Erythema chronicum migrans, acrodermatitis chronica atrophicans (ACA), and lymphocytoma cutis are all cutaneous manifestations of Lyme disease, a multisystem disorder that follows infection with Borrelia burgdorferi. An aetiological role for B burgdorferi has also been proposed for other skin disorders, including primary cutaneous B cell lymphoma (PCBCL) and morphoea. Although there is increasing evidence to support this hypothesis in the context of PCBCL, studies investigating the link between B burgdorferi and morphoea have produced conflicting results, some reports suggesting a positive association, but others not. This has led to the proposal that regional variations in B burgdorferi may be important in dictating the spectrum of clinical disease following infection, with morphoea only being caused by certain subspecies endemic to specific geographical areas.

“Studies investigating the link between Borrelia burgdorferi and morphoea have produced conflicting results, some reports suggesting a positive association, but others not”

Although Lyme disease is endemic in many areas of the UK, only two previous studies have investigated the possible link with morphoea specifically in the UK. Both gave a negative result, but this was based primarily on patients with morphoea lacking serological evidence of previous infection with B burgdorferi. However, because B burgdorferi infection may occur without the production of specific antibodies these results are inconclusive. Therefore, we used a polymerase chain reaction (PCR) technique to evaluate skin biopsies taken from patients with morphoea in the Highlands of Scotland, an area with endemic Lyme disease, for the presence or absence of B burgdorferi specific DNA. Recently, this approach has been used successfully to demonstrate a significant association between B burgdorferi and PCBCL in patients from the same region. A literature review was also performed to interpret our results in the context of the possible geographical variations that may exist in the relation between B burgdorferi infection and the subsequent development of morphoea.

MATERIALS AND METHODS

Case selection
The pathology department, Raigmore Hospital, Inverness, UK, processes all surgical pathology specimens for the Highland and Western Isles regions of Scotland, an area with a population of approximately 250 000 in which Lyme disease is endemic. By searching the surgical pathology files, Raigmore Hospital, Inverness (from 1976 to date), and reviewing case records of all patients with histology compatible with morphoea we were able to identify 16 patients for study, all with histological and clinical features typical of morphoea.

Pathological studies
Sections (5 µm thick) were cut from formalin fixed, routinely processed, paraffin wax embedded tissue blocks of each biopsy and stained with haematoxylin and eosin. Each case was assessed to confirm a diagnosis of morphoea and to approximate the stage of the disease using standard criteria.

Demonstration of B burgdorferi DNA
Sections (6 × 5 µm) cut from formalin fixed, paraffin wax embedded tissue blocks were dewaxed and DNA extracted using the Intergen EX-WAX DNA extraction kit (Intergen, Inc., Westbury, New York, USA).

Abbreviations: ACA, acrodermatitis chronica atrophicans; PCBCL, primary cutaneous B cell lymphoma; PCR, polymerase chain reaction.
with a reduced concentration of MgCl₂ (2.5 mM) and primers
the first stage product was amplified in a 50 µl reaction volume of 50 µl,
containing final concentrations of 10 mM Tris/Cl₂ (pH 8.5),
50 mM KCl₂, 3.5 mM MgCl₂ (Applied Biotechnologies), 0.2 mM
dNTP (Pharmacia Biosystems, Milton Keynes, UK), 0.5 U Taq polymerase (Applied Biotechnologies), and 0.5 µM of each
primer F1 (5′-ATT AAC GCT GCT ATT CTT AGT -3′) and F3 (5′-
GTA CTA TTC TTT ATA GAT TC-3′) (Severn Biotech, Kidderminster, UK). The thermal cycling conditions were 35 cycles of one minute at 94°C, two minutes at 41°C, and three minutes at 66°C, followed by a further extension period of five minutes at 72°C. For the nested PCR, 20 µl of a 1/5 dilution of the first stage product was amplified in a 50 µl reaction mix with a reduced concentration of MgCl₂ (2.5 mM) and primers F6 (5′-TTC AGG GTC TCA AGC GTC TTT GAC T-3′) and F8 (5′-
GCA TTT TCA ATT TTA GCA AGT GAT G-3′) (Severn Biotech). The thermal cycling conditions were 35 cycles of one minute at 94°C, one minute at 50°C, and one minute at 72°C, with a final extension of five minutes at 72°C. A positive control (~10 cultured B. burgdorferi organisms) and negative control were included in all cases. The PCR products were visualised under ultraviolet transmission of ethidium bromide stained agarose gels.

The first stage PCR was performed with 20 µl of sample in a reaction volume of 50 µl, containing final concentrations of 10 mM Tris/Cl₂ (pH 8.5), 50 mM KCl₂, 3.5 mM MgCl₂ (Applied Biotechnologies), and 0.2 mM
dNTP (Pharmacia Biosystems, Milton Keynes, UK), 0.5 U Taq polymerase (Applied Biotechnologies), and 0.5 µM of each
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Literature review
A textword Medline search was conducted for the years 1966 to date using the terms “morphea”, “morphoea”, “lyme disease”, and “Borrelia” in various combinations. Abstracts of all articles were read and papers selected for inclusion if they were published in English and used laboratory techniques to investigate the relation between B. burgdorferi infection and morphea.

RESULTS
Clinical features
There were five male and 11 female patients ranging in age from 3 to 69 years at diagnosis. According to the classification of Peterson et al., there were 13 cases of plaque morphea (12 morphea en plaque, one atrophoderma of Pasini and Pierini), two cases of linear morphea (one linear morphea, one en coup de sabre), and one case of generalised morphea. None of the patients had a history of antibiotic treatment in their hospital notes and none had a documented history of manifestations of Lyme disease.

Pathological findings
All 16 cases showed histological features consistent with a diagnosis of morphea, the principle finding being the presence of thickened collagen bundles in the reticular dermis. The histological stage of the lesion was approximated by additional changes. Three cases were interpreted as early stage lesions. All displayed a moderate lymphocytic infiltrate with fewer numbers of plasma cells arranged around blood vessels lined by swollen endothelial cells (fig 1). Six biopsies were regarded as showing features of late stage lesions. In these, there was increased eosinophilia of the thickened collagen bundles, together with homogeneous collagen in the papillary dermis, atrophy of eccrine glands with marked loss of periglandular fat, some fibrous thickening of blood vessel walls, and a minimal or absent chronic inflammatory cell infiltrate (fig 2). The remaining seven cases displayed features in keeping with lesions of an intermediate duration in which the “late” changes were either less pronounced, or only some were present.

PCR analysis
Amplifiable DNA was obtained from 14 of the 16 biopsies, as determined by PCR with primers for β globin. These 14 cases were tested using primers for the B. burgdorferi flagellin gene. In each test, there was successful amplification of control DNA extracted from cultured borrelia organisms, giving a product of 275 bp after the nested run. However, all 14 samples from patients with morphea were negative, as were the negative controls (fig 3).

Figure 1 Haematoxylin and eosin stained section (original magnification, ×100) showing early changes of morphea. A perivascular infiltrate is present in the dermis and the superficial subcutis. The reticular dermis appears pale owing to the presence of swollen collagen bundles.

Figure 2 Haematoxylin and eosin stained section (original magnification, ×20) showing later stage morphea. Thickened collagen bundles fill the reticular dermis in the vicinity of an eccrine gland that has lost the normal surround of adipose tissue.

Figure 3 Agarose gel showing results after the polymerase chain reaction with B. burgdorferi flagellin gene. Lanes 1 and 6, DNA molecular weight markers; lane 2, positive control; lane 3, negative control; lane 4 and 5, negative results from DNA extracts of two cases of morphea.
Table 1  Summary of previous studies investigating the relation between Borrelia burgdorferi infection and morphea in different geographical locations

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Direct visualisation in tissue sections of morphea biopsies</th>
<th>Serumology</th>
<th>Culture from biopsies of morpheic lesions</th>
<th>PCR analysis of DNA extracts of morphea biopsies</th>
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*Eleven of 39 cases in this study also showed an increased B burgdorferi induced proliferation of peripheral blood lymphocytes. †This represents the only positive culture where spirochaetes have been subtyped; in this instance PCR analysis confirmed B afzelii. ‡This study investigated patients from Germany, Japan, and USA; the results of each location are presented separately for clarity. §All patients in this study were selected on the basis of positive serology for borrelia infection; 7 of 7 tested also showed an increased B burgdorferi induced proliferation of peripheral blood lymphocytes.

ND, not done; PCR, polymerase chain reaction.

Literture review
Table 1 summarises the results of the literature review.14–37

DISCUSSION
The suspicion that morphea may occur as a consequence of infection with B burgdorferi was initially aroused by clinical observations of coexisting morphea and ACA, a cutaneous manifestation of chronic borreliosis.30–40 Subsequently, several different methodologies have been used to investigate this relation, often with confusing and conflicting results (table 1). Initially, studies used positive serology for B burgdorferi as evidence of previous infection and sought to correlate this with clinical manifestations of morphea. In several series, all patients with morphea tested have been seronegative, including 33 patients included in two studies from the UK.15 16 25 26 28 Conversely, other studies have found specific antibodies to B burgdorferi in between 6% and 54% of cases.14–20 22 23 25 27–29 In addition to a humoral immune response to the organism, large numbers of patients with morphea may display specific cellular immunoreactivity.15 30

Because negative serology does not exclude previous infection with B burgdorferi and because positive serology may merely represent coincidental infection, other studies sought more definite evidence of a causal link by seeking to demonstrate the organism in biopsies of lesional skin taken from patients suffering from morphea. Attempts to visualise borrelia organisms directly in histological sections after silver staining or immunohistochemistry have met with mixed success, and in only a small number of cases have spirochaetes been demonstrated.14 15 17 19 20 23 32 33 In view of problems in evaluating positive results using these methodologies, recent studies have focused on more sensitive and specific PCR techniques to demonstrate the organism. Once more the results have been contradictory. Studies reporting a positive association between B burgdorferi infection and morphea have shown evidence of the organism in between 26% and 100% of cases,14–20 22 23 whereas in a further 10 reports, including our current one, no positive cases have been identified in a total of 190 cases tested.22 23 25 26 28 31–37 Lastly, the culture of borrelia from biopsies of morphea lesions has also been attempted. Although several studies have produced completely negative results,9 19 25 26 28 success has been achieved in a small number of cases,14 16 17 20 24 and the negative results may be partly or wholly attributable to the fastidiousness of the organism in culture.

Two possible conclusions can be drawn from this diaspora of results. The less charitable argument is that all of the positive findings reported are the result of one or more of the following: clinical and/or histological misdiagnosis of ACA as morphea, because both entities share several common features; the chance occurrence of B burgdorferi infection and morphea, especially in cases from areas of endemic Lyme disease; the presence of another causative agent that may be transmitted at the same time as B burgdorferi; and contamination of samples, especially with regard to PCR studies. However, it is difficult to envisage that all positive results to date can be explained in this way. This is particularly so for one group of Austrian researchers who have consistently found positive titres for B burgdorferi in a significantly higher proportion of patients with morphea than in controls, and who have successfully demonstrated B burgdorferi in biopsies of morpheic skin by immunohistochemistry with specific antibodies, in addition to the use of culture and typing.15 30 If the positive results are taken at face value then an alternative
The particular strains of the subsequent development of morphoea. This phenomenon is already well documented for other accepted facets of borrelia infection. For example, ACA rarely occurs during the course of Lyme disease in North America where B burgdorferi sensu stricto is the dominant species, but is commonly seen in Europe where B afzelii and B garinii are more prevalent.29

“These results indicate that in the Scottish Highlands there is no association between infection with indigenous strains of Borrelia burgdorferi and the subsequent development of morphoea”

If this hypothesis is correct, then the literature suggests that B burgdorferi is implicated in the pathogenesis of at least some cases of morphoa in Austria (especially in the vicinity of Vienna). But almost certainly not in the USA or some, but possibly not all, parts of Germany.21–24 35–37 Isolated studies reporting a positive association in countries such as Italy, Switzerland, Puerto Rico, Turkey, and Japan, and a negative association in Spain, Finland, Holland, and France20 25–32 require corroboration before definite conclusions can be drawn about these geographical locations.

The results of our study can be assessed in a similar light using the Austrian studies as reference for an example of a population in which B burgdorferi plays a role in the pathogenesis of morphoea. Given the cohort of patients studied, a similar positive result should have been forthcoming if the strains of borrelia endemic in the Scottish Highlands also possessed the potential to initiate the development of morphoea, because this region has the highest incidence of Lyme disease in Scotland, and probably the UK.46 The breakdown of morphoea subtypes studied was similar to that described in one of the Austrian papers in which a positive association was also demonstrated, meaning that case selection was unlikely to have biased our results. In addition, we are confident that although PCR is prone to false negative results, our technique is sufficiently sensitive to have detected the organism were it present. Positive controls were amplified in each reaction and we have also used this technique to identify B burgdorferi in archival material from patients with PCBC1. In addition, our cases were not biased towards late stage morphoea and, coupled with the fact that B burgdorferi has been identified in biopsies of patients with morphoea up to 20 years after the onset of cutaneous lesions, this means that the age of the lesion sampled was unlikely to have produced a false negative result.21 Moreover, although not directly questioned, there was no evidence that our patients were receiving antibiotic treatment before biopsy and none had a history documenting the potential to initiate the development of morphoea up to 20 years after the biopsy.46

Whether or not this negative result is entirely attributable to the particular strains of B burgdorferi encountered in the highlands, as compared with Austria, remains to be determined. However, the data currently available do not disprove this theory. We have recently typed 12 isolates of B burgdorferi sensu lato grown from highland ticks, five as B afzelii and seven as B burgdorferi sensu stricto, and discovered two different strains of the first organism and four of the second. Of particular interest to our current study was the finding that all strain types differed from reference strains derived from mainland Europe. In fact, such genomic heterogeneity appears to be normal, both within and between geographical areas.45 In view of this, and considering the expanding spectrum of disease attributed to B burgdorferi, further studies are warranted to investigate the effect of strain type on the clinical manifestations of infection.

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REFERENCES


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Morphoea and *Borrelia burgdorferi*: results from the Scottish Highlands in the context of the world literature

J R Goodlad, M M Davidson, P Gordon, R Billington and D O Ho-Yen

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