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### ► **To cite this version:**

Pauline Trezel, Mickaël Guérin, Hugo da Ponte, Irene Maffucci, Stéphane Octave, et al.. Borrelia surface proteins: new horizons in Lyme disease diagnosis. Applied Microbiology and Biotechnology, 2025, 109, pp.156. <10.1007/s00253-025-13490-6>. <hal-05139074>

**HAL Id: hal-05139074**

**<https://hal.science/hal-05139074v1>**

Submitted on 2 Jul 2025

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# *Borrelia* surface proteins: new horizons in Lyme disease diagnosis

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Received: 7 March 2025 / Revised: 11 April 2025 / Accepted: 14 April 2025  
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## Abstract

Spirochetes of the genus *Borrelia* are the causative agents of vector-borne diseases, including Lyme borreliosis (LB). Clinical LB diagnosis is challenging due to the heterogeneous and nonspecific symptoms among patients further complicated by the potential for co-infection with *Borrelia* species and other pathogens. Current diagnosis is based on an indirect serological approach with limitations such as early-stage low sensitivity and cross reactivity. This review explores the potential of *Borrelia* surface proteins as biomarkers for a more accurate diagnosis of LB, with a particular interest in proteins involved in (i) tissue adhesion, essential for host colonization, (ii) antigenic variation, and (iii) immune evasion. By detailing the role of the distinct surface proteins, the review highlights their potential as biomarkers in order to improve the detection of LB, particularly in the early stages. In addition to their diagnostic value, they also represent potential targets for the development of new therapeutic strategies.

## Keypoints

- Various *Borrelia* surface proteins drive host colonization and immune evasion.
- Novel bacterial proteins biomarkers are needed for improved diagnosis and therapy.
- State of the art on *Borrelia* surface proteins and their associated role.

**Keywords** Lyme borreliosis · *Borrelia* · Surface proteins · Biomarker · Diagnosis

## Introduction

*Borrelia* species are known to cause several vector-borne illnesses including Lyme borreliosis (LB) (also known as Lyme disease) and relapsing fever (RF) (Stanek et al. 2012; Cutler et al. 2017). Within the *Borrelia* genus, various species have been described as the etiological agents of LB (Takano et al. 2010; Margosid et al. 2018). In North America, *Borrelia burgdorferi* sensu stricto (*Bbss*) has been identified as the predominant causative agent of LB (Mead 2022). Conversely, in Asia and Europe, *Borrelia afzelii* and *Borrelia garinii* have been observed to be more frequently associated with LB (Marques et al. 2021). Altogether, these bacteria are classified as the *Borrelia burgdorferi* sensu lato complex (*Bbsl*). A second group of *Borrelia* species, including *Borrelia miyamotoii*, has been implicated as etiological agents of RF (Warrell 2019; Burde et al. 2023).

*Borrelia*, spirochetes first described in 1907, exhibit several distinctive characteristics. In their analysis of the *Borrelia burgdorferi* B31 strain genome, Fraser and colleagues

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(Fraser et al. 1997), showed that this bacterium possesses a single large linear chromosome and numerous linear and circular plasmids, totaling approximately 1500 kbp (Casjens et al. 2000). By analyzing this genome, *Borrelia* proteins can broadly be categorized into (i) housekeeping and metabolic functions proteins, primarily encoded by chromosomal genes (Ren et al. 2023), and (ii) interaction with host and vector proteins, mainly encoded by plasmid genes, including integral outer membrane proteins (OMPs) and outer surface proteins (Osps) (Casjens et al. 2018). The *Borrelia* genus bacteria are distinguished by a unique diderm structure (Skerman et al. 1989; Kudryashev et al. 2009). This diderm, which consists of an inner membrane and an outer membrane (OM), is crucial for numerous biological functions including (i) motility driven by periplasmic flagella (Liu et al. 2009; Zückert 2019) and (ii) the dynamic interaction between the bacterium and its host. The latter is primarily driven by the OM, to which belong the numerous adhesins and Osps that mediate the attachment to the host tissues and the evasion of the immune system, a key aspect of LB pathogenesis (Wawrzyniak et al. 2020; Martin et al. 2023).

An estimated 700,000 cases of LB are reported each year in North America and Europe. The disease, transmitted by infected ticks of the genus *Ixodes*, can be divided into three stages: (i) early localized, (ii) early disseminated, and (iii) late disseminated (Skar and Simonsen 2024). The clinical manifestations of LB are various, ranging from a characteristic skin lesion known as erythema migrans (EM), localized at the initial infection site, to severe systemic symptoms such as Lyme arthritis, Lyme carditis, and Lyme neuroborreliosis (Garza et al. 2019; Miller and Aucott 2021).

Despite the clinical diagnosis of LB relies on the EM, it may not always be visible or can be challenging to differentiate from other skin lesions (Schotthoefer et al. 2022). Indeed, it is considered that EM has an incidence rate of approximately 80%, which widely varies from 27 to 90% (Bingham et al. 1995; Steere 2001; Stanek and Strle 2022). Without EM identification, diagnosing LB becomes more complex due to its broad spectrum of clinical presentations (Strle and Stanek 2009; Garza et al. 2019). The conventional method for diagnosing LB is serological testing, an indirect approach that identifies antibodies (Abs) produced by the host organism in response to the infection (Branda et al. 2013). Current diagnostic guidelines endorsed by American and European organizations rely on standard two-tiered testing and modified two-tiered testing serological assays (Molins et al. 2014; Miraglia 2016, 2020). However, these approaches suffer from significant limitations. These include (i) the inability in reliably detecting infections in their early stages (Marques 2015; Aslam et al. 2017), (ii) the capacity of *Borrelia* bacteria for immune evasion through antigenic variation, modulation of the host immune response, and intracellular survival (Haque et al. 2022; Rana et al.

2023), (iii) the occurrence of cross-reactivity and potential co-infections with other pathogens (Hu et al. 1981; Cabello et al. 2022), and finally (iv) the difficulty in distinguishing between past, resolved, and active infections (Aguero-Rosenfeld et al. 2005; Nielsen and Miller 2025).

In particular, this latter point contributes to the chronic Lyme disease (CLD) controversy advocated by the International Lyme and Associated Disease Society (ILADS) and patient groups, which argues for persistent infection requiring prolonged antibiotic therapy (Cameron et al. 2014; Shor et al. 2019). Conversely, the Infectious Diseases Society of America (IDSA) and European guidelines, including the Haute Autorité de Santé (HAS) (Wormser 2006; Lantos et al. 2021; Haute Autorité de Santé 2025), refute CLD and endorse post-treatment Lyme disease syndrome (PTLDS) for persistent symptoms (asthenia, cognitive dysfunction, polyalgia) following a proven and appropriately treated LB (Haute Autorité de Santé 2025; Fallon et al. 2025). *Borrelia* pleomorphism, which can be influenced by the presence of antibiotics, may contribute to treatment challenges (Rudenko et al. 2019; Bowman et al. 2024). These limitations and the lack of specific anti-infectious therapies for PTLDS, due to limited evidence of active infection (Zafar et al. 2024), highlight the urgent need for novel diagnostic tools.

In such a context, direct molecular diagnostic techniques are promising and attractive. Nevertheless, the ability of *Bbsl* to be localized in specific anatomical niches, makes its detection in blood or urine complex (Klempner et al. 2001; Aguero-Rosenfeld et al. 2005; Ružić-Sabljić and Cerar 2017; Branda and Steere 2021; Strnad et al. 2023). The low blood bacterial load, often below conventional detection thresholds (Das et al. 2020; Guérin et al. 2023), necessitates the use of invasive samples such as skin biopsies for EM, synovial fluid for arthritis, and cerebrospinal fluid for neuroborreliosis, to improve sensitivity (Wormser et al. 2006; Stanek et al. 2012). The use of non-conventional and less invasive fluids such as genital fluids is being explored (Middelveen et al. 2015, 2021; Rudenko and Golovchenko 2021). Finally, a pre-treatment could potentially facilitate detection by releasing circulating bacteria (Verderosa et al. 2019; Grooters et al. 2024). These observations highlight the need to broaden diagnostic approaches and identify new biomarkers to improve the management of Lyme disease.

This review aims to provide a comprehensive exploration of the several types of Lyme *Borrelia* proteins, with an emphasis on those mediating host–pathogen interactions. Although the presence of these proteins with varying degrees of sequence similarity may be possible in RF *Borrelia*, this review will primarily focus on Lyme *Borrelia*. When appropriate, the associated orthologs and paralogs are also listed. As a reminder, orthologs are proteins encoded by genes originating from a single ancestral gene in the last common ancestor of *Borrelia* genome (Koonin 2005), and

paralogs are proteins encoded by homologs genes related via duplication and generating couples of the same sequence copies in the same lineage (Phillips 2006). The potential of these proteins as biomarkers for the diagnosis of LB will be assessed. The scope of this review encompasses an overview of current diagnostic techniques and a detailed examination of (i) the proteins involved in tissue adhesion, which are critical for the initial colonization of the host; and (ii) the proteins responsible for immune evasion, allowing *Borrelia* to persist in the host despite immune defenses. Both groups of proteins include multifunctional proteins that facilitate adaptation to the host environment, enabling long-term survival and pathogenicity. Identification and analysis of the expression profiles of such key proteins could provide valuable insights into promising targets for the development of novel diagnostic tests.

## Host tissue adherence: foundations of infection and diagnostic targets

The adhesion of *Borrelia* to host tissues is a crucial step in the establishment of the infection (Antonara et al. 2011). The proteins involved in this process are called surface adhesins (Coburn et al. 2005). By virtue of their capacity to bind to a diverse array of host cell receptors and ligands, including plasminogen (Brissette et al. 2009b) and a variety of extracellular matrix (ECM) components, such as glycosaminoglycans (GAGs), fibronectin, and laminin (Verma et al. 2009; Salo et al. 2015; Niddam et al. 2017; Lin et al. 2017), adhesins underscore the importance of these interactions in leading the host's inflammatory response to infection (Oosting et al. 2016). Beyond their physiological role, these proteins offer promising targets for the development of diagnostic tests (Coburn et al. 2013; Siitonen et al. 2017).

### ECM-binding-proteins

#### Bgp: glycosaminoglycan-binding protein

GAGs are long carbohydrate chains, which are ubiquitously expressed, particularly in the extracellular matrix of the skin, neurons, and host tissue junctions (Jackson et al. 1991; Isaacs 1994). The disaccharide units, composing the GAGs typically, consist of hexuronic acid and an amino sugar linked by a glycosidic bond. Variations in these disaccharide units give rise to different classes of GAGs, including chondroitin sulfate, dermatan sulfate, heparin, heparan sulfate, hyaluronic acid, and keratan sulfate (Gandhi and Mancera 2008). The covalent attachment of one or more glycosaminoglycan (GAG) chains to a core protein results in the formation of a proteoglycan (Carrino et al. 2011). These molecules play a crucial role in tissue organization, cell adhesion, and

cell signaling (Karamanos et al. 2018). Similar to GAGs, proteoglycans are classified according to their GAG composition (Martínez et al. 2020). Several *Borrelia* proteins have been shown to interact with GAGs (Lin et al. 2017).

*Borrelia* glycosaminoglycan-binding protein (Bgp) is a prominent example of this family. Bgp, a 26 kDa surface-exposed protein of *Borrelia burgdorferi*, is encoded by the bb0588 gene localized on the linear chromosome (Table 1) (Parveen et al. 2006). Bgp binds to heparin-related GAGs (Parveen and Leong 2000) (Fig. 1), and studies have shown that its loss impairs *Borrelia* tissue adherence and colonization, resulting in reduced inflammatory manifestations (Parveen et al. 2006). Although Bgp is not essential for infectivity, it is an important virulence factor for *Borrelia burgdorferi* (Schlachter et al. 2018). In addition, Cluss and collaborators have demonstrated that Bgp, exposed on intact bacteria surface (Parveen and Leong 2000), is also secreted extracellularly, particularly during the logarithmic growth phase (Cluss et al. 2004). Considering the functions of Bgp in adhesion and in secretion, its potential as a diagnostic biomarker requires further investigation.

#### DbpA and DbpB: decorin-binding proteins

Together with Bgp, *Borrelia burgdorferi* possesses two additional proteins involved in ECM binding, the decorin-binding proteins A and B (DbpA and DbpB) (Fig. 1; Table 1). These proteins recognize connective tissue through the interaction with molecules contained in it, such as decorin, a smaller proteoglycan expressed on collagen fibrils (Zhang et al. 2006). It has been demonstrated that *Borrelia* deficient in *dbpA/B* exhibit impaired various tissue colonization and also impaired persistence of infection (Shi et al. 2008). In addition, decorin-deficient mice were found to be resistant to *Borrelia burgdorferi* infection, with reduced bacterial loads in joints and less severe arthritis (Blaho et al. 2011).

More precisely, DbpA (20-kDa) binds with greater affinity than DbpB (18-kDa) to decorin, with an estimated dissociation constant ( $K_D$ ) of  $1-4.10^{-7}$  M for DbpA and a  $K_D$  superior to  $10^{-7}$  M for DbpB (Guo et al. 1998), though both proteins are essential for optimal adhesion. With 40% sequence identity and by studying DbpA and DbpB structures using NMR, Feng and Wang demonstrated that DbpB shares the same overall topology as DbpA but possesses a shorter terminal helix. This results in a longer, unstructured C-terminal tail enriched in basic amino acids. This structural difference likely contributes to distinct mechanisms (Feng and Wang 2015), and leads to difference in DbpA versus DbpB's affinity for decorin (Guo et al. 1998). These proteins are encoded by the *dbpB/A* operon (*bba24* and *bba25* genes), localized on the linear plasmid lp54 in *Borrelia burgdorferi* B31. Interestingly, the DbpB sequence is highly conserved, while the DbpA sequence is more variable between

**Table 1** Comparative analysis of Borrelial surface proteins using BLAST: sequence identity and alternative names

Name	Synonyms	PDB code	% identity of prevalent European <i>Bbsl</i> strains associated with Lyme disease					Others <i>Bbsl</i> strains associated with Lyme disease	
			<i>Bb</i> ss	<i>B. afzelii</i>	<i>B. bavariensis</i>	<i>B. bissettiae</i>	<i>B. garinii</i>	<i>B. spielmannii</i>	
BAD16	-	-	70%	<b>100% (Pko)</b>	72%	73%	71%	95%	<ul style="list-style-type: none"> <li><i>B. japonica</i>: &lt;70%</li> <li><i>B. kurtenbachii</i>: 73%</li> <li><i>B. turdi</i>: &lt;70%</li> <li><i>B. valaisiana</i>: 71%</li> </ul>
BBK32	-	6 NIL	<b>100% (B31)</b>	71%	85%	88%	99%	70%	<ul style="list-style-type: none"> <li><i>B. carolinensis</i>: &lt;70%</li> <li><i>B. japonica</i>: &lt;70%</li> <li><i>B. kurtenbachii</i>: 73%</li> <li><i>B. kurtenbachii</i>: &lt;70%</li> <li><i>B. lanei</i>: &lt;70%</li> <li><i>B. yangtzensis</i>: &lt;70%</li> <li><i>B. yangtzensis</i>: &lt;70%</li> </ul>
BGA66	-	-	NA	<70%	<b>100% (Pbi)</b>	NA	93%	<70%	<ul style="list-style-type: none"> <li><i>B. turdi</i>: &lt;70%</li> <li><i>B. valaisiana</i>: &lt;70%</li> <li><i>B. yangtzensis</i>: &lt;70%</li> </ul>
BGA71	-	6 FMH	NA	<70%	<b>100% (Pbi)</b>	NA	89%	<70%	<ul style="list-style-type: none"> <li><i>B. tanukii</i>: &lt;70%</li> <li><i>B. valaisiana</i>: 75%</li> </ul>
BGD19	-	-	95%	70%	84%	84%	<b>100% (Pbr)</b>	<70%	<ul style="list-style-type: none"> <li><i>B. carolinensis</i>: &lt;70%</li> <li><i>B. japonica</i>: &lt;70%</li> <li><i>B. kurtenbachii</i>: 72%</li> </ul>
Bgp	-	-	<b>100% (B31)</b>	87%	85%	89%	83%	86%	<ul style="list-style-type: none"> <li><i>B. americana</i>: 96%</li> <li><i>B. andersonii</i>: 92%</li> <li><i>B. californiensis</i>: 88%</li> <li><i>B. carolinensis</i>: 89%</li> <li><i>B. chilensis</i>: 83%</li> <li><i>B. finlandensis</i>: 92%</li> <li><i>B. japonica</i>: 85%</li> <li><i>B. kurtenbachii</i>: 89%</li> </ul>
BmpA	p39	-	<b>100% (N40)</b>	88%	88%	89%	88%	NA	<ul style="list-style-type: none"> <li><i>B. andersonii</i>: 93%</li> <li><i>B. californiensis</i>: 89%</li> <li><i>B. carolinensis</i>: 90%</li> <li><i>B. finlandensis</i>: 95%</li> <li><i>B. japonica</i>: 89%</li> <li><i>B. kurtenbachii</i>: 90%</li> </ul>
CspA	CRASP-1; BbCRASP-1, BBA68, ZS7.A68, FHBP	1 W33	<b>100% (B31)</b>	NA	NA	71%	NA	NA	<ul style="list-style-type: none"> <li><i>B. americana</i>: &lt;70%</li> <li><i>B. andersonii</i>: &lt;70%</li> <li><i>B. californiensis</i>: &lt;70%</li> <li><i>B. carolinensis</i>: &lt;70%</li> <li><i>B. turdi</i>: 73%</li> <li><i>B. valaisiana</i>: 81%</li> </ul>
CspZ	CRASP-2, BbCRASP-2, BBH06	6 ATG	<b>100% (B31)</b>	85%	89%	95%	88%	84%	<ul style="list-style-type: none"> <li><i>B. americana</i>: &lt;70%</li> <li><i>B. andersonii</i>: &lt;70%</li> <li><i>B. californiensis</i>: &lt;70%</li> <li><i>B. carolinensis</i>: &lt;70%</li> </ul>
DbpA	Osp17; p18	4ONR	<b>100% (B31)</b>	NA	<70%	70%	<70%	NA	<ul style="list-style-type: none"> <li><i>B. americana</i>: &lt;70%</li> <li><i>B. andersonii</i>: 72%</li> <li><i>B. californiensis</i>: &lt;70%</li> <li><i>B. carolinensis</i>: &lt;70%</li> <li><i>B. finlandensis</i>: 84%</li> </ul>

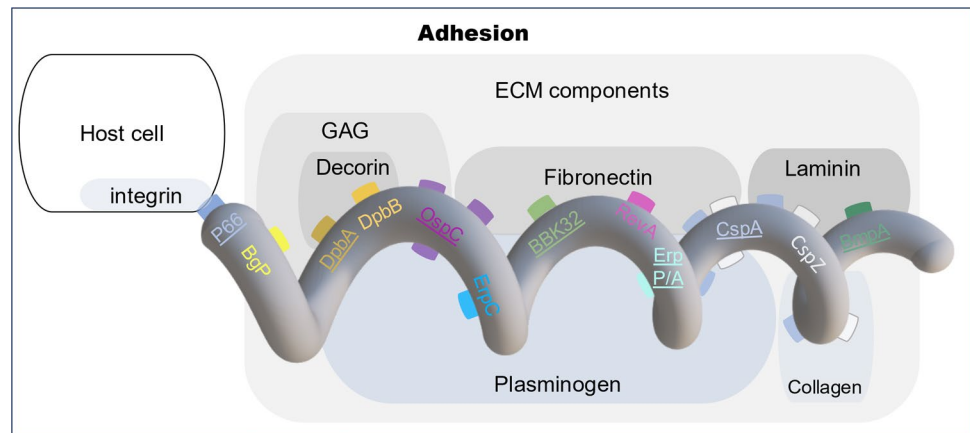
Name	Synonyms	PDB Code	% identity of prevalent European <i>Bbsl</i> strains associated with Lyme disease					Others <i>Bbsl</i> strains associated with Lyme disease	
			<i>Bb</i> ss	<i>B. afzelii</i>	<i>B. bavariensis</i>	<i>B. bissettiae</i>	<i>B. garinii</i>	<i>B. spielmannii</i>	
DbpB	-	2MVG	<b>100% (B31)</b>	<70%	<70%	77%	<70%	<70%	<ul style="list-style-type: none"> <li><i>B. americana</i>: &lt;70%</li> <li><i>B. andersonii</i>: 80%</li> <li><i>B. californiensis</i>: 73%</li> <li><i>B. carolinensis</i>: 75%</li> <li><i>B. chilensis</i>: &lt;70%</li> <li><i>B. finlandensis</i>: 95%</li> <li><i>B. japonica</i>: &lt;70%</li> <li><i>B. kurtenbachii</i>: 76%</li> </ul>

Table 1 (continued)

Name	Synonyms	PDB Code	% identity of prevalent European Bbsl strains associated with Lyme disease					Others Bbsl strains associated with Lyme disease	
			<i>Bb ss</i>	<i>B. afzelii</i>	<i>B. bavariensis</i>	<i>B. bissettae</i>	<i>B. garinii</i>	<i>B. spielmannii</i>	
ErpP	CRASP- 3; BbCRASP- 3; BBN38	4BOB	<b>100% (B31)</b>	77%	74%	70%	77%	75%	<ul style="list-style-type: none"> <li>• <i>B. americana</i>: 72%</li> <li>• <i>B. californiensis</i>: 77%</li> <li>• <i>B. finlandensis</i>: &lt;70%</li> <li>• <i>B. kurtenbachii</i>: 71%</li> </ul>
ErpC	CRASP- 4; BbCRASP- 4	4BOD	<b>100% (B31)</b>	< 70%	70%	84%	< 70%	< 70%	<ul style="list-style-type: none"> <li>• <i>B. americana</i>: 70%</li> <li>• <i>B. californiensis</i>: &lt;70%</li> <li>• <i>B. kurtenbachii</i>: 80%</li> <li>• <i>B. lanei</i>: 75%</li> </ul>
ErpA	CRASP- 5; BbCRASP- 5; ErpI; ErpN; BBP38, BBL39		<b>100% (B31)</b>	< 70%	< 70%	71%	< 70%	NA	<ul style="list-style-type: none"> <li>• <i>B. americana</i>: &lt;70%</li> <li>• <i>B. finlandensis</i>: &lt;70%</li> <li>• <i>B. kurtenbachii</i>: 72%</li> <li>• <i>B. lanei</i>: 72%</li> </ul>
OspC	-	7BML	<b>100% (B31)</b>	78%	NA	77%	77%	NA	<ul style="list-style-type: none"> <li>• <i>B. californiensis</i>: 75%</li> <li>• <i>B. carolinensis</i>: 78%</li> <li>• <i>B. finlandensis</i>: 77%</li> <li>• <i>B. japonica</i>: 76%</li> <li>• <i>B. kurtenbachii</i>: 84%</li> </ul>
p66	-		<b>100% (B31)</b>	92%	91%	95%	91%	92%	<ul style="list-style-type: none"> <li>• <i>B. americana</i>: 95%</li> <li>• <i>B. andersonii</i>: 95%</li> <li>• <i>B. californiensis</i>: 95%</li> <li>• <i>B. carolinensis</i>: 94%</li> </ul>
RevA	-		<b>100% (B31)</b>	< 70%	< 70%	86%	< 70%	< 70%	<ul style="list-style-type: none"> <li>• <i>B. americana</i>: &lt;70%</li> <li>• <i>B. californiensis</i>: &lt;70%</li> <li>• <i>B. finlandensis</i>: &lt;70%</li> <li>• <i>B. japonica</i>: &lt;70%</li> <li>• <i>B. kurtenbachii</i>: 70%</li> </ul>
VisE	-	1L8 W	<b>100% (B31)</b>	< 70%	NA	76%	NA	NA	<ul style="list-style-type: none"> <li>• <i>B. lanei</i>: 74%</li> <li>• <i>B. mayonii</i>: 76%</li> <li>• <i>B. valaisiana</i>: &lt;70%</li> <li>• <i>B. yangtzensis</i>: &lt;70%</li> </ul>

*Bbss*, *Borrelia burgdorferi* sensu stricto; NA, non-applicable as no protein expression by this *Borrelia* specie, 70%, BLAST limitations up to 70% sequences identity (Altschul et al. 2005); Bolded percentage: blasted species with specific strains in parentheses and PDB code available for crystal structure by X-RAY diffraction

**Fig. 1** Borrelial proteins implicated in host-tissue adherence and their binding targets. ECM, extracellular matrix; GAG, glycosaminoglycans. Underlined proteins are used in commercial diagnostic tests



different strains (Roberts et al. 1998; Blevins et al. 2008). These sequence variations according to the strains lead to differences in the clinical manifestations of LB. Parveen and colleagues have shown that DbpA from different strains have different affinities for decorin-forming GAGs leading to tissue tropism (Parveen et al. 1999). More in detail, the transfection of bacterial strains naturally lacking DbpA with PBr DbpA, which binds to dermatan sulfate with a  $K_D$  of 0.21  $\mu\text{M}$ , results in bacterial colonization of the heart, whereas expression of N40 DbpA, which binds to dermatan sulfate with a  $K_D$  of 3.10  $\mu\text{M}$ , results in localization to the knee (Benoit et al. 2011; Lin et al. 2014). Thus, these two proteins are primarily responsible for Lyme arthritis by mediating bacterial attachment to decorin-rich tissues such as joints, thereby creating a protective niche for *Borrelia burgdorferi* against the immune response (Liang et al. 2004a; Salo et al. 2015). In consideration of their function in Lyme arthritis, these proteins have the potential to serve as biomarkers for the diagnosis of Lyme disease in patients with musculoskeletal symptoms.

### RevA: fibronectin-binding protein

*Bbsl* possesses RevA and RevB proteins, both able to bind the fibronectin, a dimeric ECM glycoprotein of around 250 kDa essential for cell adhesion, differentiation, and wound healing (Zollinger and Smith 2017) (Fig. 1). RevA, a 19 kDa protein, is encoded by the *revA* gene, located on the plasmid cp32. This gene is prevalent and well-conserved among Lyme *Borrelia* species, with certain isolates, including B31, harboring two copies (Floden et al. 2013). Conversely, RevB, which is encoded by the *revB* gene located on the cp9 plasmid, shares 28% amino acid sequence identity with RevA, and is often missing as many isolates, including B31 and infectious wild-type *Borrelia*, lack the cp9 plasmid (Brissette et al. 2010) (Table 1). Although RevB has been identified as a fibronectin binding protein, along with RevA, its biological function remains poorly understood and

requires further investigation. Due to the loss of the cp9 plasmid (Brissette et al. 2009a, 2010), *revB* is not a relevant diagnostic biomarker for Lyme disease, while this does not appear to be the case for RevA.

RevA expression in *Bb*, modulated in response to environmental temperature and pH (Gilmore et al. 2001; Carroll et al. 2001), is downregulated shortly after tick infection and an upregulation during the tick's blood meal on a mammalian host. A study has indicated a potential role for RevA in tissue tropism through interaction with the host's ECM. More precisely, mice infected with a RevA-deficient mutant exhibited reduced infection in cardiac tissues but showed increased arthritis (Byram et al. 2015). The involvement of RevA in tissue dissemination, coupled with the ability of patient antibodies from both North America and Europe to recognize recombinant RevA, suggest that RevA may serve as a promising biomarker for the diagnosis of LB (Brissette et al. 2010). Furthermore, given the high sequence conservation of RevA among Lyme *Borreliae*, it can be considered a valuable target for preventive or therapeutic interventions. Indeed, as demonstrated by numerous studies, passive immunization with anti-RevA antibodies has been shown to have the potential to prevent infection in murine models. This renders RevA a promising candidate for vaccine development (Floden et al. 2013).

### BmpA and paralogues: laminin-binding proteins

In contrast with the previously mentioned proteins, the BmpA protein (*Borrelia* membrane protein A), also called P39 antigen (39 indicating its molecular weight of 39 kDa), together with its three paralogues BmpB, BmpC, and BmpD, exhibits binding affinity for mammalian laminin but does not bind to others ECM components such as collagen nor fibronectin (Simpson et al. 1990; Verma et al. 2009) (Fig. 1; Table 1).

The *bmpa* gene is located on the main chromosome, adjacent to three paralogous genes called *bmpb*, *bmpc*, and

*bmpd*. Together, these four genes form a complex operon called the *bmp* genes. This operon is present in the majority of *Bbsl* (Gorbacheva et al. 2000; Åstrand et al. 2019). BmpA, BmpB, and BmpD sequences are highly conserved compared with BmpC. Microscopic studies by Verma and collaborators demonstrated that antibodies targeting BmpA effectively blocked the attachment of live *Borrelia burgdorferi* to laminin, pinpointing the laminin-binding domain of BmpA to its carboxy-terminal 80 amino acids (Verma et al. 2009).

Similarly to DbpA and DbpB, BmpA and BmpB are implicated in the maintenance of mammalian infection and Lyme arthritis. Indeed, a *Borrelia* strain mutant deficient in *bmpA/B* genes displayed infectivity in murine models but lacked persistence in the joints and did not cause severe arthritis (Pal et al. 2008). BmpA has been identified as a promising candidate biomarker for the diagnosis of LB, particularly in patients with arthritis. Studies have highlighted the frequency of immune responses to the BmpA antigen during the early stages of infection, making it a marker for serodiagnosis in early Lyme borreliosis through two-tiered IgM testing (Wormser 2006). Based on immunoblot and competitive ELISA, work from Bryksin and collaborators confirm that BmpA, as well as BmpB and BmpD proteins of *Bbss* B31, are expressed in infected patients (Bryksin et al. 2005). In this context, BmpA has been engineered alongside other recombinant proteins to create divalent chimeric proteins (BmpA-BBK32 and BmpA-BBA64). These chimeric proteins have been shown to exhibit a strong reactivity with IgG from the sera of Lyme borreliosis patients, making them potential diagnostic targets for distinguishing between positive and negative sera (Grażlewska et al. 2021; Grażlewska et al. 2023). Finally, it should be noted that the Optiplex *Borrelia*, a diagnostic test using cell lysate and recombinant antigens, including the BmpA protein (p39) from *Borrelia afzelii* to detect IgM and IgG antibodies, has recently been improved (Wojciechowska-Koszko et al. 2022). In the long term, the goal is to replace the two-step serological diagnostic test, although for the current period, it remains to be used in a complementary manner (Wojciechowska-Koszko et al. 2022). The role of BmpC and BmpD remains to be elucidated, and thus their potential as biomarkers remains to be confirmed.

### BBK32: GAG and fibronectin-binding protein

Previously mentioned, the chimeric protein BmpA-BBK32 exhibited robust reactivity with sera from individuals with LB, underlining the role of BBK32 as potential diagnostic marker (Grażlewska et al. 2021; Grażlewska et al. 2023). The 47-kDa outer surface lipoprotein BBK32, previously designated as p47, is encoded by the *bbk32* gene located on the linear plasmid 36 (lp36) (Probert and Johnson 1998)

(Table 1). The expression of the BBK32 protein, initially low in unfed ticks, increases significantly during engorgement and decreases after the blood meal, with maximal expression observed during mice infection (Li et al. 2006).

BBK32 can bind to various elements of the ECM such as GAGs like heparan sulfate and dermatan sulfate, and fibronectin, specifically gelatin within the collagen-binding domain (Probert and Johnson 1998; Fischer et al. 2006) (Fig. 1). By interacting with fibronectin and GAG, BBK32 proteins show adhesive and dissemination function. Indeed, its interaction with fibronectin has been demonstrated to slow bacterial movement and enables the bacteria to anchor to vascular surfaces (Moriarty et al. 2012). Furthermore, its GAG-binding has been shown to promote joint colonization as early as 1-h post-infection (Lin et al. 2015). Recent studies have explored BBK32's fibronectin-binding capacity to develop a novel electrochemical detection method using a biomimetic flow cell system (Flynn et al. 2023). Further diagnostic assay development and potential of this biomarker will be discussed in the following section ("BBK32-mediated complement inhibition" section).

### OspC: ECM components-binding protein

Another outer surface lipoprotein, OspC (Outer surface protein C) (22-kDa), is expressed on the bacterial surface during tick feeding and in the early stages of mammalian infection, as its production is temperature-dependent (Schwan et al. 1995). However, this same protein is expressed at low level during persistent infection, illustrating the variability of expression pattern according to the disease stage (Liang et al. 2004b). OspC, which is encoded by a gene located on a 26-kb circular plasmid, is highly heterogeneous among the *Bbsl* complex (Table 1). The central portion of OspC is highly variable, exhibiting at least 8% variability between groups and 22 distinct sequence types (A to U), (Wang et al. 1999). Certain OspC types (including A, B, I, and K, as well as C, D, and N) are associated with higher invasiveness (Seinost et al. 1999; Earnhart et al. 2005). Furthermore, studies have demonstrated a correlation between sequence variations within the OspC gene and the development of disseminated infection in humans (Hanincova et al. 2013) thereby influencing the tissue tropism of *Borrelia*. Indeed, Lin and collaborators have shown that certain OspC sequence variations allow *Bbss* to colonize multiple tissues, including joints, skin, heart, and bladder, whereas *Borrelia garinii* fails to colonize the joints (Lin et al. 2020). Crystallographic structures of OspC from different strains, such as HB19 and B31, have been determined, revealing a region with a strong negative electrostatic potential that may facilitate binding to positively charged host ligands. Notably, this feature is present only in strains known to cause invasive human disease (Kumaran et al. 2001).

OspC can bind to multiple host molecules (Fig. 1). (i) As an adhesin, OspC has been demonstrated to bind to components of the ECM, including fibronectin and GAGs such as dermatan sulfate, thus facilitating tissue-specific dissemination, for example in the joints. It is noteworthy that OspC is orthologous with Vsp proteins, the variable surface proteins that are characteristic of the RF *Borrelia* (Zückert et al. 2001). Some of these Vsp proteins, such as Vsp2 from *Borrelia turicatae*, can interact with GAGs in the extracellular matrix. *Borrelia turicatae* strains expressing Vsp2 colonize blood and joints more efficiently than those expressing Vsp1, which does not recognize GAGs (Pennington et al. 1997; Magoun et al. 2000). Collectively, these observations demonstrate that the ability to bind to GAGs, a characteristic shared by OspC, plays a crucial role in the tissue tropism of *Borrelia* (Lin et al. 2020). (ii) OspC also demonstrates a plasminogen-binding potential, thereby functioning as a plasminogen receptor on the *Borrelia* surface as demonstrated by co-immunofluorescence staining assays showing immobilization of plasminogen only by wild-type *Borrelia* expressing OspC. Following activation, plasmin can promote the dissemination of the bacterium by degrading host tissue barriers (Önder et al. 2012).

OspC is among the most extensively studied external surface proteins. Highly expressed during the early phases of infection due to its role in adhesion, which is critical for the establishment of infection, OspC has emerged as a promising marker for early infection detection (Schwan 2003; Earnhart and Marconi 2007; Caimano et al. 2019; Guérin et al. 2023), and has been identified as a potential target for vaccine development (Earnhart and Marconi 2007). In this context, investigations have been carried out on OspC-based subunit chimeritope vaccinogens. These are currently used in canine LB vaccines, and potentially in humans, inducing broad antibody responses (Marconi et al. 2020; Camire et al. 2021). The potential of OspC and a derived peptide of OspC as diagnostic biomarkers will be further explored in the following section (“OspC: refined immune evasion strategies” section).

### The CRASP family

The CRASP (Complement Regulator-Acquiring Surface Proteins) family comprises CRASP- 1 to – 5. Independently of their sequence alignment, structural similarities or phylogenetic relationship, they all share a common characteristic linked to their ability to bind complement proteins. A complex nomenclature system has been developed to accommodate the multiple names assigned to each member of the family (Table 1).

CRASP- 1 and CRASP- 2, also known as CspA and CspZ respectively, are two lipoproteins encoded by *cspA*

and *cspZ* genes whose expression varies throughout the infection phases (Bykowski et al. 2008). CspA is predominantly expressed in fed and unfed nymphal ticks and at the inoculation site early in infection, being the earliest expression, which becomes undetectable after 2 weeks of established mammalian infection. In contrast, CspZ is upregulated during mouse infection but downregulated in the tick midgut (Kraiczy and Würzner 2006; Bykowski et al. 2007; Hart et al. 2018; Rego et al. 2020). It has been demonstrated that both CspA (27-kDa) and CspZ (27-kDa) have the capacity to bind to a number of ECM proteins, including collagen, laminin, fibronectin, decorin, integrin, and GAGs, helping *Borrelia*'s attachment to host tissues and contributing to the initial establishment and colonization of infection (Hallström et al. 2010; Coburn et al. 2013, 2022; Brissette et al. 2014). These lipoproteins also bind plasminogen, facilitating tissue invasion and bacterial dissemination (Hammerschmidt et al. 2014) (Fig. 1). Studies have shown that the interaction between CspA and plasminogen depends on specific lysine residue interactions, as binding is inhibited by a lysine analog. Additionally, CspA utilizes two distinct regions at its N- and C-termini for plasminogen binding (Hallström et al. 2010).

CRASP- 3 (also named ErpP or BBN38), CRASP- 4 (or ErpC), and CRASP- 5 (also known as ErpA, ErpI, ErpN, BBP38, or BBL39) have also been identified as proteins capable of binding plasminogen (Brissette et al. 2009b) (Fig. 1) and binding complement proteins (Kraiczy et al. 2001; Kenedy and Akins 2011). As these three proteins are known to be produced during all phases of infection in mammals, the acquisition of plasminogen by these proteins may play a role in long-term infection (Das et al. 1997; Miller and Stevenson 2006; Miller et al. 2006).

CRASP proteins play a central role in bacterial adhesion and invasion (Kraiczy and Stevenson 2013). By binding to both components of the ECM and plasminogen, CRASPs facilitate colonization of host tissues. Activation of plasminogen by CRASPs enables bacteria to degrade cell surface components, thereby facilitating their dissemination (Bergmann and Hammerschmidt 2007; Brissette et al. 2009b; Hallström et al. 2010). CRASPs also interact with complement regulators, thereby enabling evasion of the host immune response (Bykowski et al. 2007). This is further discussed in the following section (“CRASP family: human complement evasion strategies” section). Consequently, beyond the role of CRASPs in the pathogenicity of *Borrelia burgdorferi*, their ability to interact with extracellular components of host cells suggests that such proteins should be considered as promising targets for the development of new diagnostic tools.

## Cell-binding protein: P66 as an integrin-binding protein

In contrast to proteins that interact with the ECM, other *Borrelial* proteins have been found to be involved in attachment to host cells through direct adhesion (Antonara et al. 2011). An integral outer membrane protein, known as Oms66 or P66 due to its 66 kDa molecular mass (Kenedy et al. 2014) is involved in this process (Fig. 1; Table 1). P66, a  $\beta$ -barrel bacterial porin, is a multifunctional protein involved in mammalian host colonization and dissemination and mediates the primary bacterial entry into host cells (Kumar et al. 2015; Ristow et al. 2015). This protein contains at least two surface-exposed parts: (i) the central portion, designated p66M, that contains all necessary information for integrin recognition (Bunikis and Barbour 1999) and (ii) the immunodominant loop on its C-terminal end (Bunikis et al. 1996). Investigations into the P66 protein sequence have revealed conserved hydrophobic regions flanking a more diverse surface-exposed loop across various *Bbsl* species (Bunikis et al. 1998). Moreover, recent research has identified polymorphism within the P66 protein *Bbsl* strains, leading to their classification into three sub-clusters (Hao et al. 2021).

The P66 protein exhibits cell-surface localization, interacts with integrins (Nowalk et al. 2006), and is expressed during mammalian infection. Importantly, it is absent in unfed ticks (Cugini et al. 2003). Therefore, it appears as a promising candidate for diagnostic biomarkers of Lyme disease (Ružić-Sabljić and Cerar 2017). However, investigations have highlighted limitations in the utility of this antigen in serodiagnostic assays for LB due to the presence of numerous cross-reactive linear epitopes on P66 (Arnaboldi and Dattwyler 2015). Finally, direct molecular diagnostic techniques use real-time PCR techniques targeting various bacterial genes, including the *p66* gene (Ružić-Sabljić and Cerar 2017). These studies highlight

the need to design more specific probes and demonstrate the potential for P66 to be used preferentially in multi-genic assays.

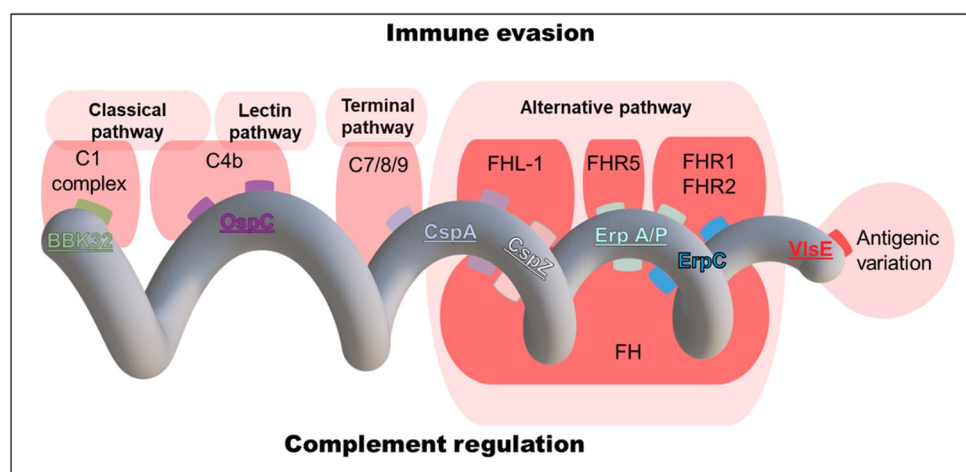
## Immune evasion: various strategies and implications for diagnosis

### VlsE: antigenic variation

*Borrelia* species responsible for vector-borne illnesses have evolved sophisticated immune evasion mechanisms to ensure their survival (Embers et al. 2017). Among these strategies, antigenic variation is one of the most extensively studied. This tactic allows the bacterium to modify the composition of its outer surface, thereby evading the host's adaptive immune response (Coutte et al. 2009). In *Bbsl*, the antigenic recombination of the VlsE protein (36-kDa) is critical for maintaining mammalian infection by evading the humoral immune response (Hudson et al. 2001). This outer surface protein features a system of approximately 16 *vls* cassettes, with the precise number depending on the strain, all located on the same plasmid (lp28 - 1) (Table 1). The process of random recombination of segments from these cassettes results in a new VlsE sequence (Norris 2014). The crystal structure of recombinant VlsE1 demonstrates that the six variable regions adopt loop conformations, enveloping the invariant, predominantly  $\alpha$ -helical regions of VlsE. This surface localization of variable amino acid segments is likely to shield the conserved regions from antibody interactions, potentially contributing to immune evasion (Eicken et al. 2002). By continuously altering its surface epitopes, VlsE prevents antibody binding, allowing the bacterium to escape immune detection and persist within the host (Norris 2014) (Fig. 2).

The antigenicity of VlsE is a key factor widely used as a biomarker in current diagnostic tests. Although

**Fig. 2** *Borrelial* proteins implicated in host immune evasion. Underlined proteins are used in commercial diagnostic tests



second-generation ELISA tests have been developed using recombinant VlsE antigens (Schulte-Spechtel et al. 2003), serodiagnosis tests often use the C6 peptide, a 23-amino acid segment of VlsE (Bacon et al. 2003). Recently, novel diagnostic immunoassays, such as MENSA (Medium Enriched for Newly Synthesized Antibodies), have been developed to measure active humoral immune responses by detecting antibodies specific to the C6 peptide (Haddad et al. 2023). The employment of enhanced detection methods targeting VlsE variants in conjunction with the development of C6 peptide-based enzyme immunoassays has the potential to improve the accuracy of LB diagnostics (Rouhiainen et al. 2021; Hoeve-Bakker et al. 2022). It has been shown that patients with PTLDS have a strong antibody response specific to the invariant N- and C-terminal domains of VlsE, in addition to its usefulness in diagnostic tests for active LB infection, particularly with the IR6 epitope of the C6 peptide (Chandra et al. 2011). These findings underscore the potential of VlsE as a valuable biomarker for both diagnosis and monitoring of Lyme disease.

## Multifunctional proteins

### BBK32-mediated complement inhibition

In addition to antigenic variation (“BBK32: GAG and fibronectin-binding protein” section), *Bbsl* is able to evade the host immune system by binding to host-derived complement proteins, that are derived from the host (Fig. 2). This ability renders it a pivotal actor in the bacterium’s niche adaptation system (Kurtenbach et al. 2002). The complement system comprises a tightly regulated network of around 30 proteins, most of which exist in the inactive form of zymogens, which are activated by three different pathways (classical, alternative, and lectin) leading to a cascade of enzymatic reactions resulting in a range of physiological responses (Sarma and Ward 2011).

Beyond its role in adhesion as previously described above (“BBK32: GAG and fibronectin-binding protein” section), BBK32 also exhibits complement-inhibitory activity by specifically inhibiting the classical pathway (CP) of the complement system through high-affinity binding to the C1 complex (Garcia et al. 2016). Indeed, structural studies have revealed that the C-terminal globular-exposed surface domain on BBK32 inhibits the CP by binding with high-affinity, with a  $K_D$  of 2.3 nM, to the serine protease domain of C1r (Garcia et al. 2016; Xie et al. 2019). It is noteworthy that orthologous proteins of BBK32 (e.g., BAD16 from *Borrelia afzelii* strain PGau, or BGD19 from *Borrelia garinii* strain IP90) also exhibit comparable complement-inhibitory potential by interacting with C1 or C1r proteins (Xie et al. 2019).

Finally, BBK32 not only binds to the ECM but also plays a crucial role in immune evasion, a dual function facilitated by its surface expression on *Bbsl*, making it a valuable target for diagnostic assays. Indeed, a cohort study showed high serological antibody responses to BBK32 in patients with borreliosis, strongly correlated with anti-VlsE antibodies, an established marker of LB (Dulipati et al. 2024). Overall, it is an indication that BBK32 is a promising biomarker with diagnostic potential across different stages of infection (Heikkilä et al. 2002; Dulipati et al. 2024).

### OspC: refined immune evasion strategies

The lipoprotein OspC, previously described for its role in adhesion (“OspC: ECM components-binding protein” section), is highly expressed during the early phase of infection. OspC exhibits antiphagocytic properties, protecting *Bbsl* from macrophage phagocytosis. This ability may help spirochetes evade mononuclear phagocytes by modulating bacterial recognition by macrophage receptors and limiting their activation, although the exact mechanisms remain to be elucidated (Carrasco et al. 2015). OspC also interacts with specific vector proteins, such as Salp15, a salivary protein from *Ixodes scapularis* ticks that helps in the initial attachment of *Bbss* during tick transmission by protecting the bacteria from antibody-mediated killing (Ramamoorthi et al. 2005).

However, OspC is significantly downregulated once the infection is established. This downregulation coincides with the rapid development of bactericidal antibodies against OspC (Mbow et al. 1999; Embers et al. 2008), demonstrating a highly sophisticated strategy of immune evasion (Şen and Sigal 2013). OspC also contributes to *Bbsl* immune evasion by inhibiting mannose-binding lectin and the CP, enhancing bacterial survival in the bloodstream. Specifically, OspC from *Bbss* competes with C2, the natural binding partner of C4b, thereby inhibiting the activity of the complement cascade activity (Caine et al. 2017) (Fig. 2).

OspC has become a well-established biomarker in serodiagnostic tests, particularly for the detection of early-stage Lyme disease (Kaiser and Rauer 1999). Numerous indirect diagnostic methods rely on the detection of antibodies against OspC using multiplexed immunoassays (Nayak et al. 2016; Joung et al. 2020). Approaches combining ELISPOT techniques utilize recombinant or synthetic OspC antigens for improved sensitivity and specificity (Jin et al. 2013; Callister et al. 2016). Emerging serodiagnostic tests also focus on C10, a peptide derived from the conserved N-terminal portion of OspC (Davis et al. 2020). OspC also serves as a critical biomarker for direct diagnosis of LB. It is commonly used as a plasmid target in traditional PCR assays, with multilocus sequence typing of the *ospC* gene helping to identify different species within the *Bbsl* complex (Margos et al.

2008; Lager et al. 2017). Advances in understanding OspC variability could lead to more tailored diagnostic approaches that consider regional and strain-specific differences in *Borrelia* infections. This will be crucial for both early detection and the development of region-specific Lyme disease management strategies.

### CRASP family: human complement evasion strategies

Like BBK32 or OspC, CRASP family proteins are multifunctional, able to bind to EC or plasminogen (“The CRASP family” section), as well as to contribute to immune evasion. Each member of this family of proteins prevents the activation of the innate immune system (Skare and Garcia 2020) by binding to the human complement regulators factor H and factor H-like protein 1 (Haupt et al. 2007) (Fig. 2). Expressed at various stages of the spirochete infection cycle (Bykowski et al. 2008), the five proteins of the CRASP family differ in their primary and tertiary structures, as well as in their binding affinities for complement regulator proteins (Siegel et al. 2010).

### CspA and orthologs

The *cspA* gene in *Bbss* B31, encoding the CspA protein, is part of the large paralogous gene family pfm54, which comprises 11 intact genes all located on the lp54 replicon (Wywiał et al. 2009; Kraiczy 2016). Orthologs of CspA have been identified in *Borrelia afzelii*, *Borrelia spielmanii*, *Borrelia bavariensis*, and *Borrelia mayonii*, (Table 1) supporting the hypothesis that these pathogens utilize similar mechanisms to evade the innate immune system of the human host. Recent research has shown that CspA orthologs have selective functions depending on host specificity, as CspA variants from different *Borrelia* genospecies, such as BGA71 and BGA66 for *Borrelia bavariensis* PBi, bind factor H from different hosts, thereby helping spirochete survival in the tick blood meal (Wywiał et al. 2009; Burgel et al. 2010; Kraiczy 2016; Hart et al. 2021). These surface proteins confer serum resistance to the bacterium by inhibiting the activation of both the CP and alternative (AP) complement pathways and by preventing the assembly of the Membrane Attack Complex (MAC) by binding to complement terminal components C7, C8, and C9 (Hammerschmidt et al. 2016) (Fig. 2).

CspA and its orthologs have potential as biomarkers that could help in the diagnosis of patients, while antibodies to CspA may indicate *Bbsl* infection and can be detected earlier than other *Borrelia* antibodies. As CspA expressions significantly increase upon entry into the mammalian host and play a critical role in the early stages of invasion (Bykowski et al. 2007, 2008), it may induce the production of specific

antibodies in the early stages of the disease. Taken together, the incorporation of these proteins into ELISA or Western blot panels could improve diagnostic sensitivity and specificity, with the potential to correlate antibody levels with disease progression or treatment response. In this context, a multiplexed vertical flow assay is being developed to detect antibodies against CspA and other *Borrelia burgdorferi* antigens (Joung et al. 2020). Finally, CspA could serve as biomarker for the detection of *Borrelia*, particularly in cases of zoonotic spillover where cross-species transmission complicates diagnosis (Plowright et al. 2017; Hart et al. 2018; Joung et al. 2020).

### CspZ

Interestingly, CspZ shares no structural homology with other CRASPs, including the Erp family (Kraiczy et al. 2004; Hartmann et al. 2006). The crystal structures of CspZ and CRASP proteins have been elucidated, revealing their factor H-binding capabilities through a conformational epitope, with CspZ exhibiting a unique fold compared to other complement-binding proteins (Cordes et al. 2005; Kraiczy et al. 2008; Brangulis et al. 2014, 2019, 2024). CspZ is highly conserved among *Borrelia* species, superior to 85% of amino acid identity (Marcinkiewicz et al. 2023) (Table 1). CspZ has been shown to interact with FH and rFHL-1 with  $K_D$  of 210 pM and 44 pM respectively, measured by SPR (Guérin et al. 2024). The binding of FH and FHL-1 to *Borrelia surface* proteins results in inactivation of C3b, allowing the bacterium to escape the human complement system and disseminate in the host (Dulipati et al. 2020) (Fig. 2). Strikingly, modified bacterial strains lacking CspZ or expressing a non-binding FH CspZ variant exhibited impaired ability to disseminate and establish infection in mice or quail. However, the virulence of these mutants was restored in C3-deficient mice (Marcinkiewicz et al. 2019).

Marcinkiewicz and collaborators have demonstrated that CspZ expression is tightly regulated in response to specific environmental cues, as demonstrated by the upregulation of its expression upon supplementation with blood components in *in vitro* cultures. These findings suggest that CspZ expression is finely tuned to the conditions encountered within a mammalian host (Bykowski et al. 2007; Kraiczy et al. 2008; Marcinkiewicz et al. 2019). Similar to anti-CspA antibodies, anti-CspZ antibodies can be detected early in the infection process (Rogers and Marconi 2007), allowing for potential early diagnosis and treatment (Kraiczy et al. 2008). It is noteworthy that antibodies against CspZ can be detected in mice as early as 2 weeks after tick infection (Rogers and Marconi 2007). Consequently, CspZ, as an immunogenic protein expressed early in the infection, offers a promising avenue for the development of sensitive and specific

diagnostic tests for Lyme disease (Hammerschmidt et al. 2014; Guérin et al. 2025).

### The Erp family members: ErpA, ErpP, and ErpC

ErpA and ErpP can bind to FH and the factor H-related proteins (CFHR1, CFHR2, CFHR5) C-terminal repetitive protein domains and are highly expressed during infection in mammals (Siegel et al. 2008). FH binding patterns differ between ErpA and ErpP depending on the mammalian reservoir host (Hovis et al. 2006) (Fig. 2).

ErpC interacts with a partially overlapping set of partners compared to ErpA and ErpP, including the factor H-related proteins CFHR1, CFHR2, CFHR5, but interacts weakly with FH (Fig. 2). By binding to these complement regulators, ErpC can also inhibit the activation of the complement cascade. However, it appears that this binding alone is not sufficient to fully protect the bacteria from complement-mediated killing (Hammerschmidt et al. 2012). The presence of an extended loop, exclusive to ErpC, within the FH binding site was correlated with a loss of binding affinity for FH. Conversely, ErpP and ErpA, which lack this extended loop, retain the ability to strongly interact with FH (Branulis et al. 2015).

The role of Erp family members in immune evasion makes them potential biomarkers of active infection. Recent studies have evaluated the specificity of synthetic peptides containing unique linear epitopes of the ErpP protein in LB seroassays, which showed high sensitivity when combined with a VlsE epitope, demonstrated high sensitivity (Arnaboldi et al. 2022). Finally, the ErpP protein, as well as CspA and CspZ, display specific immunodominant epitopes along with 120 others which are included in the novel Epitogen Lyme Detect IgG ELISA assay. The performance of this assay demonstrates high sensitivity in patients with suspected EM and offers a promising potential for LB diagnosis (Wang et al. 2024).

## Conclusion

*Borrelia* proteins play a pivotal role in adhesion, immune evasion, and persistence of infection, making them central to LB pathogenesis. The diversity of *BbsI*, encompassing variations in gene coding, protein structures, and associated functions, accounts for the complexity of its nomenclature and contributes significantly to the challenges of a comprehensive characterization. The proteins of this bacterial complex serve as promising biomarkers for the development of novel diagnostic tests with the objective of offering high sensitivity and specificity. Furthermore, their direct detection, as well as the indirect detection of their specific antibodies, could potentially allow early diagnosis and the assessment of

disease presence. Based on *Borrelia* proteins, the prospects for developing new diagnostic tests are based on different strategies. (i) The variability of the strains involved and the coinfection that are frequently associated with them, highlight the need for advanced diagnostic tests that can accurately distinguish between multiple *Borrelia* strains and from other pathogens. Thus, the use of combined biomarkers in multiplex assays should improve diagnostic sensitivity and specificity by simultaneously detecting multiple proteins (both bacterial and host or tick) (Joung et al. 2020; Häring et al. 2022). Additionally, the development of rapid point-of-care tests, combined with the use of biomarker panels and advanced technologies like chip arrays or artificial intelligence, will facilitate sensitive and specific diagnosis and will improve patient care (Nayak et al. 2016; Magni et al. 2020; Guérin et al. 2023).

While OspC is commonly used as a marker of early infection, its antigenic variability requires the development of more comprehensive assays capable of detecting different variants. Such variants may serve as useful markers for strain differentiation (Jin et al. 2013; Nayak et al. 2016; Davis et al. 2020) and consequently have epidemiologic value. Similarly, Bgp and CspA could potentially serve as early diagnostic targets due to their expression during the early stages of infection (Hallström et al. 2013; Schlachter et al. 2018). Finally, DbpA and DbpB, which are involved in tissue adhesion, are particularly interesting targets for the diagnosis of Lyme arthritis (Schulte-Spechtel et al. 2006; Arvikar and Steere 2015; Lager et al. 2017).

The disease stages can be monitored using potential biomarkers such as RevA, which is involved in tissue dissemination and is useful for diagnosing later stages of infection, or CRASP proteins, which are involved in immune evasion and may serve as markers of disseminated infection (Byram et al. 2015; Rego et al. 2020). Serological detection of anti-VlsE antibodies can track disease at each stage, from early stages to late neurological involvement (Burgel et al. 2011; Porwancher et al. 2011; Branda et al. 2017). Indeed, the evolution of specific human B cell epitopes in response to the VlsE protein during infection has been demonstrated. This highlights the dynamic nature of the immune response to VlsE, which changes over time: early in the infection, antibodies predominantly target the IR6 region of VlsE, while antibodies against the N- and C-terminal domains (such as VlsE21) appear successively (Jacek et al. 2016). These data suggest that the analysis of antibody targeting patterns could be used to stage the disease.

Tick biomarkers, such as salivary proteins like Salp15, which interacts with OspC to facilitate bacterial colonization in murine hosts, can also be explored as diagnostic probes (Ramamoorthi et al. 2005; Hovius et al. 2008). Salp15 inhibits the activation of naive CD4 T lymphocytes through its persistent binding to CD4, inducing a

long-lasting modulatory effect (Tomás-Cortázar et al. 2017). Recent research, using microscale thermophoresis and biolayer interferometry, has revealed robust and persistent interactions between three Salp15 homologs from the tick *Ixodes ricinus* (Iric1, Iric2, Iric3) and OspC proteins from four strains of *Bbsl*. Notably, the binding site of these homologs appears to be in close proximity to OspC epitopes recognized by human antibodies, which may have important biological and medical implications (Bierwagen et al. 2021).

In conclusion, the study of *Borrelia* surface proteins not only enhances our understanding of LB pathogenesis but also provides promising targets for the development of potential diagnostics. In addition to diagnostic implications, these multifunctional proteins may also provide insights into novel prophylactic or therapeutic approaches, including vaccines and immunomodulatory treatments. In this context, OspA, an outer surface protein, was the target of the first Lyme disease vaccine (LYMErix), which was effective in preventing *Borrelia* transmission from ticks to humans. Although this vaccine was withdrawn from the market, research on OspA-based vaccines continues, with new formulations aimed at improving safety and efficacy. A novel multivalent vaccine targeting the outer surface protein OspA subunit, known as VLA15, is currently in clinical trials (Thanassi and Schoen 2000; Oliveira et al. 2021; Dattwyler and Gomes-Solecki 2022; Hajdusek and Perner 2023). In addition, vaccines that target the vector (i.e., the tick), rather than the pathogen, offer a promising strategy for the prevention of tick-borne diseases (Johnson et al. 2024). Tick salivary proteins from ticks have been identified as potential vaccine targets (Trentelman et al. 2022). Indeed, anti-ticks' vaccine as Ticovac® (Baxter Healthcare) preventing tick-borne encephalitis, is already on the market (Barrett et al. 2003). Studies have shown that vaccination with Salp14 can inhibit tick feeding, and the combination of several salivary proteins may enhance the protection (Kitsou et al. 2021; Matias et al. 2021). Recent work by Matias and collaborators have demonstrated the potential to induce strong immune responses and early tick detachment (Matias et al. 2024). Thus, a combination of general preventive measures, anti-tick vaccines, and improved detection and monitoring of the LB infection should have the potential to significantly reduce the incidence of tick-borne diseases.

**Author contribution** P.T. and S.PL. conceptualized the idea for the manuscript. P.T. performed literature review and drafted the manuscript. H.D.P. and M.G. completed sections of the manuscript and elaborated the associated tables. P.T. and S.O. created the figures. I.M., S.O., B.A., and S.PL. evaluated the manuscript and improved its content. All authors contributed to the article and approved the submitted version.

**Funding** This study was supported by Hauts-de-France Region (STIMuLE, STIP, Dialyme) and the French Ministry of Higher Education, Research and Innovation (MESRI).

**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

**Disclaimer** The funding bodies played no role in the design of the study, collection, analysis, interpretation of data, nor in the writing of the manuscript.

**Clinical trial number** Not applicable.

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