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The expanding Lyme *Borrelia* complex—clinical significance of genomic species?

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Abstract

Ten years after the discovery of spirochaetes as agents of Lyme disease in 1982 in the USA, three genomic species had diverged from the phenotypically heterogeneous strains of *Borrelia burgdorferi* isolated in North America and Europe: *Borrelia afzelii*, *B. burgdorferi* sensu stricto (further *B. burgdorferi*), and *Borrelia garinii*. Whereas *B. burgdorferi* remained the only human pathogen in North America, all three species are aetiological agents of Lyme borreliosis in Europe. Another seven genospecies were described in the 1990s, including species from Asia (*Borrelia japonica*, *Borrelia turdi*, and *B. tanukii*), North America (*Borrelia andersonii*), Europe (*Borrelia lusitaniae* and *Borrelia valaisiana*), and from Europe and Asia (*Borrelia bissettii*). Another eight species were delineated in the years up to 2010: *Borrelia sinica* (Asia), *Borrelia spielmanii* (Europe), *Borrelia yangtze* (Asia), *Borrelia californiensis*, *Borrelia americana*, *Borrelia carolinensis* (North America), *Borrelia bavariensis* (Europe), and *Borrelia kurtenbachii* (North America). Of these 18 genomic species *B. afzelii*, *B. burgdorferi* and *B. garinii* are the confirmed agents of localized, disseminated and chronic manifestations of Lyme borreliosis, whereas *B. spielmanii* has been detected in early skin disease, and *B. bissettii* and *B. valaisiana* have been detected in specimens from single cases of Lyme borreliosis. The clinical role of *B. lusitaniae* remains to be substantiated.

Keywords: *Borrelia afzelii*, *Borrelia burgdorferi*, *Borrelia garinii*, clinical relevance, genomic species

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Introduction

Lyme borreliae may be considered postmodern pathogens, because the illness they cause varies, does not have a predictable incubation period or course, and is likely to have a variable response. Protean manifestations and the absence of techniques to identify the organism in cases of Lyme borreliosis lead to bizarre ideas, and fantasies [1–3].

From Lyme Spirochaete to *Borrelia burgdorferi* Sensu Lato

In 1982, after the discovery of Lyme spirochaetes in hard ticks from Long Island, NY, USA [4], the aetiology of Lyme disease was confirmed by the cultivation of these spirochaetes from skin, blood and cerebrospinal fluid (CSF) of patients [5,6]. The Lyme spirochaete was identified as a new species of the genus *Borrelia* [7]. It very quickly became evident that not only did the clinical presentation of a *Borrelia burgdorferi* infection in Europe differ somewhat from that in North America, but so did the isolates from Lyme borreliosis patients and from ticks [8–10]. It was observed that an increasing number of European isolates of Lyme borreliae from patients and ticks were phenotypically heterogeneous and differed from the American type strain of *B. burgdorferi*. Thus, it was concluded that *B. burgdorferi* may comprise different genomic species, which, however, share common epitopes that are recognized by certain monoclonal antibodies. A serotyping system based on monoclonal antibody reactivity against the outer surface protein OspA was introduced. At the subspecies level, heterogeneity was demonstrated by restriction endonuclease analysis, hybridization with whole *B. burgdorferi* DNA or specific probes, and plasmid analysis. Genetic analysis of the broad variety of phenotypically defined strains was required in order to identify genotypic clusters [11]. The first result of an approach to classify Lyme borreliae on the basis of genomic

criteria was the delineation of three DNA groups, namely of genospecies *B. burgdorferi* sensu stricto (further *B. burgdorferi*), *Borrelia garinii* sp. nov., and group VS461; all of these strains were associated with clinical Lyme borreliosis [12]. In a similar study, genomic fingerprinting by an arbitrarily primed PCR with *Borrelia* isolates predominantly from *Ixodes* species and mice from North America, Europe and Japan delineated three *Borrelia* groups [13]. These results were in complete agreement with the results of the previously cited study [12]. However, two isolates were distinct from all of the other strains in the collection but were clearly members of the genus *Borrelia* [13]. Later, group VS461 strains were identified with monoclonal antibodies and named *Borrelia afzelii*. On the basis of a small number of *Borrelia* isolates from the skin of patients suffering from acrodermatitis chronica atrophicans, a skin manifestation of European Lyme borreliosis, but also recovered from erythema migrans, it was stated that *B. afzelii* sp. nov. is the only member of this group to result in acrodermatitis chronica atrophicans [14].

Expansion of the Lyme *Borrelia* Complex during the 1990s

Genomic fingerprinting of *B. burgdorferi* sensu lato strains by pulsed-field gel electrophoresis (PFGE) showed that all isolates used in this study were recognized by one band (135 kbp), each of the *B. garinii* isolates by two bands (220 and 80 kbp), and each of the *B. afzelii* isolates by three bands (460, 320 and 90 kbp). Whilst there were differences in the PFGE patterns among *B. burgdorferi* and *B. garinii* isolates, the patterns of *B. afzelii* isolates were all similar [15]. The number of genomic species was further expanded by the characterization of borreliae isolated from *Ixodes ovatus* ticks in Japan. A new species, apparently not a human pathogen and restricted to Japan [16], was hence named *Borrelia japonica* [17]. Another study focused on the ribosomal genes of *B. burgdorferi* [18], using restriction polymorphism analysis of PCR products obtained with primers at the 3′-end of the first *rrf* gene and at the 5′-end of the second *rrl* gene. An amplicon, 226–266 bp in length, was generated from the *B. burgdorferi* strains tested. Restriction polymorphism analysis of the resulting amplicons with the nuclease *Mse*I permitted identification of the established species *B. burgdorferi*, *B. garinii*, *B. afzelii*, and *B. japonica* (formerly group F63B), and the identification of four new genomic groups. Two of these genomic groups were European strains, and the other two were North American strains. The method developed in that study could be applied for rapid screening of strain collections and for epidemiological and medical purposes [19]. With a similar approach, a new species, named *Borrelia andersonii*, was identified [20]. Genomic typing of borrelial strains isolated from *Ixodes tanuki* and *Ixodes turdus* ticks in Japan revealed two new genospecies, named *Borrelia tanukii* and *Borrelia turdi* [21].

Some researchers recognized the greater variety of *B. burgdorferi*, the sole North American aetiological agent of Lyme borreliosis, which is also present in Europe. The multiplicity of genospecies in Europe might indicate that Lyme borreliae emerged in Europe. However, according to *ospC* typing, there was a closer relationship between the European strains than between those in North America, supporting the reverse conclusion, that *B. burgdorferi* was introduced to Europe from America [22,23]. Despite this, a different view on the origin of *B. burgdorferi* has recently been published [24].

Nevertheless, more genospecies were described. *Borrelia* strains isolated from *Ixodes ricinus* ticks in Switzerland, The Netherlands, and the UK of genomic groups VS116 and M19 were carefully characterized, and their taxonomic status was assessed; as a result of this, new genospecies was proposed, *Borrelia valaisiana* sp. nov., type strain VS116 [25].

Isolates of another genomic species, PotiB2, isolated from *I. ricinus* ticks in Portugal, were studied in detail, and this resulted in the proposal of a new species, *Borrelia lusitaniae*, type strain PotiB2 [26].

Not only was diversity among European *Borrelia* strains being being re-examined, but atypical strains of North American origin, previously designated genomic group DN127, were closely analysed, and it was found that they cluster separately from *B. burgdorferi*. The conclusion was that genomic group DN127 should be referred to as a new species, *Borrelia bissettii* sp. nov., and that other related but distinct strains, which require further characterization, should be referred to as *Borrelia* spp. [27].

Up to this point, ten species within the *B. burgdorferi* sensu lato complex have been recognized, but only three—*B. afzelii*, *B. burgdorferi*, and *B. garinii*—were widely accepted human pathogens. These pathogenic *Borrelia* species were characterized by their vectors, geographical distribution, and organotropism [28].

Expansion of the Lyme *Borrelia* Complex in the New Millenium

The newly described genospecies *B. valaisiana*, a *Borrelia* species isolated from *I. ricinus* ticks in some countries of Europe [25], was also identified in specimens from wild rodents captured on Kinmen Island and from central Taiwan [29]. Borreliae were also isolated from rodents and ixodid ticks collected in southern China. Molecular characterization of