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Letter to the Editor

Prevalence of H63D, S65C and C282Y mutations of the *HFE* gene in 1120 voluntary blood donors from Antioquia region of northwest Colombia

To the Editor:

Hereditary hemochromatosis (HH, OMIM#235200) is an autosomal recessive disorder of iron metabolism caused by increased iron absorption leading to severe iron overload. At least three causative mutations of HH have been detected in the *HFE* gene: The c.C187G (H63D) and c.A193T (S65C) in exon 2, and the c.G845A (C282Y) in exon 4 [1]. Given that the early symptoms of iron overload are non-specific and undiagnosed HH might lead to severe organ damage and death, the identification of *HFE* mutations in racially different populations may play a significant role in preventive health care.

The Antioquia region in northwest Colombia has approximately 5,000,000 inhabitants. This population ("paisa community") was established in the middle of the 16th and/or early 17th century by Spanish and Sephardic Jewish population [2], and grew in relative isolation until the late 19th century. Therefore, the "paisa community" is a unique resource for the study of Spanish-related gene flow and founder mutations. The aim of the present investigation was to determine the H63D, S65C and C282Y mutation frequency of the *HFE* gene in a cohort of 1120 (673 women, 447 men) apparently healthy voluntary blood donors from Antioquia. This investigation was approved by the Human Ethics Committee of the University of Antioquia, and of the Hospital "Pablo Tobon Uribe", and was supported by Colciencias grants #1115-041-8113 and #1115-041-6337 to C.V.P. Mutations of the *HFE* gene were analyzed by rapid-cycle PCR with allele-specific fluorescent probe melting profiles performed on the Roche Diagnostics LightCycler as described elsewhere [3]. Table 1 and 2 show the results of genotype and allelic analysis, respectively. Of notice, the H63D mutation was detected in 347 out of 2240 chromosomes, resulting in an allele frequency of 0.1549. The carrier frequency was 0.2618 (one out of four). The C282Y mutation was detected in 41 chromosomes, resulting in an allele frequency of 0.01830. The carrier frequency was 0.035 (one out of 28). As to the S65C mutation, it was detected in only 2 chromosomes, corresponding to an allelic frequency of 0.0009. The carrier frequency was 0.00178 (one out of 560). Although women were more numerous than men genotyped in this study, no significant differences were found for gender percentage distribution of the *HFE* mutations.

This is the first large scale screening for the *HFE* mutations conducted in Antioquia, where 54% of the patients displayed the clinical and molecular features of classical HH associated with homozygous C282Y mutation and 8% were associated with either H63D or C282Y heterozygotes, respectively [4]. The S65C mutation of *HFE* may also play some role in the disease. Historically, Spaniards have participated fully in the massive late 16th- and early 20th-century European immigration to the Americas. This population movement adopted the "demic-diffusion" model. Hence, the prevalence of the *HFE* mutations is expected to be directly associated to the migration model and the founder effect in Antioquia. We found that the allele frequency of the H63D is similar to that reported from Madrid and Extremadura, but significantly differs from the northwest, north, northeast and southeast Spanish regions (Table 3). Therefore, the H63D mutation found in Antioquia could not be related with any specific Spaniards population or community. However, further genetic studies are required to establish the reason of such *HFE* mutation allelic variability displayed not only in Antioquia (Colombia), and Latin America, but also in Spain.

As previously reported by others, we found neither S65C homozygotes nor compound heterozygote, except H63D/S65C. Moreover, the S65C allele frequency is the lowest so far reported (Table 3). Concerning the C282Y, the allele frequency is similar to the allele frequency from some Spanish regions such as Madrid, Extremadura and Balearic Islands, but significantly differs from Northern Spanish regions [[5] and references within]. Noticeably, low C282Y allele frequency has also been reported in South America (Table 3). Taken together these observations and our data suggest that immigrants to the Americas most probably came either from the center or southern Spain. However, we do not discard the possibility that a contribution of the C282Y mutation distribution in Antioquia may have been introduced by Northern Spaniard immigrants. Although, further genetic studies associated with *HFE* gene (e.g. HLA-A3/B7 haplotype HLA analysis) are needed to fully understand these findings, the present evidence suggest that differences among the C282Y allelic frequencies in Antioquia and Latin America most probably are not only due to divergent population admixture, but also to other demographic events such as singular migration patterns, population isolation and changes in population size. Taken together these results suggest that the S65C mutation in the paisa community is extremely rare, whereas the H63D is the commonest one. Consequently, 120,000 individuals for the H63D; 1675 for the C282Y and 4 for the S65C mutation are expected to be homozygous,

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Table 1
Genotype frequencies of the *HFE* (H63D, S65C and C282Y) mutations in the Antioquia "paisa community"

H63D	S65C	C282Y	All			Men			Women		
			N	%	95% CI	*N*	%	95% CI	*N*	%	95% CI
+/+	−/−	−/−	27	2.41	1.5–3.4	12	2.68	1.1–4.3	15	2.22	1–3.4
−/−	+/+	−/−	0	–	–	0	–	–	0	–	–
−/−	−/−	+/+	4	0.35	0.1–0.9	2	0.44	0.1–1.6	2	0.29	0–1.1
+/−	−/−	−/−	291	25.98	23.4–28.6	124	27.74	23.5–32	165	24.51	21.2–27.8
−/−	+/−	−/−	0	–	–	0	–	–	0	–	–
−/−	−/−	+/−	33	2.94	1.9–4.0	12	2.68	1.1–4.3	21	3.12	1.7–4.5
+/−	+/−	−/−	2	0.17	0.0–0.6	1	0.22	0–1.2	1	0.14	0–0.8
+/−	−/−	+/−	0	–	–	0	–	–	0	–	–
−/−	+/−	+/−	0	–	–	0	–	–	0	–	–
−/−	−/−	−/−	765	68.30	65.5–71.1	296	66.22	61.7–70.7	469	69.68	66.1–73.2
Total			1120			447			673		

N, number of subjects tested; CI, 95% confidence half-interval; Genotypes: '+' indicates mutated allele and '−' indicates wild-type allele.

respectively. Taken as a whole, the present data do justify genetic testing as part of routine evaluation for hereditary hemochromatosis.

Table 2
Allele frequencies of the *HFE* (H63D, S65C and C282Y) mutations in the Antioquia "paisa community"

Allele	All		Men		Women	
	%	95% CI	%	95% CI	%	95% CI
H63D	15.49	14.0–17.0	16.66	14.2–19.2	14.56	12.6–16.5
S65C	0.09	0.01–0.32	0.11	0–0.6	0.07	0–0.4
C282Y	1.83	1.25–2.41	1.78	0.9–2.7	1.85	1.1–2.6

Table 3
Distribution of the allele frequencies of the H63D, S65C and C282Y mutations in Spain and Latin-American countries

Country region	Number of subjects tested	Allele frequency %		
		H63D	S65C	C282Y
Spain				
Madrid	1000	16.4	–	1.70
Cataluña	7142	19.78	–	3.05
Galicia	50	23.00	–	5.00
Extremadura	125	16.00	–	2.00
Basque Country	144	29.85	–	4.40
Cantabria	213	–	–	4.4
Balearic Islands	665	20.9	–	2.00
Tarragona	812	22.3	1.2	3.10
Murcia	370	27.02	2.02	0.67
Mexico				
Mexico City	2524	11.5	–	0.2
Venezuela	214	11.9	0.9	1.9
Ecuador	100	3.5	4.0	0
Chile				
Santiago	156	10.58	–	1.28
Colombia				
Antioquia	1120	15.49	0.09	1.83

What is the actual origin of the *HFE* mutations detected among the "paisa" community? Since the north regions of Spain present high C282Y allele frequency similar to the frequencies of northern European regions, this outcome has been interpreted as a Celtic legacy [6]. Therefore, it is highly probable that the C282Y *HFE* mutation found in Antioquia is originally connected with the Celtic civilization. On the other hand, the origin of the H63D and S63C mutations is less certain. Because the highest H63D frequencies have been detected in Spain, it has been hypothesized that the H63D mutation emerged in the Iberian Peninsula [7]. Alternatively, the H63D could have arisen in the Middle-East or in Northern Africa. In fact, the conquest of the former Roman province of Hispania by the Moors from about 711 AD until 1492 AD certainly marked the history, culture and most probably the genetic makeup of Spanish population [8]. It is then highly probable that the H63D *HFE* mutation identified in Antioquia is originally connected to North African population. Finally, low S65C allelic frequencies observed in Europe suggest a more recent appearance of this mutation in the history of the *HFE* gene mutations, and hence its lower value observed in Antioquia and Latin America.

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