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Borrelia burgdorferi Sensu Stricto Is Clonal in Patients with Early Lyme Borreliosis▽

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Lyme borreliosis, the most commonly reported vector-borne disease in North America, is caused by the spirochete *Borrelia burgdorferi*. Given the extensive genetic polymorphism of *B. burgdorferi*, elucidation of the population genetic structure of the bacterium in clinical samples may be relevant for understanding disease pathogenesis and may have applicability for the development of diagnostic tests and vaccine preparations. In this investigation, the genetic polymorphism of the 16S-23S rRNA (*rrs-rrlA*) intergenic spacer and *ospC* was investigated at the sequence level in 127 clinical isolates obtained from patients with early Lyme borreliosis evaluated in suburban New York City. Sixteen distinct *rrs-rrlA* and 16 distinct *ospC* alleles were identified, representing virtually all of the genotypes previously found in questing *Ixodes scapularis* nymphs in this region. In addition, a new *ospC* group was identified in a single patient. The strong linkage observed between the chromosome-located *rrs-rrlA* and plasmid-borne *ospC* genes suggests a clonal structure of *B. burgdorferi* in these isolates, despite evidence of recombination at *ospC*.

Lyme borreliosis is the most commonly reported vector-borne disease in the United States, with approximately 20,000 new cases tabulated by the Centers for Disease Control and Prevention each year (4). In the United States, Lyme borreliosis appears to be caused exclusively by *Borrelia burgdorferi* sensu stricto (hereafter referred to as *B. burgdorferi*)(5). *B. burgdorferi* exhibits a high degree of genetic diversity (1, 3, 19, 29, 30, 46), and the genetic background of the bacterium appears to affect its pathogenic and ecological properties. For example, *B. burgdorferi* genotypes can infect a wide range of vertebrate species, and there is a significant fitness variation among genotypes that can result in different epidemiological behavior of this pathogen (13, 18). Furthermore, a growing number of clinical and animal studies have shown that the genotype of *B. burgdorferi* plays a critical role in spirochete dissemination and affects the severity of clinical manifestations in mice and humans (34, 43, 44, 47). Thus, knowledge of *B. burgdorferi* population structure in ticks, reservoir hosts, and patients is essential in assessing the risk of Lyme borreliosis to humans. This information may also be applicable for the development of diagnostic assays and candidate vaccine preparations.

Population genetic studies of *B. burgdorferi* frequently involve the *rrs-rrlA* rRNA intergenic spacer (IGS), located on the chromosome, and/or the plasmid-borne outer surface protein C gene (*ospC*) (2, 3, 12, 16, 30, 34, 46). The noncoding *rrs-rrl* IGS has been shown to be a useful target for the study of bacterial phylogeny. In contrast to rRNA genes, which are well conserved throughout most bacterial species, *rrs-rrl* spacer regions exhibit sequence variation that allows intraspecies strain differentiation (9, 10, 17). Sequence analyses of *B. burgdorferi* at four genetic loci (*ospA*, *ospC*, p66, and *rrs-rrlA*) in a nymphal tick population in Connecticut confirmed the utility of *rrs-rrlA* as an important phylogenetic marker for *B. burgdorferi* and further separated the bacterial species into at least nine clonal IGS types (3). OspC, on the other hand, is an immunodominant, plasmid-encoded surface protein that exhibits significant immunological and genetic variation (39, 40, 46). To date, 16 *ospC* groups have been found in questing ticks from the northeastern United States (2, 30, 46). It has been suggested that the genomic diversity of *B. burgdorferi* in nature is maintained by balancing selection operating on OspC and that *ospC* groups represent evolutionarily stable clones (30, 31, 46).

To date, population genetic analyses of *B. burgdorferi* in human clinical specimens employing *rrs-rrlA* and/or *ospC* has been performed mainly by restriction fragment length polymorphism (RFLP) (16, 20) and single-stranded conformational polymorphism assays, respectively (34). These analytic methods, however, have limited discriminatory power when it comes to strain differentiation at the nucleotide level. Although several studies have used DNA sequencing of multiple loci, only a small number of clinical isolates was analyzed (1, 31).

In this study, the population structure of *B. burgdorferi* isolates from early Lyme borreliosis patients from suburban New York City and the phylogenetic relationship among these isolates were investigated at *rrs-rrlA* and *ospC*. This represents the largest survey of sequence diversity in human-derived strains of *B. burgdorferi* performed to date.

MATERIALS AND METHODS

Clinical isolates. *B. burgdorferi* was cultivated from erythema migrans lesions or blood of patients with early Lyme borreliosis associated with erythema migrans diagnosed at the Lyme Disease Practice of the Westchester Medical Center in Valhalla, NY, between 1991 and 2005, as described previously (33, 42, 47). One hundred twenty-seven isolates (35 blood and 92 skin isolates) from a col-

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TABLE 1. *ospC* and *rrs-rrlA* alignment features

Locus	No. of samples	No. of alleles	Aligned characters				
			No. of base pairs	No. gapped	No. (%) of polymorphisms	No. (%) parsimony informative	π^a
rrs-rrlA	127	16	812	11	63 (7.8)	47 (5.8)	0.025
ospC	127	16	522	30	272 (52.1)	205 (39.3)	0.193

[a] π, mean nucleotide diversity at each aligned position.

lection of more than 400 isolates that had been previously typed by both RFLP analysis of the *rrs-rrlA* IGS (20) and reverse line blot analyses of *ospC* (2, 30; G. P. Wormser et al., submitted for publication) were used in this study. Skin and blood isolates obtained from separate patients were chosen in order to cover the full diversity of *B. burgdorferi* genotypes found in the collection. The *rrs-rrlA* and *ospC* sequences of 11 of the isolates used in this study have been previously reported (44).

DNA extraction, PCR screening, and sequencing. DNA from low-passage (passages 1 to 5) *B. burgdorferi* cultivated in BSK-S (42) was isolated with IsoQuick (Orca Research, Bothell, WA). A 941-bp fragment of *rrs-rrlA* was amplified by PCR with primers PB and P97 as described previously (19). The nearly complete *ospC* gene was amplified by PCR with primers OspC-C and OspC-N or OC6 (+) and OC602 (−) as described elsewhere (2, 45). PCR products were sequenced on both strands (Genewiz, Inc., South Plainfield, NJ) with PCR screening primers. Isolates that produced ambiguous sequence results were cloned by limiting dilution, and sequence analyses of both loci were performed on two clones from each isolate.

Sequence alignments. Multiple sequence alignments were generated with the ClustalW algorithm and BioEdit software (11) by using default parameters, followed by manual inspection. For *ospC*, alignments were made on the translated amino acid sequences and then back-translated to nucleotide sequences to ensure in-frame nucleotide alignment.

Phylogenetic analyses. Gene trees were inferred by PAUP*4.0b10 (37) under both maximum-parsimony and maximum-likelihood criteria. Insertion and deletion (indel) polymorphisms were excluded from all but parsimony analyses of the protein-encoding *ospC* region, where gaps were considered as a fifth state. Maximum-parsimony analysis used parsimony-informative sites only, 20 replicates of random taxon addition, and tree bisection-reconnection branch swapping. Support for internal nodes of the inferred phylogeny was estimated by the nonparametric bootstrap method, with 1,000 replications under a maximum-parsimony criterion and tree bisection-reconnection branch swapping. For the maximum-likelihood analysis, parameters including the proportion of invariable sites, nucleotide frequencies and substitution rates, and the gamma shape parameter were estimated by the Akaike information criterion implemented in Modeltest 3.06 (27). Rate heterogeneity among sites was examined by assuming a discrete gamma distribution with eight rate categories. The maximum-likelihood trees were generated by 20 replicates of random taxon addition and tree bisection-reconnection branch swapping. Starting trees for branch swapping were obtained by stepwise addition. Nonparametric bootstrapping with 100 replicates was performed by tree bisection-reconnection branch swapping. For both maximum-parsimony and maximum-likelihood analyses, all branches receiving 70% or higher bootstrap values were considered well supported. The topologies of two gene trees were compared by using the Shimodaira-Hasegawa test (35) implemented in PAUP*4.0b10. For a given gene, the Shimodaira-Hasegawa test compares the difference in log likelihoods of competing tree topologies. The null distribution of differences in log likelihoods was obtained with 1,000 replicates of nonparametric bootstrapping of re-estimated log likelihoods. To avoid potential bias toward higher levels of significance due to small numbers of topologies (41), 100 random topologies were added to each test. For both data sets, the two maximum-likelihood trees plus 100 random trees separately generated for each data set were compared by using the Shimodaira-Hasegawa test. Statistical significance was set at $P = 0.05$.

Recombination analyses. Putative recombination events were detected with RDP2.0b08 (22). This program uses several methods to identify putative recombination events and breakpoints in DNA. The three methods chosen, Geneconv (32), MaxChi (23), and Chimaera (28), are based on patterns of nucleotide substitution and have been shown to be the most powerful recombination methods for divergent data sets and do not infer false-positive recombination events (28). The following settings were used for all of the methods: (i) sequences were linear, (ii) sequences in the alignment were screened in triplets, and (iii) statistical significance was set to $P = 0.001$ with Bonferroni correction for multiple comparisons. In Geneconv, the parameter GSCALE was set to 0. In MaxChi and Chimaera, a sliding window was used, the width was set to the number of polymorphic sites divided by 1.5 (28), the number of permutations was 1,000, and the permutation *P* value was 0.001. Only recombination events detected by at least two methods were considered further. The data were imported into RDP2.0b08 in nexus file format.

The reference sequences used in this study were downloaded from GenBank. The accession numbers of the *B. burgdorferi* IGS types and subtypes used are AY275189 to AY275212 (3). The accession numbers of the *B. burgdorferi ospC* groups used are AF029860 to AF029865 for *ospC* A to F, AF029867 to AF029871 for *ospC* G to K, L42899 for *ospC* L, U01892 for *ospC* M, L42897 for *ospC* N, X84778 for *ospC* O, U91796 for *ospC* P, U91790 for *ospC* Q, U91791 for *ospC* R, U91793 for *ospC* S, AF065143 for *ospC* T, and AF065144 for *ospC* U (34, 46).

Nucleotide sequence accession numbers. The sequences for each ribosomal spacer (RSP) allele reported in this study have been deposited in GenBank with accession numbers EF649781 for RSP1, EF649783 for RSP3, EF649784 for RSP4, EF649786 for RSP6, EF649787 for RSP7, EF649789 for RSP9, EF649790 for RSP10, and EU477177 to EU477185 for RSP12 to RSP20. The accession numbers for the *ospC* alleles are EU482041 to EU482056.

RESULTS

Sequence diversity of *rrs-rrlA*. Sequencing of 127 clinical isolates of *B. burgdorferi* revealed 16 distinct RSP alleles (for *rrs-rrlA*, the term allele is used to indicate a unique sequence, although this locus does not encode a protein product). The 812-bp-long alignment started at nucleotide 47 and ended at nucleotide 859 of the *rrs-rrlA* IGS region of GenBank reference strain *B. burgdorferi* B31 (accession number U03396). The lengths of individual sequences varied from 805 to 812 nucleotides due to indel polymorphism. The mean nucleotide diversity per position (π) was 0.025. Out of 812 nucleotide positions, 63 (7.8%) were polymorphic and 47 (5.8%) were parsimony informative (Table 1). No nucleotide substitutions were detected in *ileT* (encoding $tRNA^{ile}$), which was included in the alignment. The sequence identity among RSP alleles ranged from 93.8 to 99.7%. The RSP alleles obtained in this study were compared to previously reported IGS types and subtypes found in a population of field-collected questing nymphs from Connecticut (3). A majority of the RSP types (11/16) identified in patients were identical to IGS sequences found in ticks. The remaining five RSP types (RSP6, -15, -16, -17, and -19) formed well-supported clusters with existing IGS sequences. Since there were no major topological differences between maximum-parsimony and maximum-likelihood trees, only the maximum-likelihood tree is shown (Fig. 1).

Sequence diversity of *ospC*. There were 16 *ospC* allele types found among the 127 sequences obtained from clinical isolates. The 522-bp-long alignment started at nucleotide 78 and ended at nucleotide 578 of the *ospC* coding region of GenBank reference strain *B. burgdorferi* B31 (accession number AE000792). The sequence alignment contained a total of 522 characters, of which 250 were constant, 67 were variable

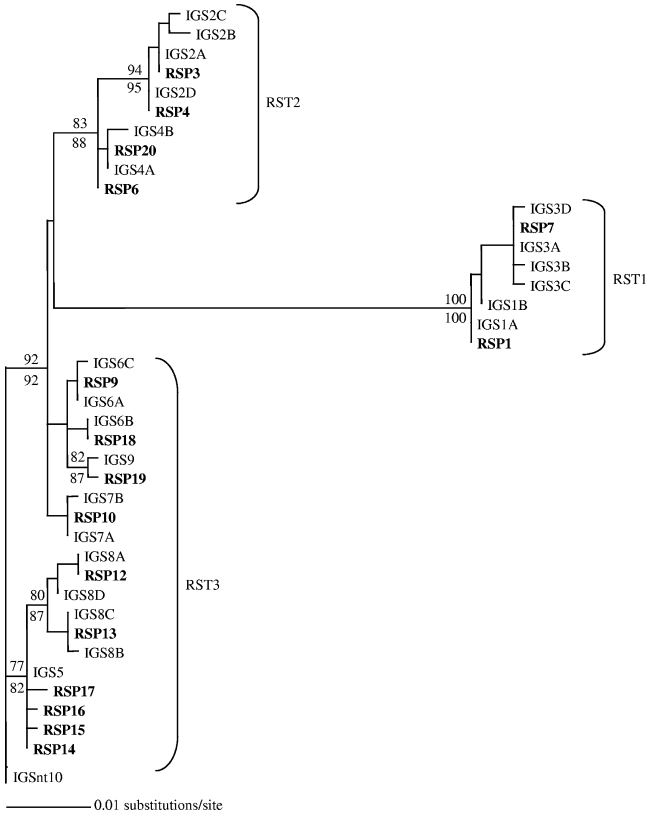

FIG. 1. Unrooted maximum-likelihood phylogenetic tree based on *rrs-rrlA* data set and GenBank reference strains. Nonparametric bootstrap values for nodes with >70% support in both maximum-likelihood and maximum-parsimony analyses are above and below the branches, respectively. *B. burgdorferi* RSP alleles identified in this study are in bold.

but parsimony uninformative, and 205 were parsimony informative. The mean nucleotide diversity per position (π) was 0.193 (Table 1). The lengths of the nucleotide sequences varied from 498 to 510, coding for 166 to 170 amino acids. Of the variable sites, 162 were at first and/or second codon positions, and 110 were at third codon positions. Pairwise nucleotide sequence identity among *ospC* alleles ranged from 74.0 to 87.6%, corresponding to amino acid identities of 63.9 and 80.2%, respectively. To assess the placement of *ospC* alleles found in this study with previously published *ospC* major groups, we compared sequence differences among our *ospC* alleles and major *ospC* groups found worldwide. It has been suggested that the members of the same *ospC* group will have a sequence difference of <2% and members of different groups >8% (46). By this criterion, 15 of the *ospC* allele types observed in this study fell into 15 major *ospC* groups previously found in the northeastern United States (Table 2). This relationship was further supported by maximum-likelihood and maximum-parsimony phylogenetic analyses that provided high bootstrap values for terminal nodes with *ospC* alleles and previously identified *ospC* groups (Fig. 2). The exception was *ospC* allele type 16 (AT16), which did not cluster with any *ospC* group and whose sequence was 85% identical to the closest *ospC* allele (*ospC* AT10) identified in this study. Furthermore, this allele was more than 8% different from any *ospC* allele submitted to GenBank to date.

Relationship between *rrs-rrlA* and *ospC* alleles and comparison with other typing methods. The partial sequences of the chromosomal noncoding *rrs-rrlA* and nearly complete sequences of *ospC*, located on circular plasmid cp26, were, without exception, strongly associated in the present data set. Sixteen unique biallelic profiles were resolved, with each profile represented by a unique combination of *rrs-rrlA* and *ospC* alleles. RFLP analyses of samples used in the present study showed that the RST1 genotype was linked with two *ospC*/*rrs-rrlA* allele profiles, RST2 with 4 and RST3 with 10 *ospC*/*rrs-rrlA* allele profiles (Table 2). RST1 and RST2 each formed well-supported clusters in the *rrs-rrlA* tree. In contrast, RST3 types were more diverse (Fig. 1). Each of the *rrs-rrlA* alleles corresponded to at least one of nine previously identified IGS types (3). The resolution of linkage among *rrs-rrlA* and *ospC* groups observed in the present study, however, was not apparent in the IGS typing framework. For example, *ospC* groups K and H were exclusively associated with RSP alleles 3 and 4, respectively, in the present study, but both belonged to the same IGS type (IGS2). Similarly, *ospC* groups U and T were exclusively associated with RSP alleles 12 and 13, respectively, but both belong to IGS type 8 (3) (Table 2).

Comparison of tree topologies. To determine whether the linkage among genetic loci could be explained by a clonal model, phylogenetic trees were constructed for each locus and examined for congruence. The Shimodaira-Hasegawa test was used to determine whether the same or different phylogenetic information was obtained from the analysis of *rrs-rrlA* and *ospC*. The *rrs-rrlA* phylogenetic tree was incongruent with the *ospC* tree (Shimodaira-Hasegawa test, $P < 0.05$) (Fig. 3). Some

TABLE 2. Comparison of *rrs-rrlA* and *ospC* alleles with different typing systems

rrs-rrlA IGS				*ospC*	
RSP[f] (no. of isolates sharing the same allele)	IGS type[a]	IGS subtype[a]	RST[b]	*ospC* AT[g]	*ospC* group[c]
1 (12)	1	1A	1	1	A
3 (25)	2	2A	2	11	K
4 (7)	2	2D	2	8	H
7 (11)	3	3A	1	2	B
6 (7)	4	NI[d]	2	6	F
20 (15)	4	4A	2	13	N
14 (4)	5	NI	3	4	D
15 (3)	5	NI	3	3	C
16 (3)	5	NI	3	10	J
17 (1)	5	NI	3	16	NT[e]
9 (7)	6	6A	3	12	M
18 (6)	6	6B	3	7	G
10 (12)	7	7A	3	9	I
12 (6)	8	8A	3	15	U
13 (2)	8	8C	3	14	T
19 (6)	9	NI	3	5	E

[a] *rrs-rrlA* typing based on reference 3.
[b] *rrs-rrlA* typing based on reference 20.
[c] *ospC* groups according to references 34 and 46.
[d] NI, not identified.
[e] NT, new type.
[f] *rrs-rrlA* typing based on this study.
[g] *ospC* typing based on this study.

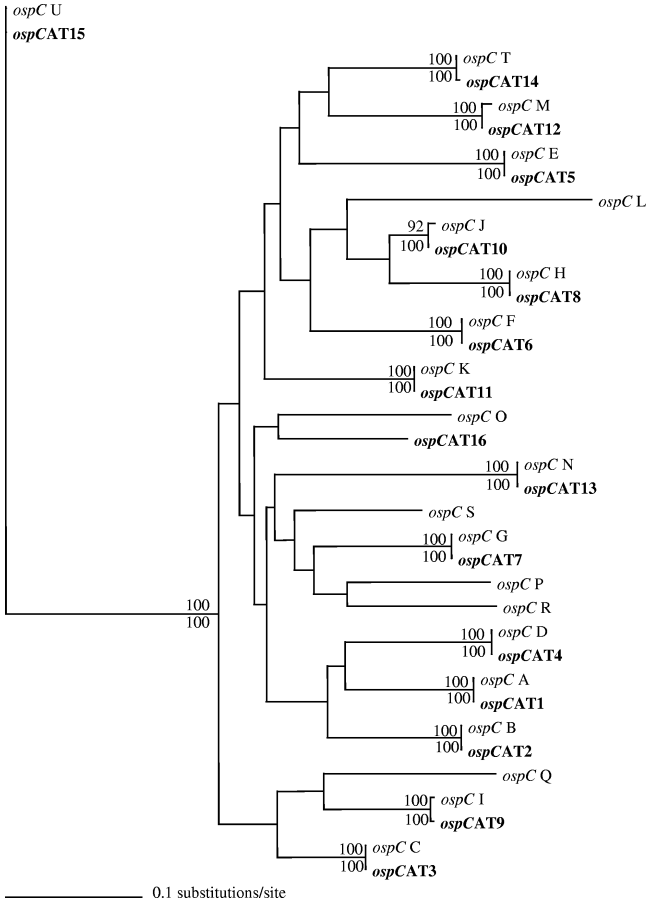

FIG. 2. Unrooted maximum-likelihood phylogenetic tree based on *ospC* data set and GenBank reference strains. Nonparametric bootstrap values for nodes with >70% support in both maximum-likelihood and maximum-parsimony analyses are above and below the branches, respectively. *B. burgdorferi ospC* alleles used in this study are in bold.

of the incongruence involved nodes with strong bootstrap support in both trees. For example, *ospC* AT10/RSP16 and *ospC* AT8/RSP4 pairing was well supported in the *ospC* tree but both grouped with different *ospC*/RSP allele profiles with >70% bootstrap support on the *rrs-rrlA* tree.

Recombination. Three recombination methods (Geneconv, MaxChi, and Chimaera) were used to assess the presence of recombination at *rrs-rrlA* and *ospC*. None of the methods detected evidence of recombination at *rrs-rrlA*. The same analyses were performed on the *ospC* alignment. To reduce the risk that the clustering of polymorphic sites at *ospC* resulted from selection rather than recombination, an alignment that contained only third codon position nucleotides was used. Three putative recombination events were detected. All of them involved *ospC* group C as a putative recipient and *ospC* group B as one of the putative donors. In addition, *ospC* groups K, M, and H were detected as possible donors with a significance level of $P < 0.001$ (Fig. 4). These observations, in addition to the overlapping positions of recombination breakpoints, however, suggest that the detected recombination events most likely represent a single recombination involving three different possible combinations of parents. There were no traces of recombination identified in the newly described *ospC* group represented by *ospC* AT16.

DISCUSSION

The present study is the first large-scale sequence-based investigation of the genetic diversity of *B. burgdorferi* clinical isolates. *rrs-rrlA* and *ospC* have previously received much attention as genetic markers in epidemiological and population studies of *B. burgdorferi*, as well as loci associated with disease severity and dissemination in mice and humans (1, 3, 30, 34, 43, 44, 46, 47). In the present data set, 16 distinct *rrs-rrlA* and 16 distinct *ospC* alleles were identified. In addition, strong linkage

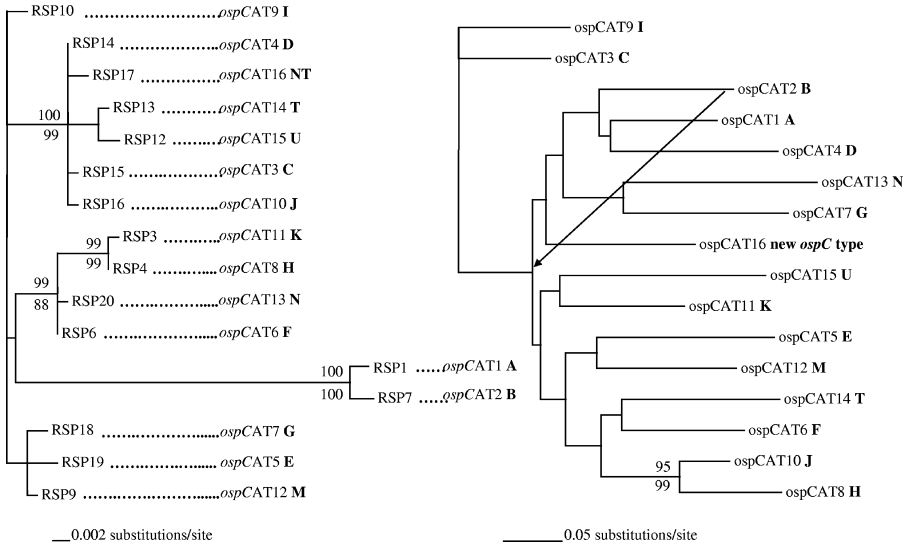

FIG. 3. Unrooted maximum-likelihood phylogenetic trees based on *rrs-rrlA* and *ospC* alleles. Nonparametric bootstrap values for nodes with >70% support in both maximum-likelihood and maximum-parsimony analyses are above and below the branches, respectively. The arrow indicates the differences between the maximum-likelihood and maximum-parsimony tree topologies supported by bootstrap values of >70% in either a maximum-likelihood or a maximum-parsimony analysis.

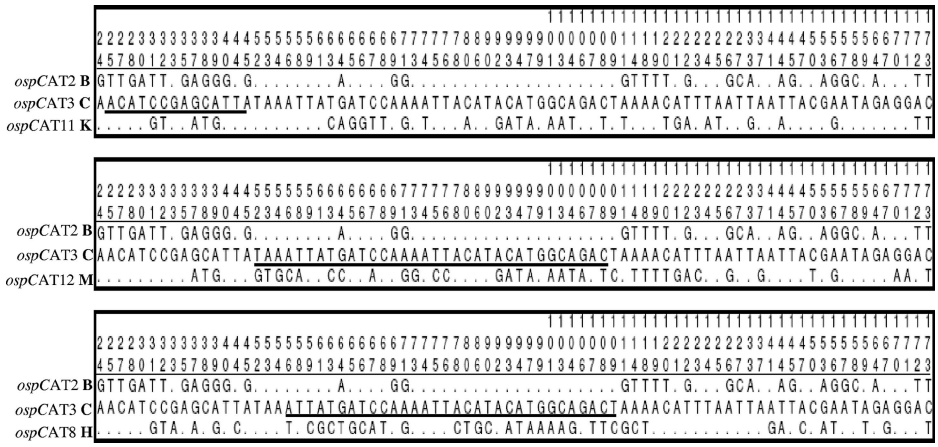

FIG. 4. Alignment of polymorphic nucleotide sites at third codon positions from *ospC* alleles involved in the putative recombination events. Three possible recombination events are shown. For each panel, the middle sequence represents a putative daughter sequence (the recombinant). The top and bottom sequences represent putative parental sequences. A dot indicates nucleotide identity with the putative daughter sequence. Underlining indicates putative recombination breakpoints identified by at least two recombination methods at $P < 0.001$.

among these loci and the presence of recombination at *ospC* were found.

Based on genetic variation, 21 *ospC* groups have been identified in *B. burgdorferi* worldwide, of which 16 have been found in the northeastern United States (30, 34, 46). We identified 15 *ospC* alleles that were representative of 15 previously defined *ospC* groups found in the northeastern United States. In addition, one unique *ospC* allele (*ospC* AT16) was found and, based on the criterion of sequence similarity, can be considered a representative of a new *ospC* group (46). Recently, *B. burgdorferi* was divided, based on *rrs-rrlA* sequence variation over a 250-bp region, into at least nine major *rrs-rrlA* (IGS) genotypes (3). Each of the 16 RSP alleles identified in the present study corresponded to one of these nine major IGS genotypes. However, three of the IGS types (2, 5, and 8) represented more than one *ospC* group. In the present analysis, an 812-bp region of *rrs-rrlA* was used. As a result of using more extensive sequence information, it was possible to link each *ospC* allele with a unique *rrs-rrlA* allele. The association of *ospC* with other genomic loci in *B. burgdorferi* has previously been reported (1, 3, 16, 30, 31, 46). It has been suggested, based on the sequence analyses of one chromosomal locus and multiple plasmid-borne polymorphic loci, that the *B. burgdorferi* population structure is dominated by balanced polymorphism at *ospC* and that the *ospC* groups could be viewed as evolutionarily stable clonal complexes (31).

In the present data set, each *rrs-rrlA*/*ospC* lineage was represented by a single *ospC* and a single *rrs-rrlA* allele, despite the fact that additional diversity of *rrs-rrlA* and *ospC* alleles was found in *Ixodes scapularis* ticks (3, 26, 46) and/or human-derived samples (34). One could hypothesize that the clonal lineages found in clinical samples of *B. burgdorferi* represent more pathogenic genotypes and therefore underestimate the genotypic diversity in the natural population and overestimate the extent of clonality (36). Alternatively, the allelic combinations found in the present study could represent the predominant clonal lineages that circulate in the ticks in the local area. It is also possible that the observed clonal selection arose from selective pressure during cultivation (20, 25).

The presence of additional *rrs-rrlA* alleles may suggest that a single *ospC* group can be associated not only with closely related *ospC* alleles (i.e., members of the same group have sequence difference of <2%) (46) but also with several different *rrs-rrlA* alleles. This phenomenon could be a result of diversification processes, such as point mutations and/or recombination, within each *ospC* clonal type that gives rise to *ospC* clonal complexes (7, 31). It is also possible that *rrs-rrlA* alleles represent different strains of *B. burgdorferi* that could share the same *ospC* group. This would be consistent with the hypothesis that balancing polymorphism may predate divergence among *B. burgdorferi* species (31). Indeed, it has been shown previously that *rrs-rrlA* alleles match putative multilocus sequence types (MLSTs) (1). However, this MLST was based on plasmid genes rather than housekeeping genes located on the chromosome. Furthermore, some of the loci included in the MLST analysis are located on plasmids that may be absent in certain *B. burgdorferi* strains (14, 38).

A number of studies have suggested extensive intra- and interspecies recombination at *ospC* among *B. burgdorferi* sensu lato species (6, 15, 21). Recombination at *ospC* was also reported in *B. burgdorferi* strains found in the tick population in the northeastern United States (3, 46). In the present study, recombination at *ospC* in *B. burgdorferi* clinical isolates was analyzed. The clonal model of bacterial evolution implies that a genome forms a single linkage group and that all loci will record the same evolutionary history (6, 8). The extent of phylogenetic congruence among *rrs-rrlA* and *ospC* phylogenetic trees was investigated. The observed discrepancy between the trees in statistically highly supported branches could be indicative of a history of recombination. Therefore, the presence of recombination at *ospC* was further investigated by analyzing the distribution of polymorphic sites within a sequence alignment. The identification of clustered polymorphism from third codon position in *ospC* group C provided the strongest evidence of recombination at *ospC* within the local *B. burgdorferi* population. Given the conservative nature of these recombination analyses, however, it is possible that the number of putative recombination events has been underestimated.

The strong linkage between *rrs-rrlA* and *ospC* appears to argue against recombination at *ospC*. Linkage disequilibrium is a well-acknowledged sign of clonality; however, it can be apparent even in recombining populations (7, 8). This might imply a low rate of recombination at *ospC* (6) that is not strong enough to disrupt the observed linkage. Since *ospC* recombinants are under strong positive selection (6), the recombination at *ospC* can be commonly detected, even if the frequency of recombination would be vanishingly small (8). Alternatively, *ospC* clones may have emerged and spread rapidly due to strong selective pressure but would be unstable over the long term (24). In addition, possible epistatic interactions among *ospC* and chromosomal loci, population bottlenecks accompanied by founder effects, and/or the presence of a strong balancing selection at *ospC* could result in the observed *ospC*-chromosome association (1, 30, 31, 46).

In the northeastern United States, *I. scapularis* nymphs are the main vectors involved in the transmission of *B. burgdorferi* to humans. The genetic diversity of *B. burgdorferi* in questing nymphs should reflect the entire spectrum of genotypes that humans might be exposed to in nature. The patients diagnosed at the Lyme Disease Practice of the Westchester Medical Center that were chosen for the present study were infected with *B. burgdorferi* genotypes that represent virtually all of the major IGS types and *ospC* groups found in the questing *I. scapularis* nymphs in this region (2, 3, 12, 30, 46). The exception was *ospC* group L, which was not identified in patients and was found only at a very low frequency in ticks, suggesting that the lack of this *ospC* group in clinical samples could simply be due to sampling error. In addition, a new *ospC* group that had not been previously described was isolated from one patient. The congruence of genotype diversity between clinical and tick-derived specimens suggests that any IGS or *ospC* genotype found in nature is capable of infecting humans.

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