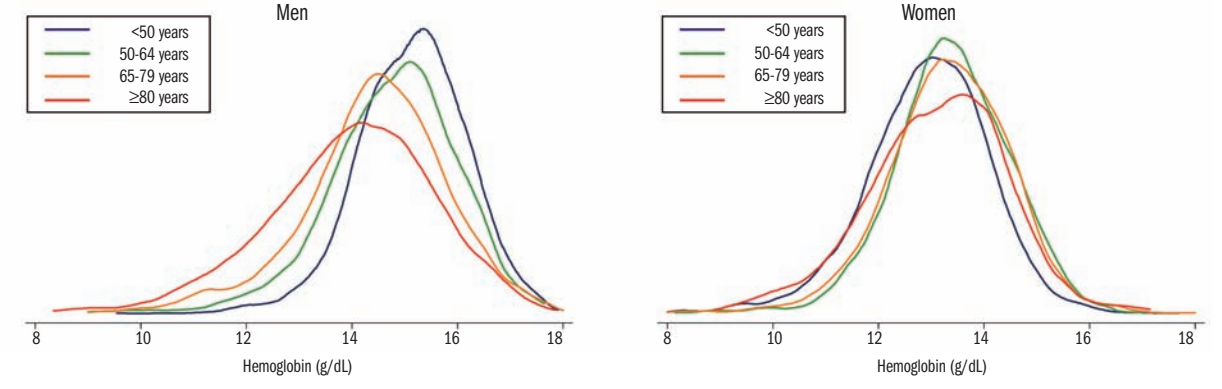

Figure 2. Distribution of hemoglobin concentration by age group and sex in the United States: 1988-1994 Third National Health and Nutrition Examination Survey (N=17,170). The mean was lower and variance was greater with each ascending age group in men, but this pattern occurred after the age of 50 in women (analyses completed by the author).

the previously reported northern to southern European difference of 0.3 g/dL.[16] To what extent these geographic differences within Italy reflect genetic differences in hemoglobin set point is difficult to assess as the authors themselves acknowledge that social, cultural, and behavioral factors might be at play. Nonetheless, it would be interesting to evaluate whether differences in the distribution of environmental factors account for geographic variability in hemoglobin and whether certain environmental exposures produce similar increments or decrements in hemoglobin concentration across the three genetically distinct isolate populations. For example, does low socioeconomic status or decreased renal function have a similar effect on hemoglobin concentration in Val Borbera as they do in Cilento or Carlantino? Differential effects, if adjusted for known confounders, could reflect genetic differences in the regulation of hemoglobin concentration. Additionally, it will be important to evaluate whether the hemoglobin concentration below which risk for adverse events (e.g., disability, hospitalization, mortality) differs geographically. That is, is the hemoglobin threshold for increased mortality risk lower in the southern than in northern isolate populations? In the United States, researchers are actively evaluating whether the proposed age-, sex-, and race-specific thresholds for defining anemia that are based on well-established population differences in hemoglobin distribution are also supported by prospective studies investigating risk for adverse outcomes.[5, 17]

The large interindividual variation in hemoglobin concentration has important implications for correctly interpreting epidemiological studies that examine clinical outcomes, for defining anemia, and for improving the treatment of anemia. Considering that hemoglobin concentration has a significant genetic component, investigating the genetic underpinnings could improve our understanding of erythropoiesis as well as the pathophysiology of anemia that occurs in later life. It is important to recognize that anemia is common in community-dwelling older adults, exceeding 20% in those aged 85 and older, and the vast majority of cases are mild (more than 90% of cases have hemoglobin >10 g/dL).[18] Further, approximately one-third of anemia cases are not explained by nutrient deficiencies (iron, B_{12}, or folate), renal insufficiency, or chronic inflamma-

tion.[18-20] Although some of these cases might result from myelodysplastic syndrome, Guralnik and colleagues estimated that at least one-quarter of the anemia cases in older adults would remain unexplained.[18] While erythropoietin levels have been shown to increase with aging,[8] suggesting that decreased bone marrow response or red cell survival contributes to anemia in older adults, Ferrucci and colleagues demonstrated that erythropoietin levels and pro-inflammatory cytokines were significantly lower in older Italian adults with unexplained anemia compared to non-anemic persons.[20] The genetic contribution to unexplained anemia in older adults has not been defined. In view of the high prevalence of anemia in older adults and its association with adverse outcomes, an expanded research effort into the role of genetics in hemoglobin variability and anemia in older adults is warranted.

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