Hemochromatosis C282Y gene mutation increases the risk of venous leg ulceration

Paolo Zamboni, MD,* **Silvia Tognazzo, BS, Marcello Izzo, MD, Francesca Pancaldi, MD, Gian L. Scapoli, MD, Alberto Liboni, MD,** and **Donato Gemmati, BS** *Ferrara, Italy*

Objective: **Chronic venous disease (CVD) is the most common vascular disorder, progressing in approximately 10% of cases toward chronic venous leg ulceration, whereas the hemochromatosis gene (HFE) C282Y mutation is the most common recognized genetic defect in iron metabolism. Because CVD leads to local iron overload in the affected legs, we investigated whether two common HFE mutations could increase the risk of chronic venous leg ulceration.**

Methods: **This was a case-control study at the Vascular Diseases Center, University of Ferrara, Italy. From a cohort of 980 consecutive patients affected by severe CVD (CEAP clinical classes C4 to C6) we selected 238 cases with the exclusion of any other comorbidity factor potentially involved in wound etiology (group A). They were subdivided into group B, including 137 patients with ulcer (classes C5 and C6: 98 primary and 39 postthrombotic cases), and group C, including 101 cases with no skin lesions (class C4). They were completely matched for sex, age, and geographic origin with 280 healthy controls (group D). A total of 518 subjects were polymerase chain reaction genotyped for HFE mutations (C282Y and H63D). We assessed the risk of ulceration by comparing the prevalence of ulcer in homogenous cases with and without the HFE variants. Other main outcome measures were the sensitivity, specificity, and predictive values of the genetic test in CVD cases.**

Results: **C282Y mutation significantly increases the risk of ulcer in primary CVD by almost seven times (odds ratio, 6.69; 95% confidence interval, 1.45-30.8; $P = .01$). Application of the HFE test in primary CVD demonstrated increased specificity and positive predictive values (98% and 86%, respectively), with negligible sensitivity and negative predictive values.**

Conclusions: **The overlap of primary CVD and the C282Y mutation consistently increases the risk of developing venous leg ulceration. These data, which have been confirmed in other clinical settings, suggest new strategies for preventing and treating primary CVD.**

Clinical Relevance: **The number of patients affected by primary CVD is so great that the vast majority of ulcers are also related to this common problem. On the other hand, there is not a reliable way for identifying in advance, from the broad base of primary CVD patients (20–40% of the general population), the high risk minority (10% of primary CVD cases) who will develop a venous ulcer. In such cases, a simple C282Y blood genetic test demonstrated an elevated specificity in predicting ulcer development (98%, CI 95%, 92.8–99.7). The genetic test could be applied starting from the C2 class, varicose veins, the most common situation observed in clinical practice. In perspective, the presence of the C282Y mutation would strengthen the indications and priorities for surgical correction of superficial venous insufficiency. (J Vasc Surg 2005;42:309-14.)**

C282Y and H63D mutations of the hemochromatosis (HFE) gene are the most common genetic defects in hereditary hemochromatosis gene (HH) in Caucasian populations, and the associated risk is increased by the homozygous condition for C282Y or by coinheritance of both variants. It was recently discovered that these genetic defects present lower penetration than previously thought and cannot be advocated alone for the development of HH. Therefore, to develop iron overload pathologies, other inherited or acquired conditions are necessary, such as environmental factors, concomitant diseases, or both.[1,2]

From the Inter-Departmental Vascular Disease Center, University of Ferrara.

Supported by the Italian Ministry for the University and the Scientific Research and by the Foundation Cassa di Risparmio di Ferrara.

Competition of interest: none.

Presented at the Seventeenth Annual Meeting of the American Venous Forum, San Diego, Calif, Feb 10-13, 2005.

Reprint requests: Paolo Zamboni, MD, Interdepartmental Vascular Disease Center, University of Ferrara, 44100 Ferrara, Italy (e-mail: zmp@unife.it).

0741-5214/$30.00

doi:10.1016/j.jvs.2005.04.003

The population frequently has C282Y and H63D heterozygous mutations, and subjects with these genetic variants are generally considered asymptomatic carriers. An even more common condition that affects the general population is chronic venous disease (CVD). Affecting 20% to 50% of the Caucasian population, CVD is the most widespread vascular pathology. In most cases, it is a minimally disabling disease, but in approximately 10% of cases it progresses toward chronic venous leg ulceration, the overall prevalence of which is 1% to 2% in the United States and Europe, with an estimated health care expenditure of $1 billion per year in the United States alone.[3,4]

Impairment of venous hemodynamics is an essential but insufficient factor in explaining the progression of the disease to the point of skin lesions. One of the critical objectives for physicians involved in evaluating and treating CVD is to identify a prognostic factor for ulcer onset. Clinical and hemodynamic parameters, including duplex scanning and plethysmographic investigations, fail to predict ulcer appearances and are insufficient for categorizing patients by the clinical severity of the disease.[5-8] For such

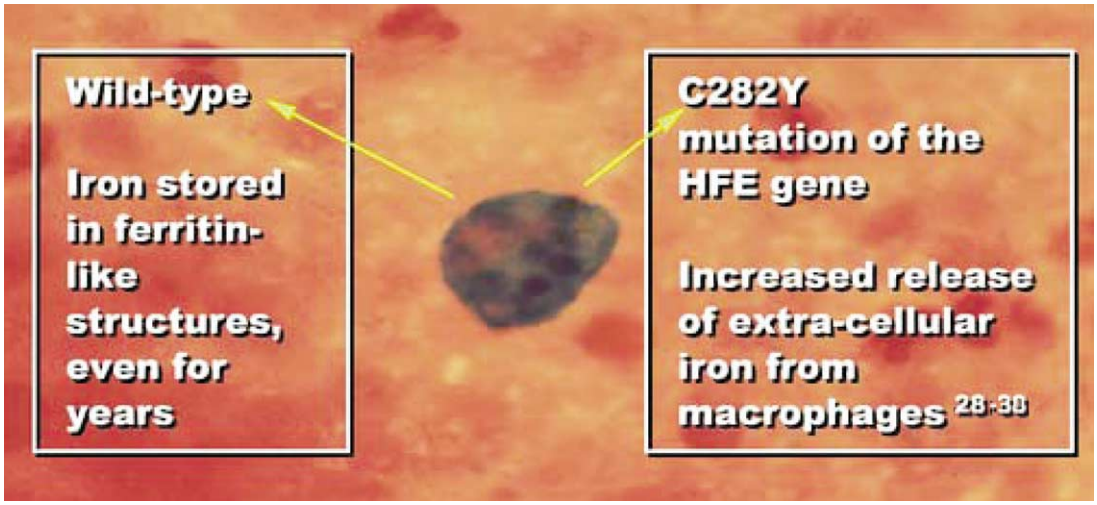

Fig 1. The iron from senescent erythrocytes migrated into the matrix in the condition of chronic venous stasis is taken up by macrophages and stored in ferritin-like structures. Such intracellular iron deposits of macrophages carrying the C282Y mutation have less stability than wild types, and the mutation is significantly associated with the risk of developing ulcers in course of chronic venous disease (Perls staining; original magnification, ×400). *HFE,* Hemochromatosis.

reasons, several adjunctive factors have been investigated during the past 20 years in an attempt to understand the etiology of venous ulceration, but none of them completely explains the entire process.[9-11]

Finally, numerous authors have demonstrated that a local iron overload exists in CVD-affected limbs.[12-16] This is due to the diapedesis of erythrocytes that enter the interstice through fenestrations in the capillaries under conditions of chronic venous stasis.[17] The heme catabolism at the interstitial level causes hemosiderin deposits at both the phagocyte and extracellular levels (Fig 1).

Local iron overload is not negligible, and hemosiderin granules have been found even in the urine of patients affected by CVD.[15] Several authors have hypothesized that this iron could be released to generate free radicals because of excessive oxidative stress, to activate a proteolytic hyperactivity on the part of metalloproteinases, or to downregulate tissue inhibitors of metalloproteinases.[14,16-20] It remains to be explained why iron overload, always visible to the naked eye in the hyperpigmentation of clinical class C4, causes lesions in some individuals, whereas in others it does not. It has recently been demonstrated that patients with ulcer have a higher concentration of iron and rate of transferrin saturation in the serum from the affected leg than do C4 patients. It seems that patients with ulcer lose the ability to counteract the iron export from inside the phagocytes, and it has been hypothesized that this inability could be genetically determined.[20] In an attempt to understand this patient-to-patient difference, we investigated whether, in the population affected by CVD, the two principal HFE mutations could play a determining role in the formation of venous ulcers.

METHODS

Selection of cases and controls. We studied 238 patients affected by severe CVD (CEAP clinical classes C4 to C6) from different areas of Italy, selected from an initial cohort of 980 patients who were referred to our Vascular Diseases Center. For purposes of patient selection, these subjects underwent clinical and duplex scanning examinations. This investigation, conducted in accordance with consensus statement criteria and a method previously described,[5-8] allowed us to separate primary from postthrombotic cases and to identify patients affected by peripheral arterial disease.

For patient selection, we strictly applied the following exclusion criteria to exclude any other comorbidity factor potentially involved in wound etiology:

1. Diabetes
2. Peripheral arterial disease or ankle-brachial index less than 0.9
3. Hemolytic anemia, iron-deficiency anemia, or malnutrition
4. Inability to walk
5. Severe cardiac, hepatic, renal, or pulmonary insufficiency
6. Chronic administration of cortisones for chronic inflammatory disease or autoimmune disease

Applying these exclusion criteria, we selected 238 cases, which constituted group A. These patients were subsequently divided into 2 groups: group B, 137 subjects with ulcers in the lower limbs of certain venous origin (clinical classes C5 and C6), and group C, 101 subjects who had never had skin lesions, of which 98 had primary and 3 had

Table I. Patient population demographics

Variable	*Group A, all CVD patients (n = 238)*	*Group B, venous ulcer patients (n = 137)*	*Group C, severe CVD; never ulcer (n = 101)*	*Subgroup F, primary ulcer (n = 98)*	*Subgroup E, postthrombotic ulcer (n = 39)*	*Group D, healthy controls (n = 280)*
Age (y)						
Mean ± SD	61.03 ± 12.95	63.7 ± 13.18	58.04 ± 13.12	63.0 ± 12.72	65.0 ± 14.12	61.5 ± 13.50
Median (range)	61 (27-85)	63 (34-85)	58 (27-84)	62.5 (34-84)	66 (41-85)	61 (27-85)
Sex, M/F	31.9/68.1	35.8/64.2	26.7/73.3	33.7/66.3	41/59	33.2/66.7
(%; n)	(76/162)	(49/88)	(27/74)	(33/65)	(16/23)	(93/187)
North/south	67.2/32.8	69.3/30.7	64.4/35.6	71.4/28.6	64.1/35.9	66.4/33.6
(%; n)	(160/78)	(95/42)	(65/36)	(70/28)	(25/14)	(186/94)

No significant differences were observed in the subgroups considered among the comparisons of computed parameters.
CVD, Chronic venous disease.

postthrombotic cases (class C4). The selected patients were comparable for distribution of sex, age, area of geographic origin, and presence of primary or postthrombotic CVD in the ulcer group, as reported in Table I.

We also considered the area of birth of both subjects and their parents because in Italy the frequency of the two principal HFE mutations is heterogeneous: whereas in the north it is similar to that of Central Europe, in the south it is much lower.[2,20-22] The boundary line between Northern and Southern Italy is usually considered to be the Apennines mountain chain, and we applied this criterion to our patient population (Table I). Finally, we subdivided the ulcer group B, in accordance with the pathogenesis and pathophysiology, into primary (subgroup F) and postthrombotic (subgroup E) ulcers.

The controls (group D) were 280 healthy subjects selected from among blood donors who were matched for sex, age, and geographic origin with the patients described previously (Table I). The total of 518 subjects (137 affected by venous ulcers, 101 affected by severe CVD, and 280 normal controls) underwent polymerase chain reaction genotyping for H63D and C282Y HFE gene mutations on chromosome 6.

DNA analysis. Genomic DNA was isolated from peripheral blood by using standard proteinase K treatment, followed by phenol-chloroform extraction and ethanol precipitation. Samples were polymerase chain reaction genotyped for C282Y and H63D HFE variants according to previous reports.[21] Genotypes were confirmed by regenotyping a random selection of samples (cases and controls) for each polymorphism investigated. There were no discrepancies between genotypes determined in duplicate.

Statistical analysis. Statistical differences between case and control populations were determined by using the χ^2 test. Where appropriate, the Yates correction or Fisher exact test was applied. P values $\leq .05$ were considered statistically significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate the risk of developing venous ulcers. Adjusted ORs for single or combined comparisons were calculated by means of logistic regression models that controlled for sex, age, geographic origin, and the H63D polymorphism. Risk assessment for ulceration was performed by comparing the prevalence of ulcer in cases with and without the HFE variants. In addition, we assessed the sensitivity, specificity, and positive and negative predictive values in CVD cases of the genetic test under study. The expected frequency of control genotypes was checked by using the Hardy-Weinberg equilibrium test. All analyses were performed by Systat version 5.0 (Systat Inc, Evanston, Ill) and the SPSS statistical package (version 10.1; SPSS Inc, Chicago, Ill).

RESULTS

HFE H63D and C282Y distribution in cases and controls. Fig 2 shows the allele and genotype distributions of the H63D and C282Y variants in the entire cohort of 238 CVD cases, in the subset groups, and in the healthy controls. In our ulcer cases (group B), the prevalence of the C282Y polymorphic allele was significantly increased compared with healthy controls (5.1% vs 2.1%; $P = .035$). When we compared group B with C4 patients without skin lesions (group C), the prevalence demonstrated a similar, although not significant, appreciable difference (5.1% vs 1.5%; $P = .063$). It is interesting to note that the prevalence of the C282Y polymorphic allele found in C4 cases of group C (1.5%) was less than that in healthy controls (2.1%); however, such a difference is nonsignificant ($P = .778$), and thus it cannot be considered a protective mechanism. Moreover, when patients affected by primary ulcer (subgroup F) were computed vs healthy controls or C4 cases, the difference in prevalence of such a variant dramatically increased (6.6% vs 2.1%, $P = .005$; and 6.6% vs 1.5%, $P = .018$, respectively). Finally, further analysis of prevalence by comparing primary ulcer vs primary C4 cases showed a highly significant difference (6.6% vs 1.02%; $P = .008$).

Although the prevalence of double carriers of the variants under study (H63D/C282Y) found in the primary CVD group was greater than among healthy controls (3.06% vs 1.07%, respectively), it was not significantly higher ($P = .357$). Very interestingly, neither in the case group (group C) without lesions nor in the subgroup with postthrombotic ulcers were double carriers identified. Finally, the frequencies of the two mutations registered in the healthy control population seemed very similar to those previously reported for the Italian population.[21-23]

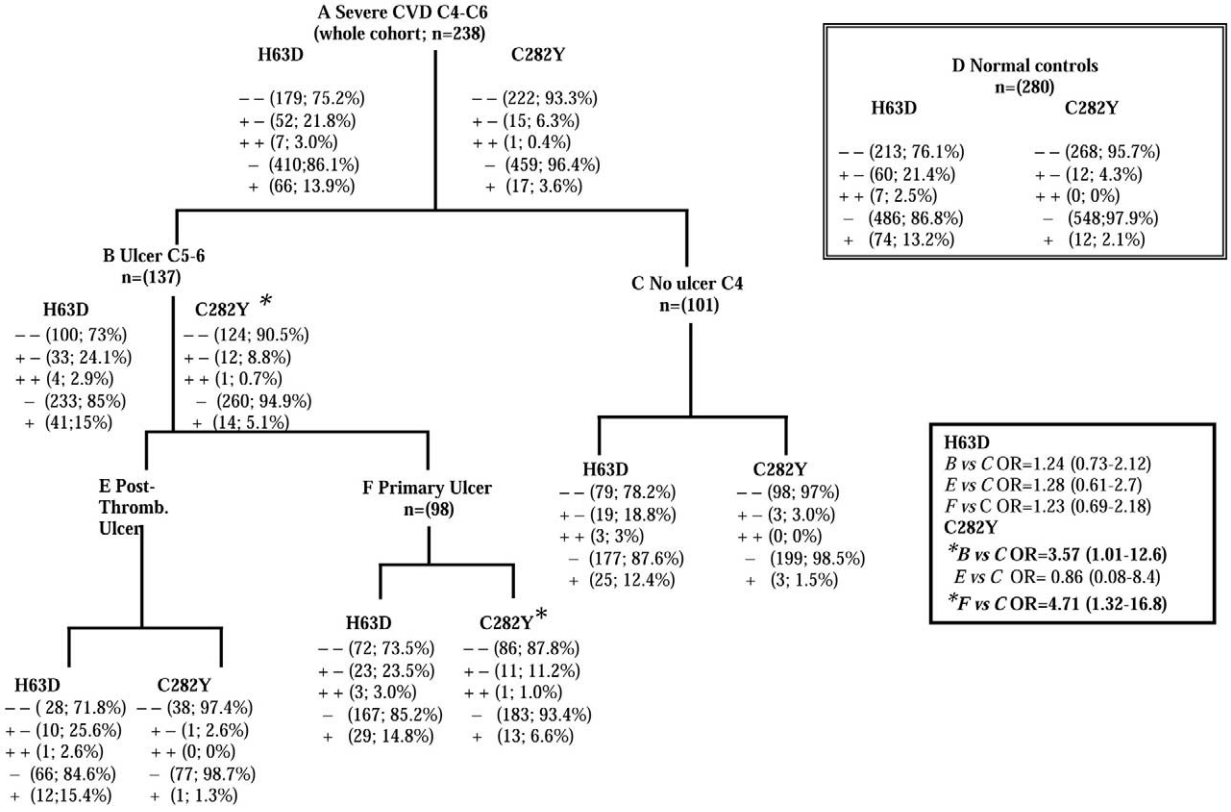

Fig 2. Allele and genotype distributions of the H63D and C282Y variants in the entire cohort of 238 chronic venous disease *(CVD)* cases, in the subset groups, and in the healthy controls. Odds ratios *(ORs)* and 95% confidence intervals are referred to polymorphic allele frequencies. *Significant results. *Post-Thromb,* Postthrombotic, ++ = homozygotes, +− = heterozygotes, −− = wild types.

Table II. Assessment of ulceration risk in CVD cases

Severe CVD; entire group (n = 238)			*Primary CVD (n = 196)*		
HFE C282Y	*Ulcer (n = 137)*	*No ulcer (n = 101)*	*HFE C282Y*	*Ulcer (n = 98)*	*No ulcer (n = 98)*
CC (n = 222)	124 (55.8%)	98 (44.2%)	CC (n = 182)	86 (47.2%)	96 (52.8%)
CY + YY (n = 16)	13 (81.3%)	3 (18.7%)	CY + YY (n = 14)	12 (85.7%)	2 (14.3%)
OR (95% CI; *P*)	3.42 (0.95-12.35; .085)		OR (95% CI; *P*)	6.69 (1.45-30.8; .01)	

CVD, Chronic venous disease; *OR,* odds ratio; *CI,* confidence interval. CC = wild types, CY = heterozygotes, YY = homozygotes.

Assessment of risk of ulceration and diagnostic predictivity. Table II shows the assessment of risk of ulceration by comparing the prevalence of ulcer in our homogeneous CVD cases carrying or not carrying the C282Y variant. The calculated ulcer risk in the entire cohort of CVD, although certainly appreciable, did not yield statistical significance (OR, 3.07; 95% CI, 0.83-11.32; $P = .085$). In contrast, in primary CVD, the risk increased by almost seven times when the C282Y variant was present (OR, 6.69; 95% CI, 1.46-30.8; $P = .01$). Consequently, in subjects affected by postthrombotic ulcer (subgroup E), the C282Y mutation did not seem to be associated with a significant risk. It is noteworthy that more than 85% of C282Y carriers in primary cases developed an ulcer, whereas ulcer occurred in only 47% ($P = .01$) of the wild types (Table II). Furthermore, we did not find any increased risk in any group regarding H63D mutation.

The specificity of the genetic test in predicting ulcer development in primary CVD cases was 98% (95% CI, 92.8%-99.7%), with a corresponding positive predictive value of 86% (95% CI, 57.1%-98.2%). On the contrary, assessment of sensitivity and negative predictive values was certainly less interesting for future clinical application (12%: 95% CI, 6.0%-20%; and 53%: 95% CI, 45.1%-98.2%, respectively).

DISCUSSION

The main finding of this study is that, in patients with primary CVD (the most common situation observed in clinical practice), the presence of the HFE C282Y polymorphic allele significantly increases the risk of developing an ulcer (Fig 2 and Table II). The consequent increased risk recorded in our population of patients affected by primary CVD was more significant than that for any other predictive parameter for venous ulcer reported to date.[5,6]

In addition, a positive test in case of primary CVD was highly predictive of ulcer development. In clinical practice, this finding suggests new prospects for the prevention and treatment of primary CVD. The proportion of CVD patients affected by primary superficial venous disease is so great that the vast majority of ulcers must also be related to this common problem. It has been demonstrated in 85% of ulcer cases that the disorder was referred to primary CVD, but with a combination of superficial and deep reflux in 32% and with a pattern of reflux confined to the superficial venous system in 53%. Isolated deep reflux, mainly in postthrombotic limbs, was found in only 15% of total ulcer cases, and these data were confirmed in other studies.[24-26]

Thus, our data are potentially applicable to the broad base of primary CVD patients because, among the many who have varicose veins (class C2), only 10% will develop a venous ulcer. This high-risk minority could be identified in advance by means of a simple blood test that would act as a genetic screening device. Then such preventive measures as elastic stockings, superficial venous surgery, and avoidance of iron-rich foods and dietary supplements could be used in a targeted program of potentially great effectiveness. Thus, primary CVD could be treated more appropriately before any lesions develop in patients with particular genetic haplotypes. Vascular surgery practice could be strongly influenced by the results of the HFE genetic test; the C282Y mutation would strengthen the indications and priorities for surgical correction of superficial venous insufficiency.

It is known that circulating neutrophils are first trapped in the venous microcirculation.[10] Red blood cell degradation products and interstitial protein extravasation are potent chemoattractants and presumably represent the initial underlying chronic inflammatory signal responsible for macrophage recruitment from trapped monocytes. Pappas et al[27] established, by means of morphometric assessment, that macrophages are the predominant cells observed in patients with CVD and severe dermal skin changes: this clearly indicates the central role of macrophages in the chronic inflammatory process of the ulcer bed.

Our results suggest a less favorable management of the iron deposits in the legs of C282Y patients with respect to wild types. It is known that the iron from red blood cells sequestered in the extracellular matrix is bound to ferritin and then to hemosiderin or taken up by phagocytes; inside these cells, the iron is arranged in ferritin-like structures, where it can be stored for years (Fig 1).[13-17,23] Such defensive mechanisms should avoid the generation of free iron and, consequently, of highly aggressive free radicals.

Even though iron metabolism is still poorly understood, some evidence in the literature suggests that intracellular iron deposits of macrophages carrying the C282Y mutation have less stability than the wild types. The mutated macrophages lose the ability to counteract the increased iron export from inside the cell.[23] In addition, it has been demonstrated that the mutated phagocytes, after having taken up the senescent red blood cells, release twice the amount of non–transferrin-binding iron and at a lower molecular weight with respect to the wild types.[28] These already-known effects of the C282Y mutation on human macrophages, if speculatively related to our findings, can lead us to assume that the mutated macrophages increase the possibility of generating free iron and free radicals, possibly leading to matrix breakdown and skin lesions.[14,16,18,19,29]

Thus, it seems that mutated macrophages potentially release more iron from inside the cell and that this effect is related to ulcer appearance. This finding is confirmed by previous studies in which a significantly higher serum iron and percentage of transferrin saturation were assessed in the legs of ulcer patients as compared with either the arm serum of the same subjects or with controls.[12,20] This gene variation could offer a plausible explanation for such a phenomenon.

In our study, the H63D mutation by itself did not prove to play the same role as the C282Y mutation in facilitating the occurrence of ulcerous lesions. However, H63D/C282Y compound carriers are overrepresented, although not significantly, in our primary chronic venous leg ulceration cases. We believe that this lack of significance is due to the relatively small number of compound carriers found overall. However, such a finding undoubtedly ascribes an appreciable role in ulcer pathogenesis to the coinheritance of the two defects, as previously demonstrated for HH.[1,2] This idea is strengthened by the fact that no compound heterozygotes were found in the postthrombotic and C4 groups.

Similarly, neither the C282Y nor the H63D mutation seems to be significantly associated with the appearance of ulcers in patients affected by CVD secondary to postthrombotic syndrome. This lack of association could be attributed to the well-known greater hemodynamic impairment in postthrombotic as compared with superficial and primary cases.[5] Finally, the slight underrepresentation of the C282Y mutation in both postthrombotic and C4 cases was probably due to a chance rather than a protective effect.

It will be interesting to determine whether our findings observed in the Italian population can be observed in other countries. In Italy and Greece, the C282Y mutation is much less frequent than in Northern Europe. In a review analyzing nine reports from Italy, allele frequencies for the C282Y were calculated to average 1.7%, with an inverted gradient from north to south (range, 4.2%-0.5%); the same analysis showed a decreased frequency in Europe from the north to the Mediterranean basin: Ireland, 9.7%; United Kingdom, 8.2%; Norway, 6.6%; Brittany and Northern France, 7.7%; Southern France, 2.6%; Germany, 4.2%; and

Greece, 1%.[21] It is noteworthy that in Northern Europe, venous ulcers also have a significant epidemiologic effect. In Italy, venous ulcers are correspondingly less frequent than in Northern Europe, although CVD has the same prevalence.[2,4,20-22,25,26,30] It could be speculated that the greater frequency of venous ulcers in Northern Europe is related to the greater frequency of HFE mutations in these countries. We speculate that the C282Y variant could play a pivotal role in ulcer risk even in areas of the United States that have high prevalences of Northern European descent, such as New England and the North Central states.

In conclusion, the overlap of primary CVD and the C282Y mutations consistently increases the risk of developing venous leg ulceration. Because this finding suggests new prospects for the prevention and treatment of primary CVD, we believe that further research in other clinical settings, including an international multicenter study, is warranted to determine whether similar results can be obtained and whether their clinical implications can be confirmed.

ACKNOWLEDGMENT

We thank Patricia Ennis for revising the manuscript.

REFERENCES

1. McCune CA, Ravine D, Worwood M, Jackson HA, Evans HM, Hutton D. Screening for hereditary haemochromatosis within families and beyond. Lancet 2003;362:897-8.
2. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of C282Y hereditary haemochromatosis mutation in USA. Lancet 2002; 359:211-8.
3. Goldman MP, Weiss RA, Bergan JJ. Diagnosis and treatment of varicose veins. A review. Dermatology 1994;31:393-413.
4. Cesarone MR, Belcaro G, Nicolaides AN, Geroulakos G, Griffin M, Incandela L, et al. 'Real' epidemiology of varicose veins and chronic venous diseases: the San Valentino Vascular Screening Project. Angiology 2002;53:119-30.
5. Nicolaides AN. Investigation of chronic venous insufficiency: a consensus statement (France, March 5-9, 1997). Circulation 2000;102:126-63.
6. Eklof B, Rutherford RB, Bergan JJ, Carpentier PH, Gloviczki P, Kistner RL, et al. Revision of the CEAP classification for chronic venous disorders: consensus statement. J Vasc Surg 2004;40:1248-52.
7. Zamboni P, Cisno C, Marchetti F, Mazza P, Fogato L, Carandina S, et al. Minimally invasive surgical management of primary venous ulcers vs. compression treatment: a long term randomized study. Eur J Vasc Endovasc Surg 2003;25:313-8.
8. Shami SK, Sarin S, Cheatle TR, Scurr JH, Coleridge Smith PD. Venous ulcers and the superficial venous system. J Vasc Surg 1993;17:487-90.
9. Browse NL, Burnand KG. The cause of venous ulceration. Lancet 1982;31:2:243-5.
10. Coleridge Smith PD, Thomas P, Scurr JH, Dormandy JA. Causes of venous ulceration: a new hypothesis. Br Med J 1988;296:1726-7.
11. Falanga V, Eaglstein WH. The "trap" hypothesis of venous ulceration. Lancet 1993;17:1006-8.
12. Zelikovski A, Podlipski A, Sternberg A, Urca I. Changes in chemical, hematological and blood gases values in chronic venous insufficiency of the lower limbs. Vasa 1977;6:26-30.
13. Ackerman Z, Seidenbaum M, Loewenthal E, Rubinow A. Overload of iron in the skin of patients with varicose ulcers. Possible contributing role of iron accumulation in progression of the disease. Arch Dermatol 1988;124:1376-8.
14. Wenk J, Foitzik A, Achterberg V, Sabiwalsky A, Dissemond J, Meewes C, et al. Selective pick-up of increased iron by deferoxamine-coupled cellulose abrogates the iron-driven induction of matrix-degrading metalloproteinase 1 and lipid peroxidation in human dermal fibroblasts in vitro: a new dressing concept. J Invest Dermatol 2001;116:833-9.
15. Zamboni P, Izzo M, Fogato L, Carandina S, Lanzara V. Urine hemosiderin: a novel marker to assess the severity of chronic venous disease. J Vasc Surg 2003;37:132-6.
16. Yeoh-Ellerton S, Stacey MC. Iron and 8-isoprostane levels in acute and chronic wounds. J Invest Dermatol 2003;121:918-25.
17. Wenner A, Leu HJ, Spycher MA, Brunner U. Ultrastructural changes of capillaries in chronic venous insufficiency. Exp Cell Biol 1980;48:1-14.
18. Abd-El-Aleem SA, Ferguson MW, Appleton I, Kairsingh S, Jude EB, Jones K, et al. Expression of nitric oxide synthase isoforms and arginase in normal human skin and chronic venous leg ulcers. J Pathol 2000; 191:434-42.
19. Herouy Y, Mellios P, Bandemir E, Dichmann S, Nockowski P, Schopf E, et al. Inflammation in stasis dermatitis upregulates MMP-1, MMP-2 and MMP-13 expression. J Dermatol Sci 2001;25:198-205.
20. Zamboni P, Scapoli G, Lanzara V, Izzo M, Fortini P, Legnaro A, et al. Serum iron and MMP-9 variations in limbs affected by chronic venous disease and venous leg ulcers. Dermatol Surg 2005;31:19-22.
21. Milman N, Pedersen P. Evidence that the Cys282Tyr mutation of the HFE gene originated from a population in Southern Scandinavia and spread with the Vikings. Clin Genet 2003;64:36-47.
22. Campo S, Restuccia T, Villari D, Raffa G, Cucinotta D, Squadrito G, et al. Analysis of haemochromatosis gene mutations in a population from the Mediterranean Basin. Liver 2001;21:233-6.
23. Piperno A, Sampietro M, Pietrangelo A, Arosio C, Lupica L, Montosi G, et al. Heterogeneity of hemochromatosis in Italy. Gastroenterology 1998;114:996-1002.
24. Nelzen O, Bergqvist D, Lindhagen A. The prevalence of chronic lower-limb ulceration has been underestimated: results of a validated population questionnaire. Br J Surg 1993;83:255-8.
25. Eberth-Willershausen W, Marshall M. Prevalence, risk factors and complications of peripheral venous diseases in the Munich population. Hautarzt 1984;35:68-77.
26. Ruckley CV, Evans CJ, Allan PL, Lee AJ, Fowkes FG. Chronic venous insufficiency: clinical and duplex correlations. The Edinburgh Vein Study of venous disorders in the general population. J Vasc Surg 2002;36:520-5.
27. Pappas PJ, DeFouw DO, Venezio LM, Gorti R, Padberg FT Jr, Silva MB Jr, et al. Morphometric assessment of the dermal microcirculation in patients with chronic venous insufficiency. J Vasc Surg 1997;26:784-95.
28. Drakesmith H, Sweetland E, Schimanski L, Edwards J, Cowley D, Ashraf M, et al. The hemochromatosis protein HFE inhibits iron export from macrophages. Proc Natl Acad Sci U S A 2002;99:15602-7.
29. Moura E, Noordermeer MA, Verhoeven N, Verheul AFM, Marx JJM. Iron release from human monocytes after erythrophagocytosis in vitro: an investigation in normal subjects and hereditary hemochromatosis patients. Blood 1998;92:2511-9.
30. Heit JA, Rooke TW, Silverstein MD, Mohr DN, Lohse CM, Petterson TM, et al. Trends in the incidence of venous stasis syndrome and venous ulcer: a 25-year population-based study. J Vasc Surg 2001;33:1022-7.

Submitted Feb 3, 2005; accepted Apr 1, 2005.