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Research Article

Chronic Nonhealing Wounds: Could Leg Ulcers Be Hereditary?

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Background. A number of well-known acquired and putative inherited etiological factors contribute to the development of venous leg ulcer (VLU). *Aim*. In this study we set out to perform a meta-analysis of putative genetic and acquired factors predisposing to VLU development. *Methods*. VLU patients ($n = 157$) were divided into three subgroups in accordance with their acquired etiological factors. The frequencies of four genetic factors were determined: the R506Q (Leiden) mutation of the F5 gene, the G20210A mutation of the F2 (prothrombin) gene, the 2451 A/G SNP of the fibroblast growth factor receptor 2 (FGFR2) 3′ UTR, and the −308 G/A SNP of the tumor necrosis factor α (TNFA) promoter. *Results*. The −308 TNFA SNP exhibited a higher frequency among VLU patients without known acquired predisposing factor in their history, than among patients with thrombosis or soft tissue infection in their history (Fisher $P = 0.0173$). *Conclusions*. This study has demonstrated that the group of VLU patients is heterogeneous in their genetic predisposing factors. Further large-scale studies are needed to delineate the associations among genetic and acquired etiological factors with regard to VLU development and to integrate the consequences of the already known genetic factors to the management of VLU.

1. Introduction

Venous leg ulcer (VLU) is multifactorial disease with well-known acquired and putative inherited predisposing factors [1–15]. Besides the characteristic acquired etiological factors, such as venous insufficiency, obesity, and deep vein thrombosis, case-control studies suggest putative inherited etiological factors, which may also contribute to the mechanism of delayed or pathological wound healing and hence to the development of leg ulcer. A delineation of the genetic susceptibility factors relating to pathological wound healing would therefore promote a better understanding of the molecular background of VLU and that could provide opportunities for developing causative treatment of therapy-resistant forms [1, 2].

The difficulties involved in such investigations are increased by the fact that these inherited factors form a complex multifactorial genetic background which does not follow the rules of Mendelien inheritance. Moreover, each genetic component contributes differently to the pathogenesis of VLU, and assessment of its individual relevance in the development of the disease is difficult. To investigate the putative genetic factors and to minimize statistical bias, we set out to form subgroups of VLU patients which were homogeneous in their clinical characteristics and to perform a meta-analysis of four genetic factors within the subgroups.

2. Methods

One hundred and fifty-seven VLU patients with therapy-resistant nonhealing VLU have been enrolled into the study. Diabetes and arterial leg ulcer were exclusion criteria. The female (48.41%) : male (51.59%) ratio was close to 1 : 1. The average duration of the VLU was 5.84 ± 5.12 years. The clinically relevant parameters and the clinically homogeneous subgroups of VLU patients are shown in Table 1.

Table 1: Clinical characteristics and subgroups of VLU patients.

Clinical characteristics of VLU patients (n = 157)		
Cardiac disease (49.04%, n = 77)		
Soft tissue infection (47.13%, n = 74)		
Deep vein thrombosis (29.94%, n = 47)		
Leg fracture (22.93%, n = 36)		
Atherosclerosis (20.38%, n = 32)		
Autoimmune disease (5.10%, n = 8)		
Subgroups of VLU patients		
	Leg fracture	Deep vein thrombosis or soft tissue infection
Group A (n = 72)	−	−
Group B (n = 33)	+	−
Group C (n = 52)	−	+

The frequency and putative interactions of several previously determined genetic factors (the R506Q [Leiden] mutation of the F5 gene, the G20210A mutation of the F2 [prothrombin] gene, the 2451 A/G SNP of the FGFR2 3′ UTR, and the −308 G/A SNP of the TNFA promoter) were earlier assessed in VLU patients [3–6]. The analysis was based on previous results of genotyping performed by either PCR-RFLP or PCR TaqMan methods [3–6]. Chi^2 tests and multinomial regression analyses performed by SPSS were used to determine frequency and genetic interactions.

The investigation was approved by the Internal Review Board of the University of Szeged. Written informed consent was obtained from all donors, and the study was conducted according to the Principles of the Declaration of Helsinki.

3. Results

The R506Q mutation of the F5 gene was detected in heterozygous form in 11 patients with an overall frequency of 7.85%, demonstrating a nonsignificant, higher presentation in group A and group C than in group B (data not shown). The G20210A mutation of the F2 gene occurred in only 3 patients in heterozygous form; all the others carried the wild-type allele (data not shown).

The distributions of the rare genotypes (AG and GG) of the FGFR2 gene polymorphism (2451A/G SNP at the 3′UTR) were highest in group A (ratio of homozygous mutants 18.84%, rare allele frequency [MAF] = 0.4638) and lowest in group B (ratio of homozygous rare alleles 8.82%, MAF = 0.3676, Fisher exact probability test P = 0.1227, Odds ratio 1.4876, CI 0.8804–1.8075; Figure 1). We have previously reported that the FGFR2 3′UTR 2451A/G polymorphism is associated with VLU [5], and the present analysis revealed a similar distribution in the various subgroups of VLU patients, suggesting an overall susceptibility role for this polymorphism in the development of the disease.

The −308 G/A SNP of the TNFA promoter likewise exhibited the highest frequency in group A (ratio of homozygous rare alleles 5.8%, MAF = 0.2246), while in groups B and C homozygous rare genotype was not detected; only the heterozygous rare genotype was present (group B MAF = 0.1765, group C, MAF = 0.1087; group A versus group B, P = 0.2711, odds ratio 1.352, CI 0.6988–2.3189; group A versus group C, P = 0.0173, odds ratio 2.3757, CI 1.0658–4.0073). It was previously demonstrated that the −308 A/G SNP of the promoter region of the TNFA gene is a factor predisposing to VLU development [6, 7]. Our present data indicate that the homozygous rare genotype of the −308 TNFA SNP occurred significantly more frequently among VLU patients without additional acquired predisposing factors in their history (group A: no thrombosis, fracture, or soft tissue infection) than among patients with other known etiological events in their history (group C: patients with previous thrombosis or soft tissue infection; group A versus group C Fisher exact probability test P = 0.0173; Figure 1). Previously we have reported that the −308 G/A SNP of the TNFA promoter is associated with VLU development in obese patients [6]. In the present study, the ratio of obese patients did not show significant difference within the subgroups of the VLU patients. In accordance with our previous results, the highest ratio (38%) was observed in group A, in which the −308 G/A SNP of the TNFA promoter also exhibited the highest frequency.

Our meta-analysis included an assessment of putative genetic interactions using the multinomial regression method. The R506Q mutation of the F5 gene and the G20210A mutation of the F2 gene were excluded from this analysis because of their low allele frequency. No interaction was found between the 2451 A/G SNP of the FGFR2 gene and the −308 G/A SNP of the TNFA gene. The 2451 A/G SNP of the FGFR2 gene proved to be a significantly (5-fold) stronger

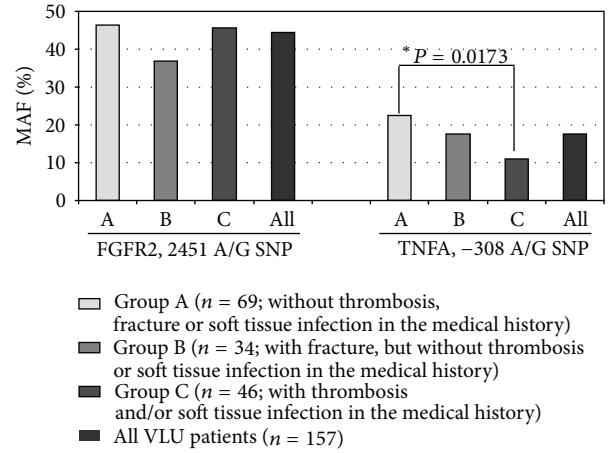

Figure 1: Rare allele frequencies of the 2451 A/G SNP of FGFR2 and the −308 A/G SNP of TNFA in the subgroups of VLU patients. The FGFR2 3′UTR 2451A/G polymorphism exhibited similar distributions among the subgroups of VLU patients, suggesting an overall role of susceptibility in the disease development. Our data also demonstrated that the homozygous rare allele of the −308 TNFA SNP occurred significantly higher among VLU patients without additional acquired predisposing factors in their history (group A) than among patients with other known etiological events in their history (group C; group A versus group C Fisher exact probability test, P = 0.0173).

Table 2: Putative genetic factors predisposing to VLU development.

	Detected genetic abnormality	Population	Author	Journal	Year
(1)	F5 gene R506Q (Leiden)*	German	Peus et al.	J Am Acad Dermatol	1996
(2)	F2 gene G20210A*	Romanian	Jebeleanu et al.	J Cell Mol Med	2001
(3)	F13A gene V34L	Italian	Gemmati et al.	Wound Repair Regen	2004
(4)	FGFR2 gene 3′ UTR A2451G*	Hungarian	Nagy et al.	J Invest Dermatol	2005
(5)	ESRB gene CA repeat D14S1026	UK	Ashworth et al.	J Steroid Biochem Mol Biol	2005
(6)	HFE gene C282Y	Italian	Zamboni et al.	J Vasc Surg	2005
(7)	TNFA gene promoter −308*	Australian	Wallace et al.	J Invest Dermatol	2006
		Hungarian	Nagy et al.	J Invest Dermatol	2007
(8)	FPN1 gene promoter −8GG	Italian	Gemmati et al.	J Vasc Surg	2009
(9)	MMP12 gene promoter −82AA	Italian	Gemmati et al.	J Vasc Surg	2009
(10)	Sex chromosome aberrations (47,XXY/48,XXXY karyotype)	Austrian	Gattringer et al.	Acta Derm Venereol	2010

* The distributions of the genotypes and the allele frequencies of these genetic factors were compared in the present study.

susceptibility factor than the −308 G/A SNP of the TNFA gene.

4. Discussion

Up to now little is known about the genetic background of VLU; however there have been several papers published in this topic. The first report on the genetic backgrounds of VLU was on the Leiden and the prothrombin gene mutation; the first findings demonstrated their association with venous thrombosis and later with postthrombotic leg ulcer development [3, 8]. The FGFR2 gene encodes keratinocyte growth factor receptor involved in the proliferation of keratinocytes and wound healing, while the TNFA gene encodes a well-known proinflammatory cytokine. The investigated SNPs of the FGFR2 and TNFA genes were previously proved to be associated with VLU [5, 6].

Other genetic factors—not investigated in this study—have been also reported to be associated with VLU (Table 2). The V34L SNP of the F13A gene was proved to be associated with the progression of VLU due to its direct effect on the activity of F13 [9]. Estrogen is a well-known accelerator of wound healing by dampening the inflammatory response; a common variant of its receptor (ESRB) increases the risk of VLU development [10]. The C282Y SNP of the HFE gene increases the risk of VLU by affecting iron protective mechanisms [11]. A DNA-array reported by Gemmati et al. (2009) revealed that the −82 A/G SNP of the MMP12 and the −8 G/C SNP of the FPN1 genes are also associated with VLU [12]. Moreover, chromosomal abnormalities have also been found in VLU patients with unusual early onset [16].

The aim of this study was to assess the relevance of already known genetic factors and their interactions in VLU development in clinically homogeneous subgroups of patients. Deep vein thrombosis, soft tissue infection, and leg fracture frequently found clinical characteristics among VLU patients, were suitable for the creation of clinically homogeneous subgroups within our study population. Cardiac disease was also frequent, but displayed a very similar distribution in the VLU patient subgroups. Of the four investigated genetic factors, the 2451 A/G SNP of the FGFR2 gene proved most relevant.

Our data further emphasize the importance of clinically homogeneous subgroups of patients for the analysis of putative genetic factors in order to assess mutual relevance, to create hierarchy, and to measure potential interactions. Further larger-scale studies are needed to assess the contributions of different putative genetic factors to the variable appearance of VLU phenotypes. Such analyses could hold the key to the understanding of VLU development. They might also serve a crucial role in the development of future causative treatment strategies through the creation of cost-effective investigation techniques for routine diagnostic assessment of putative genetic factors and causative treatment options.

Conflict of Interests

The authors have declared no conflicting interests.

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