Influence of gene polymorphisms in ulcer healing process after superficial venous surgery

Donato Gemmati, MS,[a,b] Silvia Tognazzo, MS,[a] Linda Catozzi, MS,[a] Federica Federici, MS,[a] Massimiliano De Palma, MD,[b] Sergio Gianesini, MD,[b] Gian L. Scapoli, MD,[a,b] Monica De Mattei, MS,[a] Alberto Liboni, MD,[b] and **Paolo Zamboni, MD,[b]** *Ferrara, Italy*

Objective: **Role of superficial venous surgery in reducing the time it takes for ulcers to heal is still controversial, although all studies confirm a significant reduction in ulcer recurrences. Recently, the *HFE-C282Y* and *FXIII-V34L* gene variants demonstrated a role in the risk of venous ulceration in primary chronic venous disorder (CVD) and in modulating lesion size in chronic venous ulcer (CVU), respectively. This study was conducted to investigate the role of *HFE-C282Y* and *FXIII* (*V34L* and *P564L*) gene variants in ulcer healing time after superficial venous surgery, by assessing the outcome of a cohort of homogeneous CVU patients.**

Methods: **The study selected 91 patients affected by primary CVU (CEAP C6, Ep, Asp, Pr), with the exclusion of any other comorbidity factor involved in delayed healing process, who underwent surgery. We assessed the ulcer area and the healing time. Patients were genotyped by polymerase chain reaction for *FXIII* (*V34L* and *P564L*) and for *HFE-C282Y* substitutions.**

Results: **Globally, CVU cases had a postoperative mean healing time of 8.5 ± 5.7 weeks. For the subset of cases above and below the median value (M = 8.0 weeks), *FXIII-V34L* genotype distribution significantly differed ($P < .0001$). In addition, Kaplan-Meier analysis yielded specific healing time profiles for the different *FXIII-V34L* classes of genotype ($P = .00001$), with an increased risk of delayed healing for the *FXIII-VV* genotype (hazard ratio, 4.14; 95% confidence interval, 2.1 to 8.2; $P = .00005$). Although *FXIII-P54L* genotype distributions did not differ, homozygous *564LL* cases ($P = .005$) and double carriers for both *FXIII* variants ($P < .0001$), had a significantly reduced healing time vs wild types. No differences in healing time were observed between carriers and noncarriers of the *HFE-C282Y* variant, whereas when these cases were stratified by *FXIII-V34L* genotypes, the *L34* carriers had a significantly shorter healing time, irrespective of the *HFE* genotype.**

Conclusion: **The *FXIII-34L* variant was significantly associated with shorter healing time after superficial venous surgery, suggesting a role in the healing and tissue regeneration phases. Conversely, *HFE-C282Y*, despite its role in ulcer establishment, did not affect the postoperative healing time. In perspective, the identification of patients with a poor prognosis may give clinicians the opportunity to modify management and to target tailored therapies in the view of a new and alternative concept of treatment based on pharmacogenomics. (J Vasc Surg 2006;44:554-62.)**

Chronic venous leg ulcers (CVU), account for a significant proportion of lower extremity wounds. CVU is a severe clinical manifestation of chronic venous disease (CVD), a widespread pathologic condition in developed countries.[1,2] CVD accounts for about 70% of CVU, and although it is an essential comorbidity factor, it is insufficient for skin lesion appearance.[2-4] The prevalence of CVU in the United States and Europe is 0.5% to 2.0%, with very high health-service and socioeconomic costs owing to the frequent recurrences and repercussions on the patients' capabilities to work.[5]

The treatment of choice for CVU is compression bandaging combined with wound bed preparation. For those patients who do not improve with conventional care, skin grafting or biologic dressing are useful.[2,6] Whilst it is controversial if the addition of superficial venous reflux correction by surgery has positive effects in reducing ulcer healing time, there is wider agreement in the associated reduction of recurrences.[7,8] The Comparison of Surgery and Compression with Compression Alone in Chronic Venous Ulceration (ESCHAR) study compared healing and recurrence rates in CVU patients treated with compression, with or without surgery.[9] It concluded that superficial venous surgery confers no added benefit over compression in healing rate but does lead to a reduction in recurrence, according to other nonrandomized studies.[8]

A recent systematic review that evaluated the overall rates of clinical outcomes in patients with severe CVD concluded that surgery, subfacial endoscopic perforator surgery included, with or without saphenous ablation, leads to an 88% chance of ulcer healing and a 13% chance of recurrence.[10] The authors concluded that randomized controlled trials were needed to define the effective contributors of compression, superficial surgery, and subfacial endoscopic perforator surgery in CVU treatment.

Finally, we published a randomized study that compared the effects on healing of minimally invasive surgical intervention vs compression, exclusively reserved for pa-

From Center Study Hemostasis and Thrombosis[a] and Center for Vascular Disease,[b] University of Ferrara.

Competition of interest: none.

This study was supported in part by the Italian MIUR funds and by a grant from Fondazione Cassa di Risparmio di Ferrara, and Fondazione Cassa di Risparmio di Cento, Italy.

Presented at the Eighteenth Annual Meeting of the American Venous Forum, Feb 22-26, 2006, Miami, Fla.

Reprint requests: Donato Gemmati, University of Ferrara, Center Study Hemostasis and Thrombosis, Center for Vascular Disease, C.so Giovecca 203, I-44100 Ferrara, Italy; e.mail: d.gemmati@unife.it

0741-5214/$32.00

doi:10.1016/j.jvs.2006.05.011

tients affected by primary and superficial venous reflux and in absence of other significant comorbidities.[11] We concluded that this kind of hemodynamic correction, respecting the proposed indications, improves healing even in terms of time, recurrence, and hemodynamics.

Regardless of the clinical strategy, a great variation exists in the clinical outcome among patients with similar disease pattern and treatment. Owing to the multifactorial etiology of longstanding ulcer, recognizing appropriate risk factors affecting healing may be a choice strategy to early select those patients that will have an unfavorable outcome. A recent study identified among a number of routinely assessed variables, increased patient age and ulcer chronicity, and slow healing time, but not surgically treated superficial venous reflux, as independent risk factors, respectively, for healing and recurrence.[12] In addition, larger wound area, duration of the wound, and fibrin on >50% of the lesion surface were associated with failure to heal.[13] Other good candidate risk factors, such as diabetes, rheumatoid arthritis, or popliteal vein incompetence, were not found to be significantly associated.[12] This clarifies the current lack of understanding in venous ulcer pathogenesis. On the other hand, many patients with CVD do not progress towards skin lesion, suggesting that interindividual variability is warranted. Genetics may have a role in subject individuality, although evidences for this and for a mode of inheritance are lacking.

We recently recognized that the common gene polymorphisms *HFE-C282Y* and *FXIII-V34L* have a role, respectively, in increasing the risk of venous ulceration by establishing local iron overload in primary CVD patients,[14,15] and in modulating the lesion size by means of positive effects on fibroblast cells and extracellular matrix components in CVU patients.[16]

The *HFE-C282Y* variant in the coding region of the hemochromatosis gene affects iron metabolism. Heterozygous subjects are generally considered asymptomatic carriers unless other inherited or acquired conditions may contextually determine iron overload.[17]

FXIII-V34L, in the coding region of the coagulation factor XIII gene, significantly increased the transglutaminase activity of the molecule in a gene-dosage effect. Basically, the *FXIII-V34L* variant decreases the risk of thrombosis, and recent in vitro and in vivo studies ascribed to *FXIII,* or to its variants, a key role in wound healing, tissue repairing/remodelling and angiogenesis.[18] Other *FXIII* gene variants, such as *FXIII-P564L*, may affect the molecule properties, but very few and conflicting data have been reported.[19] Therefore, in the present study we investigated whether these recognized gene factors might have effects on the healing time of CVU patients after superficial venous surgery.

PATIENTS AND METHODS

Patient population. We studied 91 patients (65% women) with a mean age of 60.5 ± 14.5 years who were affected by primary CVU involving exclusively the superficial and perforator vein system. Patients were selected from an initial cohort of 215 patients referred to our Vascular Diseases Center. For the purpose of patient selection, these patients had a clinical history recorded and a duplex scanning examination. These investigations, conducted in accordance with consensus statement criteria and a methodology previously described,[11,22,23] allowed us to carefully separate primary superficial from secondary cases, and within the latter, those affected by post-thrombotic venous insufficiency, and also to identify patients with peripheral arterial disease.

The following exclusion criteria for patient selection were strictly applied to exclude any other comorbidity factor that might potentially be involved in delayed wound healing: diabetes, peripheral arterial disease or an ankle-brachial index <0.9, or both; hemolytic anemia, iron-deficiency anemia, or malnutrition; inability to walk, severe cardiac, hepatic, renal, or pulmonary insufficiency; long-term administration of cortisones for chronic inflammatory disease or autoimmune disease, duration of the skin lesion <6-months, and post-thrombotic syndrome.

After applying the criteria, 91 patients were selected, described by the following CEAP algorithm:C6s, Ep, As2 3 4 5-p 17 18, Pr. The area of the ulcer was digitally acquired and then assessed by means of software that was able to calculate any irregular area (Visitrak Capture, Smith & Nephew, London, UK). Finally, patients underwent preoperative air plethysmography assessment of venous function.

Surgical treatment. The selected patients underwent hemodynamic correction of superficial venous reflux according to a technique previously reported and validated by a long-term randomized trial of venous ulcer treatment.[11] On admission, the operation was planned for a week later and the patients, while waiting, were immediately treated with compression bandaging. Operations were performed in the Day-Surgery Unit with local anesthesia and required preoperative duplex mapping. Surgical débridement was performed in the same session.

In the case of patients with infected wounds, the antimicrobial susceptibility test was ordered and wide-spectrum antibiotics were administered; they were replaced after 5 days by a specific antibiotic as indicated by the test results.

The primary cover of the ulcer was an advanced dressing (Actisorb plus 25, Johnson & Johnson, Berkshire, UK, or Hyalofil, Fidia Advanced Biopolymers [FAB], Padova Italy). The bandaging was changed every 3 to 5 days during the first month of treatment and then every 7 days. Once the ulcers were healed, the patients were fitted for elastic stockings exerting 20 to 30 mm Hg of compression at the ankle.

Surgical procedure details. Two different minimally invasive techniques were performed, according to the location of the superficial opening of the re-entry perforator vein (RPV). The RPV is defined as a perforator in which finger compression above its opening is able to eliminate the reflux wave in the saphenous trunk. Such an opening can be located either on the long saphenous vein-short saphenous vein main trunk (type I shunt) or on a long

saphenous vein-short saphenous vein tributary (type II shunt). A duplex assessment, which was performed for both procedures, can easily differentiate between the two main hemodynamic presentations.[20,21]

The operation for the type I presentation was a classic high ligation of the saphenofemoral junction or saphenopopliteal junction plus flush ligation and division from saphenous trunk of the insufficient T (Fig 1, *A*). It was performed in 72 cases (79.1%). Patients began to walk 1 hour after the procedure. The ulcer was covered by an advanced dressing, and the patient wore an elastic stocking exerting 20-30 mm Hg of ankle pressure.

The operation for the type II presentation was a flush ligation and division from the saphenous trunk of the insufficient T, which contains the RPV (Fig 1, *B*). The postoperative course was the same as described for the type I shunt.

Measurements. The clinical result assessment was made in a blinded fashion to the genotype of patients and according to the following parameters:

- Healing process. The process of healing was assessed by measuring changes in area of the lesions at the subsequent visits, as described above. The rate of recurrence was also assessed whenever appropriate.
- Duplex scanning. To assess reflux recurrences, complete venous duplex scanning was performed 6 months later with the patient standing and the reflux elicited by calf manual squeezing.
- Venous function. The selected patients underwent air-plethysmography (APG) (ACI Medical, Sun Valley, Calif), an evaluation of total venous volume (VV), venous filling index (VFI), ejection fraction (EF), and residual volume fraction (RVF) preoperatively and 6 months postoperatively at the same hour and temperature condition (between 8:00 AM and 10:00 AM at 23° C).

DNA analysis. Genomic DNA was isolated from peripheral blood by using standard proteinase-K treatment, followed by phenol-chloroform extraction and ethanol precipitation. Polymerase chain reaction was used to genotype samples for *FXIII-V34L*, *FXIII-P564L*, and *HFE-C282Y* gene variants according to previous reports.[24-26] Genotypes were confirmed by re-genotyping a random selection of samples for each polymorphism investigated. No discrepancies were found between genotypes determined in duplicate. Genotyping was done in a blinded fashion relative to the clinical phenotype and the healing progress of patients.

Statistical analysis. The statistical significance for the differences between case groups was performed by Student's *t* test for parametric data and χ^2 for nonparametric data. Where appropriate, the Yates correction or Fisher's exact test was applied. $P \leq .05$ was considered statistically significant. To estimate the risk of having a poor clinical outcome in terms of healing time greater than the median value, hazard risk (HR) and 95% confidence intervals (95% CI) were calculated between different classes of genotypes by means of Cox proportionate hazards modeling, with and without the enclosure of confounding factors such as sex, age, and mutual genotype adjustments. Healing time profiles for cases with different classes of genotype were similarly obtained by Kaplan-Meier analysis, and differences were determined by using a log-rank test. All analyses were performed with Systat 5.0 (Systat Inc, Evanston, Ill) and the SPSS 10.1 statistical software (SPSS Inc, Chicago, Ill).

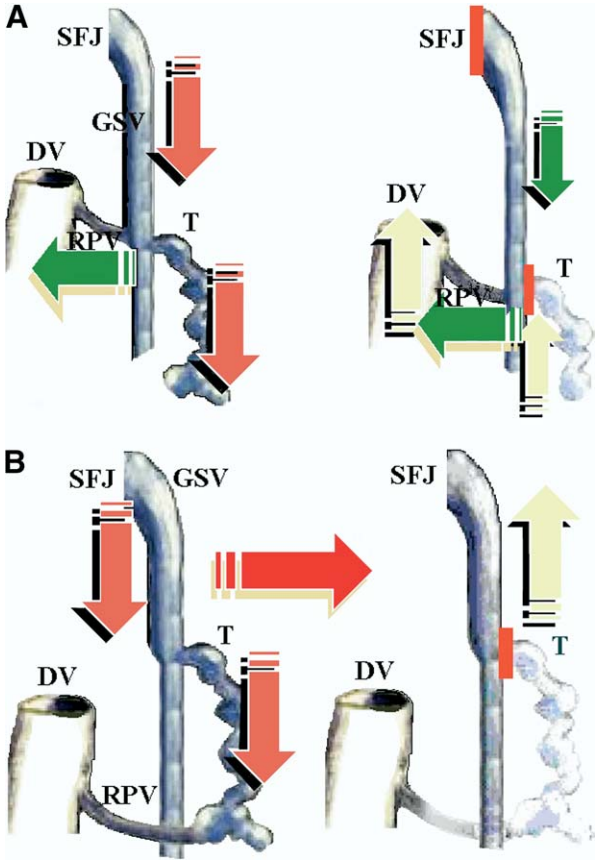

Fig 1. A, Hemodynamic correction procedure performed for type I presentation. *Left panel:* reflux from the saphenofemoral junction *(SFJ)* flows downward along the main saphenous trunk *(GSV)* to the varicose tributary *(T)* and to the re-entry perforating vein *(RPV)*. *Right panel:* operation consisted in flush legation and disconnection of the GSV from the femoral vein, and of the T from the GSV. We eliminated in this way the reflux point with change of compartments from deep to saphenous compartment, and from saphenous compartment to the superficial one, respectively. In the GSV, we so obtained a reverse flow from the junction to the RPV, and a forward flow from the foot to the RPV. Air-plethysmography parameters assessed the effectiveness of the procedure.[20] **B.** Hemodynamic correction procedure performed for type II presentation. *Left panel:* reflux from the SFJ flows downward along the GSV to the T without re-entry perforating vein detectable along the GSV. *Right panel:* operation consisted in flush legation and disconnection of the T from the GSV. In the GSV, we also obtained a forward flow. Air-plethysmography parameters assessed the effectiveness of the procedure.[21]

Table I. Air-plethysmography parameter assessment

APG assessment	*VV mL/air*	*VFI mL/s*	*EF % of VV*	*RVF % of VV*
Pre-op	170 ± 54.6	6.7 ± 3.4	48 ± 12.5	40 ± 15.7
6 months post-op	134 ± 44.1* (−25%)	3.0 ± 51* (−44%)	57.0 ± 18.1	29 ± 15.1* (−31%)

APG, Air-plethysmography; *VV,* total venous volume; *VFI,* venous filling index; *EF,* ejection fraction; *RVF,* residual volume fraction.
Preoperative and 6 months post-operative APG parameters expressed as mean ± SD. In parenthesis are reported the rate of postoperative variations.
*Postoperative parameters significantly different compared with preoperative assessments ($P < .001$).

Table II. Comparison of polymorphism genotype distributions between chronic venous ulcer patients with healing time above and below the median value.

FXIII-V34L		P	*FXIII-P564L*		P	*HFE-C282Y*		P
HT ≥M (n=52)			HT ≥M (n=52)			HT ≥M (n=52)		
VV	36 (69.2)		PP	29 (55.7)		CC	47 (90.4)	
VL	11 (21.1)		PL	21 (40.4)		CY	5 (9.6)	
LL	5 (9.6)		LL	2 (3.8)		YY	0 (0)	
HT < M (n = 39)		<.0001	HT < M (n = 39)		.29	HT < M (n = 39)		.885
VV	12 (30.7)		PP	24 (61.5)		CC	34 (87.2)	
VL	24 (61.5)		PL	11 (28.2)		CY	5 (12.8)	
LL	3 (7.7)		LL	4 (10.2)		YY	0 (0)	

HT, Healing time; *M,* median.
Data are numbers and percentages (%)

RESULTS

Clinical results. The 91 ulcerated lower extremities that were related to a primary and superficial venous disorder had the hemodynamic correction procedure in our Day-Surgery Unit. Patients were discharged ≤3 hours and were seen twice a week for the first week and then weekly until the ulcer was healed. Patient follow-up lasted about 6 months. The healing rate was 100% in the 6-month period.

Duplex assessment. Duplex imaging allowed the correctness of the operations for type I and type II presentations to be assessed according to the schema presented in Fig 1, *A* and *B*.

Venous function. All the APG parameters (VV, VFI, and RVF), with the exception of EF, significantly improved 6 months after the operation, confirming the surgical restoration of venous hemodynamics (Table I).

***FXIII-V34L* and *FXIII-P564L* gene variants and ulcer healing time.** Table II summarizes the genotype distribution of the *FXIII-* and *HFE-* gene polymorphisms stratified above and below the median healing time value of 8 weeks. The *FXIII-V34L* distribution was significantly different ($P < .0001$), accounting for the significant overrepresentation of *L34* carriers found in patients with shorter healing times (27/39, 69.3%) vs those with longer healing times (16/52, 30.7%, $P < .0001$) (Fig 2). In explorative analysis, this yielded an overall crude estimate of the risk to have a delayed healing time of about five times for the *VV34* genotype vs *L34* carriers in our population (HR, 5.06; 95% CI, 2.06 to 12.4; $P < .0001$). When confounding variables and the other gene variants were entered in the Cox regression model, the risk estimate did not change the output (HR, 4.14; 95% CI, 2.08 to 8.23; $P < .0001$).

The evaluation for differences in the healing time profiles for the two classes of *FXIII-V34L* genotype was done by Kaplan-Meier analysis. It showed significant differences and ascribed to the *FXIII-VV* genotype the worst clinical outcome in terms of greater percentage of cases with healing times above the median (log-rank test, $P = .00001$) (Fig 3). No significantly different distributions were found with the *FXIII-P564L* gene variant.

Together with the significant overrepresentation in the number of *L34* carriers found in the group with healing times below the median, a significantly shorter mean healing time was also found in the *L-34* carrier group (ie, 6.2 ± 3.4 weeks vs 10.6 ± 6.5 weeks, $P < .0001$, respectively, for the *L34* and the *VV34* carriers; see Table III). In these two subgroups of patients, we also assessed the dimension of the ulcer area, and as expected, we found that it was significantly smaller in the *L34* carriers than in the *VV34* carriers (6.0 ± 6.2 cm^2 vs 13.1 ± 24.5 cm^2, $P = .029$; respectively; see Table III).

Results with a similar trend were found with the *FXIII-P564L* gene polymorphism but with nonsignificant or borderline values (Table III). In explorative analysis computing merely the *564LL* homozygotes, they had both mean healing time (5.7 ± 1.9 weeks, $P = .005$) and ulcer area (2.83 ± 1.3 cm^2, $P = .003$) significantly lower than that found in the wild types (data not shown).

To minimize the predictable effects of ulcer dimension on healing time and to better assess and define the effective genotype contribution, we considered patients above and below the median healing time as two separate groups. We found significant results only in the subgroup with a larger ulcer area. In particular, patients with

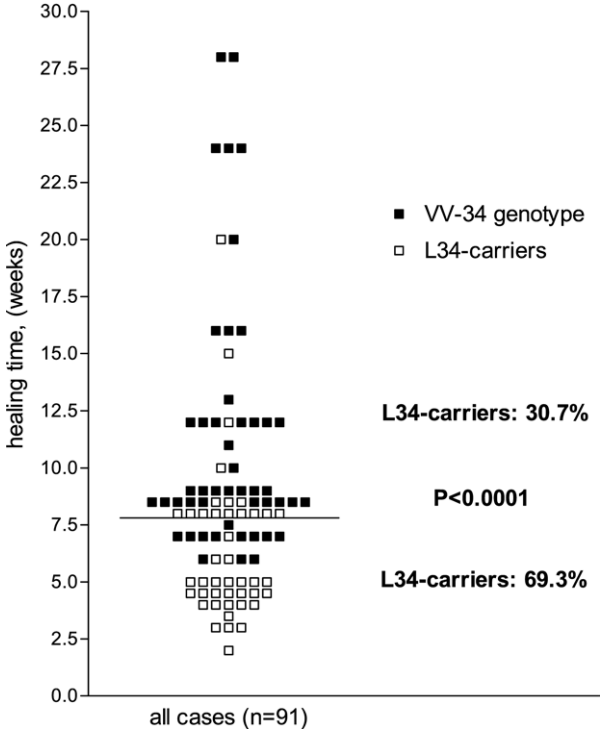

Fig 2. Healing time and *FXIII-V34L* polymorphism distribution in the whole cohort of patients with chronic venous ulcer investigated in the study. *L34* carriers indicate cases with *FXIII-VL34* or *FXIII-LL34* genotype. The line indicates a median healing time of eight weeks. The *P* value indicates the statistical significance between the percentage of *FXIII-L34* carriers in cases with healing time above and below the median.

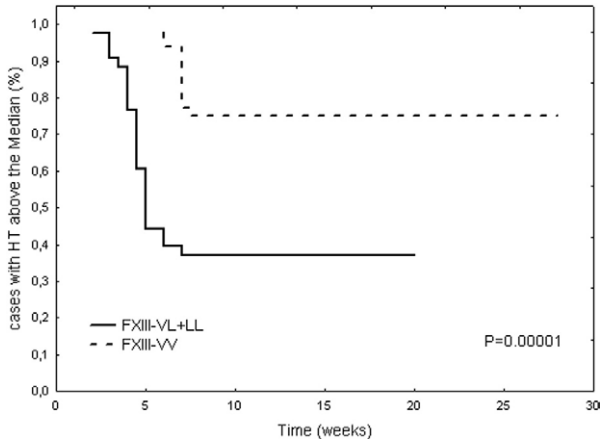

Fig 3. Kaplan-Meier analysis for chronic venous ulcer patients stratified by *FXIII-V34L* genotypes (log-rank test, $P = .00001$).

the *VV34* or *PP564* genotype had the longer healing time (Table IV, top). It is noteworthy that also in the absence of significant differences in mean ulcer size between patients with the *FXIII-V34L* genotype ($P = .08$), patients carrying the *L34* allele had a significantly shorter healing time ($P = .017$; see Table IV, top). With the *FXIII-P564L* variant, borderline significant values were obtained between different genotypes for both mean healing time and ulcer size (Table IV, top). Conversely, absolutely no significant results or trend were obtained in the group with smaller ulcer area (Table IV, bottom). Fig 4 clearly summarizes the relationships between mean healing time and different *FXIII* genotypes.

In addition, to investigate possible combined effects on healing time of the two *FXIII* gene polymorphisms, the 18 subjects carrying both gene variants were compared with the 28 subjects null for both the polymorphic alleles. The different healing time distributions of the two subgroups of cases are shown in Fig 5, with significantly shorter mean healing times found in double carriers compared with wild types (5.8 ± 2.1 weeks vs 10.9 ± 6.9 weeks, $P < .0001$).

***HFE-C282Y* gene variant and ulcer healing time.** The genotype distribution of the *HFE* gene polymorphism stratified above and below the median healing time was not significantly different (Table II). Similarly, the mean healing time and the mean ulcer area were both nonsignificantly different comparing *HFE-C282Y* carriers vs noncarriers in the whole group of cases, as well as in the subset groups that were analyzed (data not shown). Finally, stratifying these cases by *FXIII-V34L* genotypes, the *L34* carriers conserved the significative shorter healing time, irrespective of the *HFE-C282Y* genotype (Table V).

DISCUSSION

Many of the limitations in the treatment of CVU are due to the lack of knowledge of molecular wound healing mechanisms. To identify a number of independent inherited or required risk factors responsible for chronic wound pathogenesis is an essential step for the diagnosis and prevention of such a complex disease and for the design of new treatment strategies as alternatives or ancillary to the standard procedures. Apart from CVD, increasing age and female gender,[2-4] clear evidences for genetic predisposition to CVU are still limited. Candidate genes may be those involved in inflammatory processes, fibroblast growth factors, angiogenesis, or apoptosis. Very few data on single nucleotide polymorphisms and CVU are available in the literature.[27-29] We recently recognized that the common single nucleotide polymorphisms *HFE-C282Y* and *FXIII-V34L* have a role, respectively, in the risk of venous ulceration in primary CVD and in the modulation of the lesion size of CVU patients.[14-16]

The main finding of our study was that among our cohort of patients affected by primary CVU who underwent superficial venous surgery, the *FXIII-VV34* genotype was a predictor for poor clinical outcome in terms of longer healing time, and confirmed also in combination with the *FXIII-PP564* genotype. In explorative monovariate analysis, the risk was about five times (odds ratio, 5.0; 95% CI, 2.06 to 12.4) for *VV34* patients, and it also remained significant in multivariate analysis (HR, 4.14; 95% CI, 2.08 to 8.23).

Table III. Healing time and ulcer area dimension in the whole cohort of cases stratified by *FXIII* genotypes

FXIII Variant	*FXIII genotype*	*n*	*HT weeks*	*Ulcer area (cm²)*	P*	P†
FXIII-V34L	VV	48	10.6 ± 6.5	13.1 ± 24.5	<.0001	0.029
	VL + LL	43	6.2 ± 3.4	6.0 ± 6.2		
FXIII-P564L	PP	53	8.9 ± 6.2	12.0 ± 23.4	0.19	0.064
	PL + LL	38	7.8 ± 4.7	6.7 ± 7.0		
Total		91	8.5 ± 5.7	9.7 ± 18.5		

Data are mean ± SD.
Note: the mean HT (5.7 ± 1.9 weeks) and mean ulcer area (2.83 ± 1.3 cm²) of the *564LL* homozygotes were both significantly lower than those found in the wild types ($P = .005$ and $P = .003$, respectively).
*Healing time *(HT)* comparison.
†Ulcer area comparison.

Table IV. Healing time and ulcer area dimension in cases with healing time above and below the median value stratified by *FXIII* genotypes

FXIII variant	*FXIII genotype*	*n*	*HT weeks*	*Ulcer area (cm²)*	P*	P†
HT ≥ median value (8.0 weeks)						
FXIII-V34L	VV	36	12.5 ± 6.2	16.5 ± 21.7	0.017	0.08
	VL + LL	16	9.5 ± 3.4	9.6 ± 8.3		
FXIII-P564L	PP	29	12.7 ± 6.0	19.0 ± 30.2	0.051	0.043
	PL + LL	23	10.2 ± 4.9	8.9 ± 8.6		
Total		52	11.6 ± 5.6	14.4 ± 23.5		
HT < median value (8.0 weeks)						
FXIII-V34L	VV	12	4.3 ± 0.7	4.1 ± 3.2	0.33	0.23
	VL + LL	27	4.0 ± 0.1	3.4 ± 1.7		
FXIIIP-564L	PP	24	4.0 ± 0.4	3.6 ± 2.5	0.37	0.42
	PL + LL	15	4.1 ± 0.5	3.7 ± 1.9		
Total		39	4.1 ± 0.5	3.61 ± 2.3		

Data are mean ± SD.
*Healing time *(HT)* comparison.
†Ulcer area comparison.

We also found that those patients had a larger wound area, a risk factor previously described as being associated with failure of wounds to heal.[13] In that study, authors observed that the failure of a wound to heal ≤24 weeks of limb compression was associated with the initial area of the lesion along with other risk factors. This could partly affect our findings. However, the clearly significant shorter healing time ($P = .017$) in the cohort of our patients who were *L34* carriers, also in the presence of nonsignificant different mean wound areas between the opposite genotypes ($P = .08$), improves our outcome.

On the other hand, the protective role of this gene marker was evident only in the larger lesions, which could partly be explained by the coexistence in the smaller wounds of whole constellations of stronger and unknown protective factors or lack stronger risk factors. Both of these situations might simply overcome the gene marker role. Conversely, larger wounds might possess biologic agents that may inhibit wound healing but also prevent systemic infections, and thus, the individual escapes systematic illness.[13] All of this could allow the lesion to progress and the gene to fully express its action. Basically, this may have a clinical sense considering that a complete linear relationship does not exist between wound area and healing failure. The magnitude of the nonhealing risk initially increases rapidly for large wounds, but it diminishes with the largest ones.[13]

Also of importance is that all of the patients in our survey were completely hemodynamically corrected by surgery. Therefore, the essential but insufficient factor for skin lesion establishment was eliminated. This could account in part for the following objection: why a genetic factor (congenital by definition) should be protective (ie, *FXIII-V34L* on healing time) only after a lesion becomes larger and not prevent it from happening in the first place? Similarly, one could ask why a gene risk factor for ulcer establishment (ie, *HFE-C282Y*) did not affect healing time or lesion size in our survey of postoperative patients?

One answer can well explain both objections: once superficial venous reflux was corrected and a normal circulation was restored, many proliferating factors can reach the débrided wound. Among these, *FXIII* strengthens the extracellular matrix against unrestrained matrix metalloproteases proteolysis, favoring in turn fibroblast migration and proliferation and promoting neoangiogenesis and healing.[15,30-34] The quality and degree of these actions may be strictly dependent on the associated gene variants.[16] It could be hypothesized that a significantly higher *FXIII* activity (ie, that of the *L34*

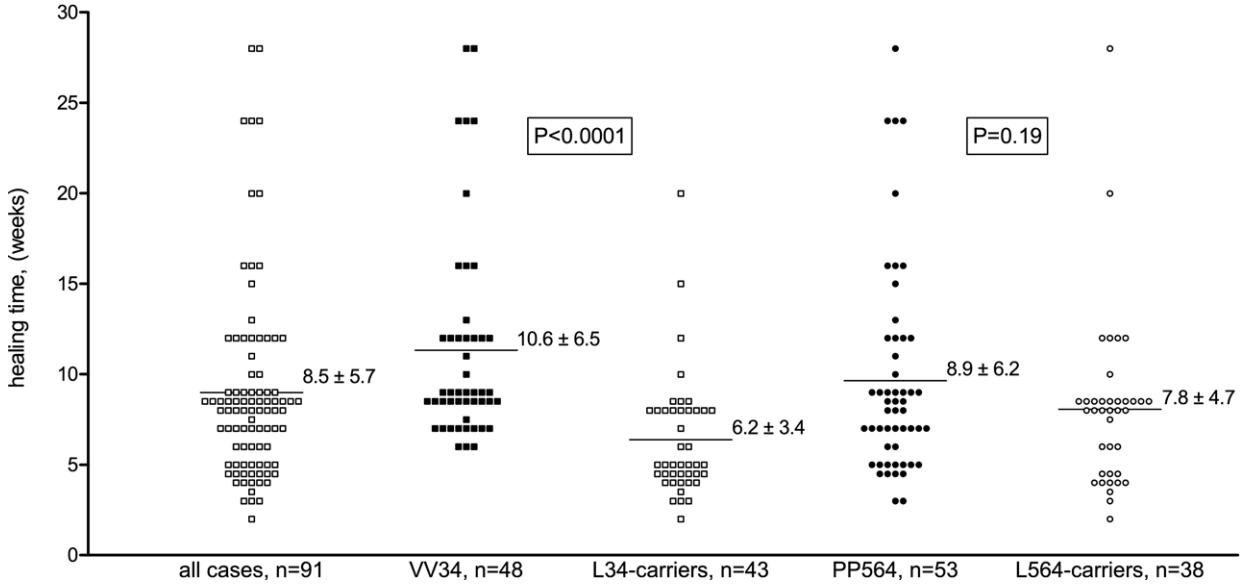

Fig 4. Relationships between mean healing time and different *FXIII* genotypes. The whole cohort of patients with chronic venous ulcers is shown and the subgroups carrying the *FXIII-V34L* and *FXIII-P564L* genotypes. *L34* carriers indicate patients with *FXIII-VL34* or *FXIII-LL34* genotype, and *L564* carriers indicate patients with the *FXIII-PL564* or *FXIII-LL564* genotype. The lines and values shown indicate the different mean healing times and standard deviation. *P* values indicate the statistical significance between mean healing times for each specific *FXIII* genotype.

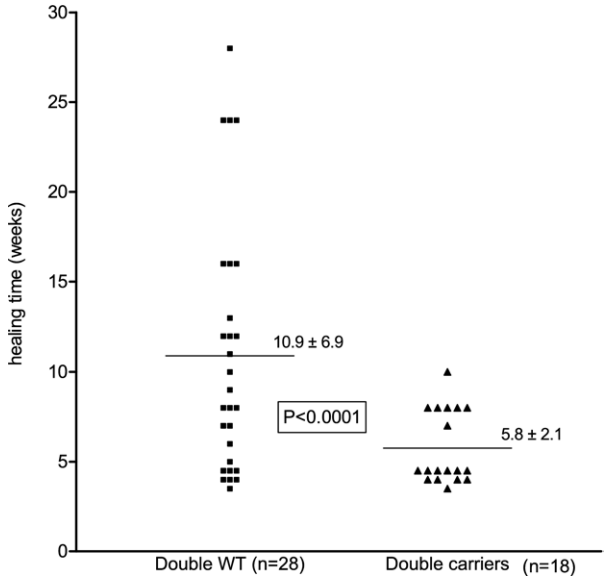

Fig 5. Healing time distribution in two subgroups of patients with chronic venous ulcers carrying the *FXIII-VV34/PP564* double wild type *(WT)* genotype, and the *FXIII-VL/LL34+ PL/LL564* genotype (double carriers), respectively. The line and value shown indicate the different mean healing times and standard deviation. The P value indicates the statistical significance between the two mean healing times.

variant) at the wound site could better promote wound healing by improving the above-mentioned tasks and thus shortening the wound closure time. Contextually, a restored venous drainage gets rid of local iron overload,

Table V. Combined effects of *HFE-C282Y* and *FXIII-V34L* polymorphism on ulcer healing time

HFEC282Y *(n = 91)*	*FXIII-V34L healing time (weeks)*		P
	VV	*VL+LL*	
CC	10.1 ± 6.2	6.44 ± 3.9	.0014
n = 81 (89%)	n = 45	n = 36	
CY	14.6 ± 10	7.4 ± 5.8	.08
n = 10 (11%)	n = 3	n = 7	

drastically lowering the possibility that *HFE*-mutated phagocytes, losing the ability to counteract the intracellular iron export, generate locally free iron and free radicals and leading to matrix breakdown and skin lesions.[14,15,35] This would avoid both the detrimental effects of free radicals and hyper-proteolysis on extracellular matrix components contrasting, in turn, cell death.[15,31]

CONCLUSIONS

Although the number of actors involved in the etiology of chronic wounds is extremely high and the above explanations may just partly account for the complex etiology of CVU, our study furnishes a good example of gene-gene and gene-environment interactions for this multifactorial disorder. Our results, for the first time, could pave the way to single out in advance categories of patients at increased risk and to address tailored strate-

gies of treatment, such as priority for surgical correction of superficial venous reflux.

Future multicenter molecular investigations could identify groups of molecular markers responsible for chronic inhibition of a wound to heal, allowing clinicians the opportunity to develop tailored therapies, design prevention programs, and target specific surgical interventions in the view of a new and alternative concept of treatment based on pharmacogenomics.

AUTHOR CONTRIBUTIONS

Conception and design: DG, PZ
Analysis and interpretation: DG, ST, AL, PZ
Data collection: ST, LC, FF, MdP, SG, GS, MdM, AL, PZ
Writing the article: DG, PZ
Critical revision of the article: DG, ST, LC, FF, MdP, SG, GS, MdM, AL, PZ
Final approval of the article: DG, ST, LC, FF, MdP, SG, GS, MdM, AL, PZ
Statistical analysis: DG, ST
Obtained funding: PZ

REFERENCES

1. Bergqvist D, Lindholm C, Nelzen O. Chronic leg ulcers: the impact of venous disease. J Vasc Surg 1999;29:752-5.
2. Abbade LP, Lastoria S. Venous ulcer: epidemiology, physiopathology, diagnosis and treatment. Int J Dermatol 2005;44:449-56.
3. Abbade LP, Lastoria S, de Almeida Rollo H, Stolf HO. A sociodemographic, clinical study of patients with venous ulcer. Int J Dermatol 2005;44:989-92.
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. Nelzen O. Leg ulcers: economic aspects. Phlebology 2000;15:110-4.
6. van Geest AJ, Veraart JC, Nelemans P, Neumann HA. The effect of medical elastic compression stockings with different slope values on edema. Measurements underneath three different types of stockings. Dermatol Surg 2000;26:244-7.
7. De Palma RG, Kowallek DL. Venous ulceration: a cross-over study from nonoperative to operative treatment. J Vasc Surg 1996;24:788-92.
8. Barwell JR, Taylor M, Deacon J, Ghauri AS, Wakely C, Phillips LK, et al. Surgical correction of isolated superficial venous reflux reduces long-term recurrence rate in chronic venous leg ulcers. Eur J Vasc Endovasc Surg 2000;20:363-8.
9. Barwell JR, Davies CE, Deacon J, Harvey K, Minor J, Sassano A, et al. Comparison of surgery and compression with compression alone in chronic venous ulceration (ESCHAR study): randomised controlled trial. Lancet 2004;363:1854-9.
10. Tenbrook JA Jr, Iafrati MD, O'Donnell TF Jr, Wolf MP, Hoffman SN, Pauker SG, et al. Systematic review of outcomes after surgical management of venous disease incorporating subfascial endoscopic perforator surgery. J Vasc Surg 2004;39:583-9.
11. 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 randomized clinical trial. Eur J Vasc Endovasc Surg 2003;25:313-8.
12. Gohel MS, Taylor M, Earnshaw JJ, Heather BP, Poskitt KR, Whyman MR. Risk factors for delayed healing and recurrence of chronic venous leg ulcers—an analysis of 1324 legs. Eur J Vasc Endovasc Surg 2005; 29:74-7.
13. Margolis DJ, Berlin JA, Strom BL. Risk factors associated with the failure of a venous ulcer to heal. Arch Dermatol 1999;135:920-6.
14. Zamboni P, Tognazzo S, Izzo M, Pancaldi F, Scapoli GL, Liboni A, Gemmati D. Hemochromatosis C282Y gene mutation increases the risk of venous leg ulceration. J Vasc Surg 2005;42:309-14.
15. Zamboni P, Izzo M, Tognazzo S, Carandina S, De Palma Massimiliano, et al. The overlapping of local iron overload and HFE mutation in venous leg ulcer pathogenesis. Free Radic Biol Med 2006;40: 1869-73.
16. Gemmati D, Tognazzo S, Serino ML, Fogato L, Carandina S, De Palma M, et al. Factor XIII V34L polymorphism modulates the risk of chronic venous leg ulcer progression and extension. Wound Repair Regen 2004;12:512-7.
17. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G →A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-8.
18. Inbal A, Lubetsky A, Krapp T, Castel D, Shaish A, Dickneite G, et al. Impaired wound healing in factor XIII deficient mice. Thromb Haemost. 2005;94:432-7.
19. Bereczky Z, Katona E, Muszbek L. Fibrin stabilization (factor XIII), fibrin structure and thrombosis. Pathophysiol Haemost Thromb. 2003-2004;33:430-7.
20. Zamboni P, Marcellino MG, Cappelli M, Feo CV, Bresadola V, Vasquez G, et al. Saphenous vein sparing surgery: principles, techniques and results. J Cardiovasc Surg 1998;39:151-62.
21. Zamboni P, Cisno C, Marchetti F, Quaglio D, Mazza P, Liboni A. Reflux elimination without any ablation or disconnection of the saphenous vein. A haemodynamic model for venous surgery. Eur J Vasc Endovasc Surg 2001;21:361-9.
22. Nicolaides AN. Investigation of chronic venous insufficiency: a consensus statement (France, March 5-9, 1997). Circulation 2000;102: 126-63.
23. 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.
24. Gemmati D, Serino ML, Ongaro A, Tognazzo S, Moratelli S, Resca R, et al. A common mutation in the gene for coagulation factor XIII-A (Val34Leu): a risk factor for primary intracerebral hemorrhage is protective against atherothrombotic diseases. Am J Hematol 2001;67: 183-8.
25. Kohler HP, Futers TS, Grant PJ. Prevalence of three common polymorphisms in the A-subunit gene of factor XIII in patients with coronary artery disease. Thromb Haemost 1999;81:511-5.
26. 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.
27. Ashworth JJ, Smyth JV, Pendleton N, Horan M, Payton A, Worthington J, et al. The dinucleotide (CA) repeat polymorphism of estrogen receptor beta but not the dinucleotide (TA) repeat polymorphism of estrogen receptor alpha is associated with venous ulceration. J Steroid Biochem Mol Biol 2005;97:266-70.
28. Nagy N, Szolnoky G, Szabad G, Bata-Csorgo Z, Dobozy A, Kemeny L, et al. Single nucleotide polymorphisms of the fibroblast growth factor receptor 2 gene in patients with chronic venous insufficiency with leg ulcer. J Invest Dermatol 2005;124:1085-8.
29. Wallace HJ, Vandongen YK, Stacey MC. Tumor necrosis factor-alpha gene polymorphism associated with increased susceptibility to venous leg ulceration. J Invest Dermatol 2006;126:921-5.
30. Zamboni P, De Mattei M, Ongaro A, Fogato L, Carandina S, De Palma M, et al. Factor XIII contrasts the effects of metallo-proteinases in human dermal fibroblast cultured cells. Vasc Endovascular Surg 2004; 38:431-8.
31. Zamboni P, Scapoli G, Lanzara V, Izzo M, Fortini P, Legnaro R, et al. Serum iron and matrix metalloproteinase-9 variations in limbs affected by chronic venous disease and venous leg ulcers. Dermatol Surg 2005; 31:644-9.
32. Dardik R, Loscalzo J, Inbal A. Factor XIII (FXIII) and angiogenesis. J Thromb Haemost 2006;4:19-25.
33. Dardik R, Loscalzo J, Eskaraev R, Inbal A. Molecular mechanisms underlying the proangiogenic effect of factor XIII. Arterioscler Thromb Vasc Biol 2005;25:526-32.

34. Chung SI, Lee SY, Ryogin U, Kamemitsu K. Affects of F XIII in wound-healing—Fibrin stability in tissues and cross linking of angiogenesis modulator, osteonectin to fibrin. Rinsho Byori 1997;Suppl 104:50.
35. Moura E, Noordermeer MA, Verhoeven N, Verheul AF, Marx JJ. Iron release from human monocytes after erythrophagocytosis in vitro: an investigation in normal subjects and hereditary hemochromatosis patients. Blood 1998;92:2511-9.

Submitted Feb 22, 2006; accepted May 9, 2006.