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Chapter 6

EVIDENCE FOR FREQUENT OSPC GENE TRANSFER BETWEEN 
BORRELIA VALAISIANA SP. NOV. AND OTHER LYME DISEASE SPIROCHETES

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SUMMARY

Molecular polymorphism of the ospC gene has been reported in Borrelia burgdorferi sensu stricto, Borrelia garinii and Borrelia afzelii, the spirochetes causing human Lyme borreliosis. To assess the genetic relationship between ospC genes from the recently described Borrelia valaisiana sp. nov. and other B. burgdorferi sensu lato species, the ospC genes from eight B. valaisiana isolates were amplified by PCR, cloned and sequenced. The ospC genes of three B. valaisiana isolates were identical, but clearly distinct from ospC genes from other Borrelia species. Four B. valaisiana isolates possessed ospC genes more related to those of B. garinii, and fell into a cluster representing B. garinii species in the phylogenetic tree. One isolate had an ospC gene encoding a protein identical to that of B. afzelii strain. Since 5 of the 8 (62.5%) B. valaisiana isolates contained a gene highly homologous or even identical to ospC genes found among B. garinii and B. afzelii strains, our findings indicate that ospC gene transfer occurs between B. valaisiana and other Lyme disease spirochetes.

INTRODUCTION

Lyme borreliosis (LB) is the most common vector-borne disease in the northern hemisphere. In Europe, five Borrelia species associated with LB, Borrelia burgdorferi sensu stricto, Borrelia garinii, Borrelia afzelii (1), Borrelia valaisiana sp. nov. (17), and Borrelia lusitaniae sp. nov. (7), have been documented. B. burgdorferi sensu stricto, B. garinii and B. afzelii have been associated with human LB (16). In addition, nine Borrelia isolates cultured from patients in Slovenia showed genetic and phenotypic similarity to a North American isolate belonging to Borrelia bissettii sp. nov. (14). To date, B. valaisiana has been cultured or detected in Ixodes ricinus ticks and avian reservoirs in a number of European countries (8, 13, 17). Although B. valaisiana has not been cultured from patients with LB, DNA specific for this species has been detected in skin biopsies from two patients with LB in the Netherlands (12). All B. valaisiana isolates studied so far formed an independent cluster in the phylogenetic trees based on 16S rRNA gene sequences and randomly amplified polymorphic DNA (RAPD) fingerprinting analysis (17, 18). However, the reactivity of these B. valaisiana isolates with a number of monoclonal antibodies (MAbs) against Borrelia outer membrane proteins, including outer surface protein C (OspC), was variable (17).

OspC, an immunodominant major protein of B. burgdorferi sensu lato, is highly heterogeneous. The amino acid sequence identity of the OspC proteins among different B. burgdorferi sensu lato species ranges from 63 to 82% (4). Lateral gene transfer between ospC genes from Borrelia strains within species, and between B. burgdorferi sensu stricto, B. garinii and B. afzelii has been suggested (9, 11). In order to elucidate the genetic relationship between ospC genes from B. valaisiana and other B. burgdorferi sensu lato species, and to evaluate the degree of conservation of the amino-terminal sequences of the OspC in B. valaisiana, the ospC genes from eight B.
valaisiana isolates were cloned, sequenced and compared to those from various B. burgdorferi sensu lato species. Our results indicate that frequent ospC gene transfer had occurred between B. valaisiana and Borrelia garinii, as well between as B. valaisiana and Borrelia afzelii.

MATERIALS AND METHODS

Borrelia isolates and culture conditions

Eight B. valaisiana sp. nov. isolates, of which the 16S rRNA gene were determined previously, were selected and used in this study. The biological and geographic sources of these isolates as well as those of B. burgdorferi sensu lato isolates used are listed in Table 1. The spirochetes were cultivated in modified BSK II medium at 33°C as described previously (16).

Table 1. B. burgdorferi sensu lato strains used in this study

<table>
<thead>
<tr>
<th>Species and strain</th>
<th>Biological source</th>
<th>Geographic location</th>
<th>GenBank Accession no.</th>
<th>Reference</th>
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<tbody>
<tr>
<td>B. burgdorferi</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>sensu stricto</td>
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<tr>
<td>B31</td>
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<td>United States</td>
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<td>B. garinii</td>
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<td></td>
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<td>(4)</td>
</tr>
<tr>
<td>PBr</td>
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<td>19857</td>
<td>Rabbit kidney</td>
<td>United States</td>
<td>L42864</td>
<td>(9)</td>
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<td>Borrelia sp.</td>
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<tr>
<td>25015</td>
<td>Ixodes pacificus</td>
<td>United States</td>
<td>L42898</td>
<td>(9)</td>
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</table>

*aCSF: cerebral spinal fluid; EM: erythema migrans; ACA: acrodermatitis chronica atrophicans;

DNA preparation and PCR amplification

Genomic DNA from B. burgdorferi sensu lato was extracted as described previously (16). The ospC genes of B. valaisiana isolates were amplified by PCR with
the use of primers OspC-N (5′ CACAAATTAAT-GAAAAA-GAATACA 3′) and OspC-C (5′ CCAGTTACTTTTTAA-AACAAATTA 3′). PCR was carried out in a 50 µl mixture containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.1 mg/ml of bovine serum albumin (BSA), 0.2 µM dNTP, 2.5 U of Taq polymerase (Qiagen, Leusden, The Netherlands), and 0.1 µM of each primer (Perkin-Elmer Applied Biosystems, Cheshire, UK) under the following conditions: an initial step of denaturation at 95°C for 5 min, followed by 30 cycles consisting of denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1.5 min, with a final extension step at 72°C for 8 min.

**Cloning and DNA sequencing of ospC gene**

PCR amplicons of ospC genes from all eight B. valaisiana strains were cloned directly into the PCR2.1 vector and transformed into E. coli INV′F′ competent cells by following the manufacturer's instructions (Invitrogen BV, Leek, the Netherlands). Subsequently, recombinant plasmid DNAs were prepared from four colonies of each transformation by using a Wizard plus minipreps DNA purification system (Promega, Leiden, The Netherlands). DNA sequence was determined by the dideoxy chain-termination technique in an ABI 373A sequencer using either the dye terminator or the dye primer cycle sequencing kit (Applied Biosystems Inc., Foster, Calif). At least two DNA preparations obtained from different colonies of each transformation mixture were sequenced on both strands.

**Phylogenetic analysis**

Nucleotide sequences of ospC gene and deduced amino acid sequences of B. valaisiana were aligned with sequences from different B. burgdorferi sensu lato species retrieved from GenBank with PC/GENE software (IntelliGenetics, Inc., Calif.). Phylogenetic analysis was performed with the Molecular Evolutionary Genetics Analysis (MEGA) program (5). Neighbor-joining method was used to construct the phylogenetic tree, and bootstrap analysis was performed up to 500 replications.

**RESULTS AND DISCUSSION**

**OspC sequence analysis**

Evidence for lateral transfer and recombination in ospC variants has been found at both intraspecies and interspecies level among B. burgdorferi sensu stricto, B. afzelii, and B. garinii, the three Borrelia species responsible for human disease in Europe (9, 11). However, the frequency of such an event between these species is relatively low (9). A DNA fragment containing 630 to 636 bp of the complete ospC gene was amplified by PCR from the eight B. valaisiana isolates. The similarity of the nucleotide sequences ranged from 74.0 to 100% among these B. valaisiana isolates. No difference was identified between the sequence of isolate VS116 determined in this study and the previously published one (9). Nucleotide sequences from three B.
**ospC Gene Transfer of B. burgdorferi**

B. *valaisiana* isolates (VS116, M19 and M53) were identical. The *ospC* sequence from AR-2, UK, M7 and M49 contained 74.6, 74.0, 81.1 and 74.9% identity to the sequence of isolate VS116, respectively. Isolate M52 had an *ospC* gene identical to that of AR-2. These two isolates, in contrast to 6 other *B. valaisiana* isolates used in this study, did not react with an OspC specific MAb L22 1F8, as shown earlier by SDS-PAGE and immunoblot analysis (17).

Considerable variations were observed among the OspC amino acid sequences of the eight *B. valaisiana* isolates. Only 68% of the positions were identical in the 8 isolates. The deduced amino acid sequences of *B. valaisiana* were aligned and
| Species and strain* |  
| B. burgdorferi ss B31 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. garinii 20047 | 72.4 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. garinii PHei | 70.5 | 73.4 | 100.0 | 89.6 | 93.3 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. garinii TN | 57.1 | 58.9 | 60.1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. garinii T25 | 59.4 | 59.7 | 54.9 | 71.2 | 70.3 | 62.7 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. garinii FBr | 55.2 | 59.9 | 57.5 | 69.1 | 71.2 | 62.7 | 75.1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. garinii FKe | 67.2 | 65.1 | 60.1 | 59.2 | 64.6 | 62.2 | 62.2 | 59.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. afzelii Orth | 70.3 | 66.1 | 62.2 | 61.8 | 63.3 | 59.6 | 62.7 | 60.1 | 76.3 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. afzelii ACA1 | 67.7 | 68.8 | 64.2 | 67.0 | 64.6 | 56.5 | 65.8 | 60.1 | 77.3 | 79.4 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. valaisiana VS116 | 63.0 | 82.0 | 53.9 | 57.6 | 57.2 | 52.3 | 61.7 | 55.4 | 66.5 | 63.9 | 67.9 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. valaisiana UK | 57.3 | 55.2 | 55.4 | 73.3 | 74.0 | 73.6 | 60.6 | 62.2 | 59.3 | 56.2 | 56.5 | 62.8 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. valaisiana AR-2 | 59.4 | 52.1 | 52.8 | 70.2 | 71.4 | 73.6 | 65.8 | 64.8 | 60.8 | 57.2 | 55.4 | 53.9 | 76.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. valaisiana M7 | 67.7 | 68.8 | 64.2 | 67.0 | 64.6 | 56.5 | 64.8 | 60.1 | 77.3 | 79.4 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| B. valaisiana M49 | 55.7 | 64.1 | 59.6 | 63.9 | 60.4 | 69.4 | 64.2 | 75.6 | 64.9 | 62.9 | 62.7 | 56.5 | 63.0 | 60.6 | 62.7 | 100.0 | 100.0 | 100.0 |
| B. andersonii 19857 | 65.6 | 57.8 | 58.0 | 56.5 | 63.5 | 54.4 | 61.1 | 54.9 | 61.9 | 64.4 | 64.8 | 61.3 | 55.2 | 56.0 | 64.8 | 56.3 | 100.0 |
| Borrelia sp. 25015 | 60.9 | 65.1 | 65.3 | 66.0 | 62.5 | 61.1 | 59.6 | 59.6 | 66.0 | 67.0 | 72.0 | 61.8 | 60.9 | 58.5 | 72.0 | 66.1 | 66.0 | 100.0 |

* B. burgdorferi ss: B. burgdorferi sensu stricto.
Gene Transfer of *B. burgdorferi* compared with the published OspC sequences from other *B. burgdorferi* sensu lato species (Fig. 1). The amino acid sequence similarity matrix of the 8 *B. valaisiana* isolates and 13 representative strains of other *B. burgdorferi* sensu lato species are presented in Table 2. Of interest are the sequences from amino acid 23 to 34 in the known *B. burgdorferi* species-specific motif region (2, 9, 19), since the 8 *B. valaisiana* isolates studied exhibited four different motifs in this region. Isolates VS116, M19 and M53 showed the *B. valaisiana* species-specific sequence described previously by Livey et al (9). However, isolate M7 had a *B. a/ze/n*-specific motif, whilst the three isolates UK, AR-2 and M52 exhibited *B. garinii* species-specific sequences. The motif of isolate M49 is highly unusual and showed similarity to *B. afzelii* strains at the amino terminus, followed by a deletion of three amino acids and ending with a *B. valaisiana*-specific amino acid (Fig1). Surprisingly, the predicted OspC amino acid sequence of *B. valaisiana* M7 and of a Danish *B. afzelii* isolate ACA1 were completely identical (Fig. 1), and showed more than 98% identity to the OspC sequences of *B. afzelii* isolates DK5 (AB009893) and PLe (accession no. S69932) from Denmark and Germany, respectively (data not shown). Since isolates M7 and ACA1 definitely belonged to *B. valaisiana* and *B. afzelii*, respectively, based on their 16S rRNA gene sequence (15, 17), MseI restriction patterns of the 5S-23S rRNA intergenic spacer (10, 17) and OspA serotype (20), our present study indicates that transfer of the *ospC* gene between *B. valaisiana* M7 and a *B. afzelii* isolate closely related to isolate ACA1 had occurred. Transfer of the *ospC* gene from *B. afzelii* to *B. valaisiana* M7 seems to be most probable, since the OspC sequence of *B. valaisiana* M7 clustered well within all *B. afzelii* OspC sequences so far analyzed (Fig. 2). This is the first report indicating transfer of a DNA sequence encoding a complete protein between *Borrelia* species. It is not clear whether also the promotor regions of the *ospC* gene or the whole plasmid containing the *ospC* gene was transferred between *B. valaisiana* M7 and *B. afzelii*.

**Phylogenetic analysis of OspC**

A phylogenetic tree was constructed based on the amino acid sequence similarity matrix of the OspC proteins using a Neighbor-joining method (Fig. 2). In this phylogenetic tree, isolates belonging to *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* clustered into respective branches corresponding to each of the species. As expected, *B. valaisiana* VS116, M19 and M53 constituted an independent branch in the phylogenetic tree. However, isolates M7, and UK, AR-2, M52 and M49 fell into the clusters of *B. afzelii* and *B. garinii*, respectively, rather than into the cluster of *B. valaisiana*. Thus, lateral transfer and recombination may have occurred between *B. valaisiana* and *B. garinii*, as well as between *B. valaisiana* and *B. afzelii*.

Four *B. valaisiana* isolates (AR-2, M52, UK and M49) clustered together with *B. garinii* in the phylogenetic tree. Three of them showed *B. garinii*-specific sequences in the species-specific motif region. The amino acid sequences of *B. valaisiana* isolates AR-2, M52 and UK were 74% identical to that of a *B. garinii* isolate (TN), but only 54-64% identical to the OspC sequence of the type strain of *B. valaisiana* VS116. Sequence differences between these three isolates and TN were mainly found in the
Fig. 2. Phylogenetic tree based on the amino acid sequences of the OspC proteins from different B. burgdorferi sensu lato species. The accession numbers for ospC gene of each strain are listed in Table 1. Numbers at the branch nodes indicate the results of the bootstrap analysis.

hypervariable region V1 and the C-terminal part of V2 (Fig. 1) (9). In all regions, their homology with the B. valaisiana consensus sequence was lower than the homology with the B. garinii TN sequence. This finding suggests that most likely ancestors of AR-2, M52 and UK acquired a complete ospC gene from B. garinii strains, and that these events were followed by mutations in the variable regions of the gene. Alternatively, these ospC genes could have evolved by a series of partial gene transfer events from B. garinii to B. valaisiana. Isolate M49 exhibited 76% identity to B. garinii PBr but only 57% identity to isolate VS116. At all, it is likely that 5 of the 8 (62.5%) B. valaisiana isolates studied acquired a partial or entire ospC gene from other LB spirochetes. The overall frequency of such an interspecies gene transfer is rather high compared to that reported between B. burgdorferi sensu stricto, B. garinii and B. afzelii (4, 9). It is thought that intragenic recombination between different strains requires double infection in the tick or host environment (9). Simultaneous infection of B. valaisiana and B. garinii or B. afzelii in Ixodes ticks (6, 8), as well as in
the avian reservoirs (3) has been reported repeatedly and may partially explain the high frequency of gene exchange between B. valaisiana and other B. burgdorferi sensu lato species. Our data suggested a ospC gene transfer between B. valaisiana and the clinical isolate of pathogenic Borrelia species had occurred. Further studies on the genetic characteristics of B. valaisiana are required to appreciate the role of this species in animal and human LB.

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REFERENCES


