

# Human Parvovirus B19 (B19V) Infection in Systemic Sclerosis Patients

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## Key Words

Parvovirus B19 · Erythrovirus genotype 1 and 2 persistence · Systemic sclerosis

DNA, in the simultaneous persistence of 2 genotypes and in the pattern of viral expression among SSC patients and controls.

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## Abstract

**Background:** Our previous reports suggested a possible association between parvovirus B19 (B19V) infection and systemic sclerosis (SSc), based on higher prevalence of B19V DNA in SSc patients in respect to controls. **Methods:** In the present study, to further evaluate the differences in the pattern of B19 infection in SSc, skin biopsies and bone marrow samples from patients and controls were analysed for B19V DNA detection, genotyping and viral expression. **Results:** B19V DNA was detected in skin biopsies from 39/49 SSc patients and from 20/28 controls. Bone marrow showed positive in 17/29 SSc patients, 5/21 haematological patients and 0/10 healthy controls. Genotype 1 was more frequent in skin and bone marrow from patients than from controls. Simultaneous persistence of 2 genotypes was detected in SSc skin and bone marrow samples, never in controls. Viral mRNA for capsid protein was detected in the skin of genotype 1-positive patients and not in control skins. **Conclusion:** The results outline some differences in the rate of persistence of B19V

Human parvovirus B19 (B19V) is a member of the erythrovirus genus known to cause a variety of diseases including erythema infectiosum (fifth disease of childhood), acute or chronic arthropathies, aplastic crisis in patients with chronic haemolytic anaemias and non-immune hydrops foetalis as a consequence of transplacental infection during pregnancy. B19 virus may cause chronic infections with persistent DNA in either immunocompromised or in apparently immunocompetent individuals [1–4]. In these last hosts, the clinical consequences of such persistence have not yet been clarified [1, 5].

Recently, it has been shown that the genetic diversity of B19V is higher than previously expected, resulting in the subdivision of the species into 3 distinct genotypes [6]. B19-related viruses were classified as genotype 1 (prototype strain Au) [7], K71-related as genotype 2 (prototype strain LaLi) [8] and V9-related as genotype 3 (pro-

**Table 1.** Detection of genotype 1 and 2 DNA in skin and bone marrow samples from parvovirus B19 antibody-positive individuals

| Geno-<br>type | Skin              |                | Bone marrow       |                |                |
|---------------|-------------------|----------------|-------------------|----------------|----------------|
|               | SSc P<br>(n = 49) | HC<br>(n = 28) | SSc P<br>(n = 29) | HC<br>(n = 10) | HP<br>(n = 21) |
| 1             | 18 (37%)          | 9 (32%)        | 5 (17%)           | 0              | 2 (10%)        |
| 2             | 11 (22%)          | 11 (39%)       | 3 (10%)           | 0              | 3 (14%)        |
| 1+2           | 10 (20%)          | 0              | 9 (31%)           | 0              | 0              |
| Total         | 39 (80%)          | 20 (71%)       | 17 (59%)          | 0              | 5 (24%)        |

SScP = SSc patients; HC = healthy controls; HP = haematological patients.

totype strain V9) [9]. Little is known about their distribution and pathogenetic significance. Genotype 2 DNA has been demonstrated in skin biopsies, synovia, tonsils, liver [10] and bone marrow samples [11]. Genotype 3 has been reported mainly in peripheral blood from Ghanaian blood donors [12] and in bone marrow samples from Brazilian haematological patients [11] while seldom in European blood samples [6, 10].

Our previous reports suggested a possible association of B19 persistent infection and systemic sclerosis (SSc) based on a high prevalence of B19V DNA persistence in skin and bone marrow of SSc patients in respect to controls [13–15]. The PCR used in these earlier studies to detect viral DNA was, however, limited to amplify genotype 1. In this study, bone marrow and skin biopsies, collected in Italy between 1999 and 2007, were analysed to assess the prevalence of genotypes 1, 2 and 3 in SSc patients and a control group. The viral load and the mRNAs expression pattern in the infected tissues were also evaluated. Screening of clinical samples for the presence and typing of B19V DNA was performed using 2 consensus PCR assays followed by RFLP analysis of the amplification products and by 2 type-specific PCRs.

A first group of specimens comprised 77 skin biopsies: 49 from patients with SSc from the Rheumatology Unit and 28 derived from healthy adults. The skin biopsies from SSc patients were taken from diseased skin regions. A second group consisted of 60 osteomedullar biopsies: 29 from SSc patients from the Rheumatology Unit, 10 from healthy donors and 21 from haematological immunodepressed patients. The mean age of the skin biopsies subjects was 52 years (range 20–70, median 54) for the SSc patients and 55 years (range 30–70, median 56) for the

control group. The mean age of the bone marrow subjects was 50 years (range 18–70, median 52) for the SSc patients and 52 years (range 22–69, median 52) for the control group. The male:female ratio for SSc patients was 0.17, whereas for controls it was 0.86. Tissue biopsies were collected from 1999 to 2007 in Italy from anti-B19V IgG-positive and IgM-negative individuals. Some patients and controls from both groups have been previously described [4, 13].

Total DNA and RNA were extracted from clinical samples using TriPure Isolation Reagent (Roche) according to the protocol of the manufacturer. All samples tested positive for  $\beta$ -globin gene [16], ensuring the presence of intact DNA material and the absence of Taq DNA polymerase inhibitors. For B19V detection and genotyping, all samples were analysed by 2 consensus PCRs followed by RFLP of amplification products [6] and by 2 genotype-specific PCRs as previously described [4]. Real-time PCR was performed as previously reported [4].

RT-PCR was used to detect spliced parvovirus mRNA species for the structural protein VP1, as previously described [17], with the following modifications: (1) the reverse transcription was performed using the QuantiTect Reverse Transcription Kit (Qiagen), (2) for PCR the anti-sense primer was slightly modified (5' CCA CGA TGC AGC TAC AAC TT 3') and 45 cycles of amplification were carried out, using TaqGold DNA polymerase (Applied Biosystems). All RNA samples tested positive for  $\beta$ -actin RT-PCR.

Table 1 shows the distribution of B19V genotypes in skin and bone marrow from SSc patients and controls. B19V DNA was detected in 39/49 (80%) skin biopsies from the SSc patients compared to 20/28 (71%) in the control group. Genotype 1 was more frequent in the skin from SSc patients (28/49, 57%) than in controls (9/28, 32%); however this difference was not statistically significant. Genotype 2 spread in patients and controls resembled one another: 21/49 (43%) and 11/28 (39%), respectively.

As regards bone marrow samples, B19V DNA was detected in 17/29 (59%) SSc patients compared to 0% in healthy donors and in 5/21 (24%) in the group of haematological patients ( $p < 0.05$ ). Such B19 DNA distribution was due to a higher prevalence of both genotypes in patients than in controls.

No correlation was observed between the age of patients and genotype detected, likely due to the high mean age of subjects included in this study.

Simultaneous persistence of genotypes 1 and 2 was detected in skin from 10/49 (20%) and in bone marrow from

9/29 (31%). No individuals from the control groups had a mixed infection.

From 6 SSc patients, both skin and bone marrow biopsies were available. In 3 cases, only skin biopsies tested positive for B19V DNA, in 1 subject simultaneous presence of genotypes 1 and 2 was detected in both bone marrow and skin, 2 patients had double infection by genotypes 1 and 2 in the bone marrow while only genotype 1 was present in the skin sample.

The viral load in the bone marrow and in the skin varied from  $1 \times 10^2$  to  $7 \times 10^4$  per  $10^6$  cells both in patients and controls. Genotype 3 was absent in the skin and bone marrow from both patients and controls. All serum samples from patients and controls tested B19/PCR negative.

To evaluate the replicative activity of B19V in infected tissues from SSc patients and controls, the VP1 mRNA expression was determined by RT-PCR as described previously [17]. The positive samples gave the characteristic electrophoretic pattern corresponding to amplified alternatively spliced viral products (262 and 125 bp). Altogether, 12 skin biopsies from SSc patients (8 typed as genotype 1, 3 as genotype 2 and 1 sample as genotype 1 + 2) and 15 from controls (6 containing genotype 1 and 9 containing genotype 2) were available for the RNA searching. Viral mRNA for capsid protein was detected in the skin of 7 SSc patients (6 typed as genotype 1 and 1 as genotype 1 + 2), while all control samples proved negative by RT-PCR.

In accordance with previous observations [13–15, 18], the occurrence rate of parvovirus B19 DNA revealed in the present study was higher in the skin and particularly in the bone marrow biopsies from SSc patients than in controls.

In all tested samples, only genotypes 1 and 2 were detected, thus confirming data reported in the literature about very limited B19V type 3 circulation in European countries [6, 10].

In both skin and bone marrow of SSc patients the genotype 1 prevalence was higher compared to the control group and the difference concerning bone marrow was statistically significant.

Simultaneous persistence of 2 genotypes was fairly common in skin (20%) and bone marrow (31%) of SSc patients, while completely absent in the control group. The higher susceptibility to B19V persistence as well as the higher prevalence of coinfections or superinfections by 2 antigenically similar B19V genotypes could be attributed to the altered immune status versus this virus in the subjects affected by SSc.

Interestingly, in a proportion of genotype 1 DNA-positive skin samples from the SSc patients, viral mRNA for capsid protein was found, while no viral messengers were detected in the controls.

Although the number of specimens investigated was rather small, the results could suggest that the persistence patterns of B19V in the skin of SSc patients and of healthy subjects could be somewhat different.

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