Variation of hemoglobin levels in normal Italian populations from genetic isolates

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ABSTRACT

Normal hemoglobin levels vary greatly according to genetic and acquired factors. As a consequence there is no general agreement on the definition of anemia in terms of hemoglobin levels. Here we compare the hemoglobin levels of subjects recruited from normal genetically isolated Italian populations whose medical history, life style habits and results of laboratory tests are available. After the exclusion of pathological samples we analyzed the hemoglobin levels of 3,849 subjects (1,661 males and 2,188 females) and evaluated the hemoglobin heritability. Normal subjects of different age groups from a northern Italian isolate have significantly higher hemoglobin levels when compared to matched subjects of southern Italian isolates. The estimated heritability of hemoglobin levels ranges from 0.34 to 0.42 in the different isolates. Our study provides a dataset of hemoglobin levels for normal subjects of different geographical origin and indicate that hemoglobin levels are substantially influenced by heritable components.

Key words: hemoglobin, anemia, genetic isolates.

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Introduction

The hemoglobin (Hb) level of normal subjects is greatly variable and is a complex trait determined by both inherited and environmental factors. Besides age and sex acquired factors such as diet, smoking, body weight, hypoxia, infections/inflammation influence the Hb levels, whereas genetic determinants remain largely unknown. The evidence for a genetic basis of Hb concentration derives from twin heritability studies.[1,2] Garner *et al.*[2] showed that additive genetic effects account for 37 and 42 % of Hb and RBC count respectively. Further evidence derives from the observation that normal Caucasians have higher Hb levels than black individuals matched for age and sex.[3-5] Controversy surrounds the definition of *normal* Hb ranges, an issue relevant to the definition of anemia, which is usually based on WHO criteria (Hb <12 g/dL in females with Hb <13.0 g/dL in males). These criteria were established several years ago, based on limited epidemiological data and on different measurement techniques. Recent discussion has questioned WHO limits[6,7] and emphasized the more general problem of establishing normal Hb levels according to the population studied and the individual age.[7] The analysis of large databases of normal subjects available in the US led to a proposal of new limits for the definition of anemia in white and black subjects, an issue also relevant for prognostic implications.[8]

Reports of normal Hb levels in large samples of European populations are limited. A study carried out with the aim of understanding the role of mutations of the HFE hemochromatosis gene in protecting from iron-deficient anemia found

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that: individuals of southern European origin have at least 0.28 g/dL lower Hb as compared to northern subjects.[9] This difference was ascribed to the prevalence of anemia associated with thalassemia genotypes in southern and of hemochromatosis HFE genotypes, associated with iron overload[9] in northern Europe. However, the series from southern Europe cannot be considered *normal* because carriers of thalassemia were not excluded from the analysis and may have influenced the results. Another study in a limited number of northern populations of different ethnic origin, Danish and Greenlander's showed lower Hb levels in the latter population.[10]

In all populations studied a trend towards lower Hb levels with aging is a common observation. Defining limits of normal in the elderly is critical, given the relevance of the anemic state on the outcome of common and age-related disorders, such as cardiovascular diseases and cancer.

We exploited ongoing studies on the genetic susceptibility to complex diseases in genetically isolated Italian populations of three different geographical areas to define reference Hb levels of normal subjects, to evaluate possible differences among the populations studied and the heritability of Hb levels. Genetic isolates are defined as populations that for geographical, ethnic, religious and/or other reasons are characterized by the presence of inbreeding and a limited gene pool variation as compared to outbred populations. Due to these features, subjects within each isolate share a common genetic background and are especially suitable to study the genetic components of complex and quantitative traits such as common biochemical/hematologic variables, including Hb levels.

Here we show that normal males and females of different ages from a northern Italian isolate have significantly higher Hb levels compared to populations of southern Italian isolates. Moreover, we show that within each isolate Hb levels are influenced by genetic components.

Design and Methods

Genetic isolates studied

The location of the isolates studied is shown in the *Online Supplementary Figure 1*. All isolates have until recently been prevalently rural areas. The villages of Campora, Gioe and Cardile are located in a remote hilly area of the Cilento National Park in Southern Italy. The features of one of these villages was previoulsy reported.[11] Carlantino village is in the south-eastern part of the Appennines in a hilly area of Apulia. The Val Borbera isolate includes seven small villages in the northern part of the Appennines. Large pedigrees are reconstructed for all isolates. The mean altitude of the isolates is similar at around 500-600 meters.

Data collection

All the populations of the genetic isolates were invited to participate in projects aimed at studying the genetics of complex disorders. All subjects willing to participate in the different isolates provided a detailed medical history, collected through a structured questionnaire, and underwent physical examination and blood sampling in the local outpatient departments. The study protocol was approved by the ethical committees of the three different institutions. All participants in the study gave their informed consent for medical data collection, laboratory analysis, storage and use of biological material. Data were collected in different periods from January 2005 to October 2006. Hb and blood cell counts were obtained by automated cell counters in three different laboratories close to the isolates. Data of individuals belonging to the Cilento villages were pooled together to obtain a unique study sample that was comparable in size to the population samples of Val Borbera and Carlantino. In order to verify the consistency of the measurements, a quality control assessment was organized between two laboratories (Val Borbera and Cilento) with the exchange of 20 samples collected in duplicate. The mean interlaboratory variability of Hb levels was ± 0.15 g/dL.

To strengthen the value of comparison results between Val Borbera and Carlantino, we confirm the statistical difference on two separate population samplings from the latter isolate.

Data analysis

Subjects with evident anemia (Hb <10 g/dL in both sexes) due to any cause or polyglobulia (Hb >18 g/dL in males and >17 in females) were excluded from the analysis. Other exclusion criteria were: MCV < 80 fl or >100 fl, creatinine >1.4 mg % mL, an elevated CRP level and/or any acute inflammatory condition at the time of the study.

Statistical analysis was carried out using one-way Anova for multiple comparison when Hb distribution was normal. Non-parametric (Sum-Rank) tests were used to assess differences when Hb levels were non-normally distributed. The heritability of Hb was estimated using the variance component method, after pedigrees breaking, as implemented by SOLAR program.[12]

Results and Discussion

Only adult subjects aged >18 years were analyzed. The characteristics of the populations studied in the three geographical areas are summarized in Table 1, which also includes the numbers of the samples excluded and the reason for exclusion. The higher exclusion rate of cases with low MCV in southern isolates is likely due to beta-thalassemia, a trait more frequent in southern than in northern Italy (Table 1).

We observed greater Hb levels for all the age decades in Val Borbera individuals compared to the southern Italian cases: the difference was significant in both sexes and was greater in older than younger age groups (*data not shown*). In subjects over 80 years of age, there was a similar trend, but a significant difference was not reached likely because of the limited number of cases.

Since no statistical difference of Hb levels was

Table 1. Population studied and causes for exclusion.

Cases	*All*	*Val Borbera*	*Cilento*	*Carlantino*
Total	4240	1298	1830	1112
Males	1843	565	814	464
Females	2397	733	1016	648
Total analyzed	3849	1209	1648	992
Males	1661	517	731	413
Females	2188	692	917	579
Excluded	391 (9.2 %)	89 (6.9%)	182 (9.9%)	120 (10.8%)
MCV <80 fL	224	26	116	82
MCV >100 fL	85	32	33	20
Creatinine >1.4 mg %mL	56	21	24	11
Hb >18 g/dL (M) or >17 g/dL (F)	12	8	1	3
Hb <10 g/dL	14	2	8	4

Table 2. Mean Hb in young and old subjects, divided by sex.

	Val Borbera		*Cilento*		*Carlantino*	
Females' age	*N*	*Hb (g/dL)*	*N*	*Hb (g/dL)*	*N*	*Hb (g/dL)*
18-49	234	13.55±0.81[a]	415	13.25±0.95[b]	297	13.07±1.0
50-79	391	13.96±0.89[a]	427	13.50±0.96	266	13.53±1.0
≥80	67	13.42±1.2	75	13.17±1.0	16	13.14±1.3
Total	692	13.77±0.93	917	13.37±0.98	579	13.28±1.06
Males' age						
18-59	289	15.54±0.93[a]	477	15.26±1.0[c]	283	14.89±1.0
60-79	192	15.34±1.0[a]	210	14.76±1.1	122	14.91±1.15
≥80	36	14.71±1.4	44	13.80±1.5	8	14.11±1.78
Total	517	15.41±1.03	731	15.03±1.15	413	14.88±1.07

[a]$p<0.0001$ *vs. Cilento and Carlantino;* [b]$p<0.01$ *vs. Carlantino;* [c]$p< 0.0001$ *vs. Carlantino.*

Table 3. Low Hb limits for normal (young and old) subjects of the three isolates in comparison with results from reference 7.

	Val Borbera Hb g/dL	*US whites* Hb g/dL	*Carlantino* Hb g/dL	*Cilento* Hb g/dL	*US blacks* Hb g/dL
Females' age					
18-49	12.21	12.2	11.42	11.67	11.5
50-79	12.50	12.2	11.88	11.90	11.5
>80	11.44			11.42	
Males' age					
18-59	14.00	13.7	13.24	13.62	12.9
60-79	13.66	13.2	13.01	12.86	12.7
> 80	12.40			11.26	

observed among females within the age deciles 18-29, 30-39, 40-49 on one side and 50-59, 60-69, 70-79 on the other side, data were pooled together for young (18-49 yrs) and old (50-79 yrs) women, broadly corresponding to premenopausal and postmenopausal women respectively. Since up to the age group >80 years no significant difference of Hb levels is observed in males, we classified as young subjects those aged <60 and as old those >60 years, as proposed.[7] Subjects older than 80 years were analyzed separately in both sexes.

Mean Hb levels ± SD in females and males of different ages are shown in Table 2. Old women have significantly higher Hb levels than young women in all cases. Old males have significantly lower mean Hb than young males in two out of three isolates. Hb levels of Val Borbera participants are statistically higher than those of the other two isolates both in young and old participants. Overall, people from Carlantino were usually more anemic than people from Cilento villages: the difference was statistically significant in young but not in old subgroups. The trend towards higher Hb levels in Val Borbera as compared to the other isolates was maintained even in the elderly samples, although the difference did not reach statistical significance.

Differences in Hb levels could be ascribed to a general difference in way of life (climate, diet, smoking habits among others) or to different genetic components between northern and southern Italian populations. Hb levels may increase in heavy smokers. Information on smoking habits showed an increased percentage of smokers in the southern isolates, especially among males and young females. In addition, mean Hb level differences among the isolates were maintained when we considered only non-smokers (*data not shown*) and, when numbers were adequate, also among smokers. To define the proportion of Hb variation attributable to genetic variation, since the family relationships of the participants in each isolate were known, we measured the heritability of the trait. The heritability coefficient (h^2) of Hb levels was 0.42 for Val Borbera ($p<0.0001$), 0.34 and 0.35 for two villages of Cilento ($p<0.0001$) and 0.41 for Carlantino ($p<0.0001$).

In all age groups considered, except >80 years, Hb was normally distributed. Calculating the mean–1.65 SD we obtained a value in good agreement with the 5th percentile observed in our dataset (*data not shown*). Based on these concordant observations ranges of normal Hb levels were calculated and low limits shown in Table 3. Our populations are prevalently healthy, since samples with evident anemia or disorders potentially causing anemia were excluded from the analysis. We emphasize that the exclusion rate for anemia was remarkably lower (<10%) compared to other studies.[7] For this reason, this is a valuable example of normal population, relevant to define reference Hb values.[5] The populations we examined are from three different Italian geographical areas but each has a uniform genetic background. Comparison of Hb levels among them allowed us to establish normal differences in Hb levels and differences that might be due to genetic components.

Since the Hb levels do not differ significantly within the age group 20-59 in males and 20-49 in females we have considered together young (20-49 year old women; 20-59 year old males) and old (>50 year old women; >60 year old males) adults.[7] However, due to

the high proportion of elderly in our study we analyzed separately individuals aged >80 years, who have significantly lower Hb levels compared to the other groups.

We observed that individuals from the southern isolates have lower mean Hb levels than individuals from Val Borbera. This is in agreement with the lower exclusion rate for anemia and higher exclusion rate for polyglobulia in Val Borbera compared to southern isolates. Evident environmental determinants such as altitude, smoking habits and socioeconomical conditions were excluded. Our analysis had removed all the MCV<80 fL and thus presumably most beta thalassemia trait - whose frequency can be higher in southern Italy - and a proportion of alpha thalassemia and of iron deficient anemia. Lower limits in southern isolates for women are similar to those proposed for black people (11.5 in both groups).[7] Limits in Val Borbera are similar to those proposed for white women.[7] The slightly higher Hb level found in old women in our study is likely explained by having considered separately subjects aged > 80 years, who have lower Hb levels. Lower Hb limits in Italian men of northern and southern origin are slightly higher than the corresponding values in whites and blacks respectively. The differences we observed should be confirmed on other larger samples in Italy. However, this difference and the well known Hb difference observed in US black compared to white subjects might suggest that genes associated with different Hb levels have been selected in different areas as an adaptation to (unknown) environmental pressure. Genetic studies might lead to an understanding of whether this is the case.

Normal Hb levels in the elderly cannot be taken to be conclusive, because multiple concomitant pathological conditions in this age group make it difficult to define *true* normals and also because of the limited number of cases examined.

Finally we observed that Hb levels are substantially heritable by heritability estimates which range from 34 to 42 %, similar to values reported in the twin study.[2] Beside their utility for establishing normal reference Hb levels, considering the genetic structure of the populations studied, our results are fundamental to a future genetic investigation of determinants that influence Hb levels in these populations and to define their genetic difference.

Authorship and Disclosures

CS performed the analysis and co-wrote the manuscript; MC, PG and DT designed the experimental work and co-wrote the manuscript; CL, TN, SB, P d'A, RM collected data, performed the analyses and analyzed the data; CC designed research and wrote the manuscript. All the authors approved the final version of the manuscript. Tables 1-3 and Online Supplementary Figure 1S were created by CS. The authors reported no potential conflicts of interest.

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