Infectious Agents as Potential Drivers of α-Synucleinopathies.

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Word count: 4999

Running title: Infections in alpha-synucleinopathies

Keywords: dementia with Lewy bodies, multiple system atrophy, Parkinson's disease, alpha-synuclein, infections

Financial Disclosure/Conflict of Interest concerning the research related to the manuscript: None.

Funding sources for study: None.

Abstract:

Alpha-synucleinopathies, encompassing Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy, are devastating neurodegenerative diseases for which available therapeutic options are scarce, mostly due to our limited understanding of their pathophysiology. Although these pathologies are attributed to an intracellular accumulation of the alpha-synuclein protein in the nervous system with subsequent neuronal loss, the trigger(s) of this accumulation is/are not clearly identified. Among the existing hypotheses, interest in the hypothesis advocating the involvement of infectious agents in the onset of these diseases is renewed. In this article, we aimed to review the ongoing relevant factors favoring and opposing this hypothesis, focusing on i) the potential antimicrobial role of alpha-synuclein, ii) potential entry points of pathogens in regard to early symptoms of diverse alpha-synucleinopathies, iii) pre-existing literature reviews assessing potential associations between infectious agents and Parkinson's disease, iv) original studies assessing these associations for dementia with Lewy bodies and multiple system atrophy (identified through a systematic literature review) and finally v) potential susceptibility factors modulating the effects of infectious agents on the nervous system.

Abbreviations:

AD: Alzheimer's disease, AMP: antimicrobial peptide, aS: alpha-synuclein, CI: confidence interval, CNS: central nervous system, DLB: dementia with Lewy bodies, EBV: Epstein Barr virus, ENT: ear, nose, and throat, HIV: human immunodeficiency virus, HTLV: human T-cell leukemia virus, KO: knock-out, MSA: multiple system atrophy, LB: Lewy bodies, OR: odds ratio, PD: Parkinson's disease, WNV: West-Nile virus.

I- Introduction

Alpha-synucleinopathies, encompassing Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), are devastating neurodegenerative diseases characterized by a progressive onset of motor, autonomic and/or cognitive dysfunctions to varying degrees [1–3]. Although MSA is considered a rare disease [2], both PD and DLB are disorders commonly affecting the elderly [4,5]. However, available therapeutic options are scarce and limited to symptomatic treatment and palliative relief due to our limited understanding of their pathophysiology.

Although these pathologies are attributed to an intracellular accumulation of the alpha-synuclein protein (aS) in the nervous system resulting in subsequent neuronal loss, the trigger(s) of this accumulation is/are not clearly identified. Some genetic and environmental risk factors have been recognized [6] but fail to provide a complete explanation for the pathophysiology underlying these diseases. Deciphering potential causes should also help explain the heterogeneity of alpha-synucleinopathies in terms of types and locations of aS deposits and of types of affected cells, whether they are mainly neurons (in PD and DLB) or oligodendrocytes (MSA) [6].

Although a controversial assumption at first, conventional infectious agents have long been suspected to have an implication in the onset of alpha-synucleinopathies. Currently, interest in this hypothesis appears to be renewed, particularly when considering studies suggesting an initiation of pathology in the periphery [7,8] and the major role of neuroinflammation in the pathogenesis of these diseases [9].

In this article, we aimed to review the ongoing relevant factors favoring and opposing this hypothesis (illustrated in Figure 1) by focusing on i) the potential antimicrobial role of aS, ii) potential entry points of pathogens in regard to early symptoms of diverse alpha-synucleinopathies, iii) pre-existing literature reviews assessing potential associations between infectious agents and PD, iv) original studies assessing these associations for DLB and MSA (identified through a systematic literature review) and finally v) potential susceptibility factors modulating the effects of infectious agents on the nervous system.

II- aS, an antimicrobial peptide?

Encoded by the SNCA gene, the aS protein is mainly expressed in the central nervous system (CNS) [10]. It predominately binds to vesicle-forming membranes in the presynaptic nerve terminals, resulting in its proposed potential implication in synaptic plasticity and neurotransmitter/vesicle transport [10]. However, this hypothesis remains debated, and aS is also reportedly present in other compartments (nucleus, cytoplasm, mitochondria, extracellular space, etc.) [11], as well as in other tissues (red blood cells, heart, etc.) [10] where its role is even more ambiguous. Indeed, in addition to a membrane-anchored form, aS also exists as a soluble cytosolic natively-unfolded protein that is, depending on the environment, more or less prone to aggregation and to the formation of Lewy bodies (LB) and neurites or glial cytoplasmic inclusions, which are neuropathological hallmarks of synucleinopathies [12]. In light of these elements, a recent hypothesis seems particularly interesting to discuss: aS might be an antimicrobial peptide (AMP) [13].

Briefly, AMPs are ancient players of the innate immunity that are found in vertebrates, invertebrates and plants, preserving a high level of conservation during evolution [14,15]. A wide variety of AMPs have been identified, and diverse tissues have their own signature "cocktail of AMPs". AMPs are small peptides whose structural features mediate their antimicrobial and immunomodulatory roles. These positively charged peptides bind to negatively charged membranes, including those of microorganisms and phospholipid-rich membranes. Characterized by secondary structures such as α-helices and β-sheets, AMPs are amphiphilic and self-assemble into oligomers, protofibrils and fibrils. Their antimicrobial activity can thus be achieved through numerous mechanisms, such as membrane pore formation, binding to intracellular nucleic acids, inhibition of pathogen adhesion, and entrapment of pathogens into fibrils [14–16]. Interestingly, the self-assembled AMP protofibrils and fibrils seem to act as a signal to boost the immune system [16] and some AMPs seem to interact with the complement system [17–19].

Intriguingly, several studies have reported both structural and functional similarities [20] between aS and AMPs.

- Structurally, aS is also a small protein that is highly conserved among vertebrates [21]. Unstructured in its soluble form, it adopts an α-helical conformation when binding to negatively charged membranes and binds preferentially to small diameter vesicles,

potentially enabling viral transport [22]. It also self-assembles into β-sheet-containing structures, such as oligomers and fibrils [10,11,22].

- Functionally, aS expression is also induced by infections and seems to intervene in antimicrobial and immunomodulatory activities.
    - In both in vitro models and rodents, aS aggregation has been successfully induced in neurons of either the central or enteric nervous system following inoculation with different pathogens (including H5N1 virus, H1N1 virus, West Nile virus (WNV), Western equine encephalitis virus, curli-producing *Escherichia Coli*, lipopolysaccharide-producing *Proteus mirabilis* or lipopolysaccharides injected separately) [23–29]. More recently, inoculation of SARS-CoV-2 in nonhuman primates has led to the formation of LB in the brains of most infected subjects ([30], preprint) whereas no LB were observed in non-infected controls. In humans, increased expression of aS was detected in postmortem brain samples from patients with histories of human immunodeficiency virus (HIV) infection or WNV encephalitis compared to controls [24,31], and using repeated duodenal biopsies obtained from subjects with an intestinal transplant, increased aS expression in enteric neurons was evidenced following an episode of Norovirus infection. Notably, the observed aS deposits persist several months after infection [32].
    - Moreover, an in vitro study showed that aS possesses antimicrobial properties against several bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, etc.) and fungi (*Candida albicans*, etc.) [13], even if the mode of action of aS remains to be clarified [13,33,34]. Studies performed on SNCA knockout (KO) mice reported more severe outcomes after inoculation with pathogens compared to their wild-type littermates. After WNV inoculation, SNCA KO mice had a higher intracerebral viral load and intracerebral concentration of cleaved caspase 3 (indicating neuronal apoptosis) and a higher mortality rate due to infection (95% vs. 25%) [24]. In the same study, a protective effect of aS was also recorded upon the inoculation of an attenuated form of the Venezuelan equine encephalitis virus [24]. SNCA KO mice were also more vulnerable to severe reovirus and *Salmonella typhimurium* infections, with a positive correlation observed between aS expression (depending on the number of SNCA alleles expressed) and their survival rates [35].

- Finally, aS appears to modulate innate and adaptive immune responses [36], including the recruitment and/or activation of the complement system, microglial cells, monocytes and T cells [25,26,32,33,37–39]. For example, a study using duodenal biopsies from 42 children presenting gastroduodenal inflammation showed that the severity of intestinal wall inflammation was positively correlated with the degree of aS deposition within enteric neurons [32]. Thus, despite the potential antimicrobial role of aS, its accumulation in the nervous system might be deleterious [40,41], potentially due to the concomitant neuroinflammation it induces.

While these studies remain few in number and must be replicated, other amyloid peptides implicated in the development of neurodegenerative disorders also present similarities with AMPs [16,20,42], including the aβ peptide involved in Alzheimer's disease (AD) [20,43–54,54], Tau protein associated with AD and frontotemporal lobar degeneration [20,55,56], TDP-43 associated with amyotrophic lateral sclerosis, frontotemporal lobar degeneration and AD [57], and ultimately the prion protein [58]. These data could argue in favor of a pathophysiological mechanism partially shared by these various neurodegenerative diseases and thus provide a potential explanation for their coexistence in the brains of subjects with dementia [59].

III- Early symptoms as markers of the infection entry point?

Given the potential role of aS in antimicrobial defense, early aS deposits in the peripheral nervous system and cranial nerves may reflect the potential effect of infectious agents on triggering synucleinopathies when in contact with nerve endings in the mucous membranes. Early "peripheral" symptoms may thus serve as clues to identify the potentially responsible pathogen via its entry point [8]. Regarding the early DLB and PD symptoms, predominant hyposmia and gastrointestinal issues [60–63] (reflecting early aS deposits in the olfactory bulb and/or the enteric plexus [7,64,65]) suggest an entry point of the pathogen in the ENT (ear, nose, and throat) and/or gastrointestinal tract [66]. Regarding MSA, Tulisiak et al. [8] suggested that the spread of lesions is different [67]: the precocity of neurogenic urinary symptoms or erectile dysfunction in some patients [68–70] (related to early aS deposits in the sacral part of

the spinal cord [71] and in nerve terminals in detrusor and external urethral sphincter [72]) might argue for a urogenital entry point.

After initiation in the periphery, aS pathology might spread in a cell-to-cell manner to the CNS through neuronal networks, as suggested by i) neuropathological staging [7] and ii) results from studies on animal models highlighting the capability of aS to propagate from different entry points (including olfactory bulbs, gastrointestinal and urinary tracts [62,66,72,73] via the olfactory tract [35,74], vagal nerve [66] or spinal cord [72]). Notably, similar hypotheses (implicating a propagation of pathogens via the cranial nerves to the CNS) have also been proposed for other neurodegenerative diseases [74], and a secondary spread of the pathology from the CNS to other structures of the peripheral nervous system cannot be excluded.

However, *why* would aS spread? A first hypothesis that has been explored by numerous research teams is that aS may *itself* be an infectious agent, namely, a prion protein [75,76]. This concept (obviously incompatible with the hypothesis of an antimicrobial role of aS) is supported by the similarities between aS and PRNP, based on the existence of "misfolded" aS forms and their seeding properties observed throughout numerous in vitro and in vivo studies (covered in the reviews of [77,78]) and in human subjects [79]. Nevertheless, this hypothesis is still highly debatable [80–82], particularly regarding the lack of evidence on aS infectivity in humans. An alternative possibility (compatible with a potential antimicrobial and immunomodulatory role of aS) might be that, secondary to its peripheral induction by pathogen entry, aS serves as a warning signal for neighboring cells [22]. From this perspective, a question would remain: would the presence of infectious agents in the periphery suffice to induce the progressive spread of aS to the CNS, or would the propagation of aS forestall the progressive spread of *infectious agents* between neurons? The former option would incriminate intracellular pathogens able to propagate transsynaptically [83], a property shared in particular by various viruses [84–86]. Moreover, one could hypothesize that the diverseness of the potentially threatening pathogens might also explain the heterogeneity among aS "strains" [87,88], as well as their particular tropism for either neuronal or oligodendroglial cells.

IV- **Do studies in humans incriminate a particular pathogen?**

In this section, we will address that question by i) discussing the *pre-existing* literature reviews that investigated this question in individuals with PD and ii) presenting the results of the systematic literature review we performed apropos of DLB and MSA.

1. Previous literature regarding PD

A significant number of literature reviews have recently been published regarding the link between certain pathogens and the onset of PD [89–110]. Consequently, the objective here is not to provide an exhaustive description of the currently existing results but to provide a brief summary of the pathogens investigated while presenting the most recent and relevant reviews. These references will provide the reader with some discussion on the plausibility of a causal association, the various potential underlying mechanisms, and possible therapeutic perspectives.

Following the hypothesis of "gut-brain axis" involvement in PD [111], the gut microbiota has received special attention in recent years (reviewed in [92–98]), and one can cite two recent meta-analyses in particular. The first study (14 studies published before August 2020 and including 959 patients with PD and 744 controls) highlighted some changes in the gut microbiota detected in the feces of subjects with PD, with lower abundance levels of *Prevotellaceae*, *Faecalibacterium*, and *Lachnospiraceae* and higher abundance levels of *Bifidobacteriaceae*, *Ruminococcaceae*, *Verrucomicrobiaceae*, and *Christensenellaceae* [93]. Animal model studies, like the one by Choi et al. [29], may help identify a single causative infectious agent (if one exists). The second (11 studies published before February 2021, 692 patients with PD and 281 controls) showed a higher prevalence of small intestinal bacterial overgrowth, as measured using either lactulose or glucose hydrogen breath tests, in patients with PD than in controls (pooled odds ratio (OR)=5.22 95% confidence interval (CI) [3.33-8.19]) [95]. In addition, several reviews have specifically focused on the potential role of the bacterium *Helicobacter pylori* [90,99–102]. The most recent meta-analysis [90] highlighted an increased risk of PD in subjects infected with this bacterium (pooled OR=1.65 95% CI [1.43-1.92], 9 studies published before 2019). Nevertheless, the benefit of its eradication on parkinsonian symptoms remains uncertain [100], in particular in view of a recent randomized clinical trial showing no significant improvement in clinical outcomes at 12 and 52 weeks post-treatment [112]. In contrast, few studies have investigated the roles of viral or fungal agents also present in the digestive system [103,113,114].

Several reviews have also assessed the potential effects of diverse *neurotropic* infectious agents on the onset of PD. In two meta-analyses of seven studies published up to 2018 and including approximately 1000 participants, no significant association was found with the infection caused by the parasite *Toxoplasma gondii* [104,105]. Moreover, given the many cases of postencephalitic parkinsonism following viral infections, different neurotropic viruses have been suspected to participate in the pathophysiology of PD [106–108]. Among these, the influenza virus was first suspected due to an exceptionally high number of postencephalitic parkinsonism cases in survivors of the 1918 Spanish flu [115]. Although the involvement of the virus has not been formally confirmed in these historical cases, it paved the way for subsequent investigations of the role of viral infections in PD development. Wang et al. [90] performed a meta-analysis of studies published until 2019 and highlighted an increased risk of PD among subjects chronically infected with hepatitis C virus (pooled OR=1.20 95% CI [1.01–1.41], 7 studies) [90,109,110]. Conversely, the pooled results revealed no increased risk associated with hepatitis B, herpes simplex, varicella-zoster, mumps, rubella or measles viruses (6, 4, 3, 3, 2 and 2 studies, respectively), and no pooled analysis was performed for cytomegalovirus [116], Epstein-Barr virus (EBV) [116], human herpes virus 6 [117], HIV [118], poliovirus or coxsackie virus [119]. Notably, in this meta-analysis, Wang et al. [90] also assessed nonviral agents and identified an increased risk of PD among patients infected with the fungus *Malassezia* (pooled OR=1.68 95% CI [1.37–2.10], 2 studies) or with pneumonia (pooled OR=1.60 95% CI [1.02–2.49], 2 studies), but not scarlet fever or pertussis (2 studies each). No pooled analysis was performed for *Borrelia burgdorferi*, [116,120,121], *Nocardia asteroides* [122], *Chlamydia pneumoniae* [116], tuberculosis [123] or diphtheria [124]. Finally, in the current context of the SARS-CoV-2 pandemic, concern about the effect of the virus on the onset or evolution of PD is increasing [91,103,108,125–127], and long-term effects should be carefully monitored.

2. Systematic literature review of human studies assessing a potential link between infectious agents and the occurrence of MSA or DLB

As no previous systematic review has been published on this subject, we conducted a systematic literature review aiming to identify human studies assessing the potential involvement of conventional infectious agents in the occurrence of DLB or MSA. Using PubMed and Scopus databases, we searched for original studies written in English and

published before June 2021 (see Appendix 1 for more details). After screening 1113 articles, 23 articles were finally included in our review (see the flow chart in Supplemental Figure 1 and the list of identified articles in Supplemental Table 1). Most of these studies examined patients with MSA, with only 8 incorporating subjects with DLB. The results are presented by infectious agents in the next sections.

Nocardia asteroides

In the early 2000s, based on the results from in vitro and animal studies suggesting the involvement of *Nocardia asteroides* bacterium in DLB, Chapman et al. [128] assessed the presence of this bacterium in 35 human substantia nigra specimens. Their study included 24 specimens with LB (from subjects with a neuropathological diagnosis of PD, DLB and/or AD with LB) and 11 specimens without LB (combining 5 healthy controls and 6 subjects with other neurodegenerative disorders). Using an in situ hybridization technique, nocardial 16S ribosomal RNA was detected in 9 samples containing LB (37.5%), but not in samples without LB. Specifically, in this study, hybridization reactions were mainly intracellular and located within inclusions resembling LB. Nevertheless, when attempting to replicate these findings in a larger subsample of substantia nigra specimens, Lu et al. [129] recorded discordant results. Indeed, of the 125 brain specimens examined (from 28 subjects with PD, 21 with DLB, 32 with other neurodegenerative disorders and 44 healthy controls), they detected an in situ hybridization reactivity for *Nocardia asteroides* in only 3 (2.4%) samples from subjects with a diagnosis of PD, DLB and AD. Despite the efforts to standardize the protocols, the interstudy reproducibility was poor, since the results for the 5 samples common to both studies were discordant. Moreover, in the study by Lu et al., the presence of *Nocardia* was not recorded in any of the samples on which two additional detection techniques were performed (PCR and Gram staining). Overall, even if we cannot completely exclude the fact that *Nocardia* is removed from the CNS after causing neuronal damage, thus preventing its detection, sufficiently potent arguments concerning the involvement of *Nocardia asteroides* in DLB are not available.

E*BV*

Using immunohistochemical techniques to detect the EBV latent membrane protein, Woulfe et al. [130] observed important immunostaining of LB and dystrophic neurites in brain samples from 5 patients with PD and 5 patients with DLB, as well as glial cytoplasmic inclusions and neuronal intranuclear inclusions in brain samples from 2 patients with MSA, while no staining

was detected in sections lacking aS inclusions. Further experiments clarified that the staining was in fact due to cross-reactivity of the anti-EBV antibody with aS. Interestingly, another study also reported this cross-reactivity, but this time between aS and Herpes simplex virus type 1 [131]. Although the significance of these results remains unclear, their interpretation regarding the potential antimicrobial role of aS may be of interest.

WNV

Recently, Segers et al. [132] reported the case of a 66-year-old man who developed probable DLB a few months after an episode of encephalitis due to WNV. Since symptoms such as rapid eye movement–sleep behavior disorder and constipation were present before the onset of encephalitis, the authors assumed that DLB was probably already developing and questioned a potential accelerating role of neurotropic WNV on the evolution of the disease. Indeed, as discussed above, following inoculation of WNV in mice, Beatman et al. documented an increase in aS production, which seemed to exert a protective effect against the infection [24].

Lyme disease

Gadila et al. [133] reported the case of a woman with a history of Lyme disease who secondarily developed dementia clinically consistent with DLB. After her death, analyses of brain and spinal cord tissues confirmed the presence of pathological markers of DLB and identified persistent *Borrelia burgdorferi* using PCR and immunofluorescence staining, raising the question of whether *Borrelia* may trigger the onset of DLB. Some cases of DLB [134] and MSA [135] associated with an intrathecal synthesis of anti-Borrelia antibodies were also reported. While this association is intriguing, the existence of a causal relationship is far from being confirmed. Indeed, in the study by Blanc et al., only 20 patients displayed a positive index among 1594 patients with dementia examined (1.25%), and only 4 of these subjects were diagnosed with DLB.

Human T-cell leukemia viruses

Two studies conducted in the 1990s focused on a possible role of human T-cell leukemia virus (HTLV) type 1 in MSA development. Although HTLV-1 is primarily known to cause a relatively rare neurodegenerative disease called HTLV-1-associated myelopathy or tropical spastic paraparesis [136], Kano et al. [137] reported a case of a patient with high HTLV-1 antibody titers in serum and cerebrospinal fluid whose symptoms were consistent with those reported for MSA. Following this publication, Yokota et al. [138] performed HTLV-1 plasma

serology in 28 patients diagnosed with MSA, detecting only one positive case (3.9%). This patient also presented a high HTLV-1 antibody titer in the cerebrospinal fluid, suggesting a possible causal relation between HTLV-1 and the patient's symptoms. Another study focused on the less common and known HTLV-2 infection [139]. With the aim of identifying possible complications following HTLV-2 infection, Hjelle et al. studied an American Indian population in which this infection is endemic and reported the case of two sisters infected who presented MSA symptoms. In summary, these studies argue against HLTV viruses as main causes of MSA but suggest that they might be involved to a certain extent in the development of *some* MSA cases. Notably, these studies probably suffer from a lack of specificity due to the limitations of the diagnostic criteria used in the 1990s [140].

Gut microbiota

More recently, as in the field of PD research, research on MSA seems to have focused on the potential effect of the gut microbiota. On the one hand, we identified 5 articles published between 2016 and 2019 comparing the composition of fecal microbiota between subjects with MSA and healthy controls [141–145]. Notably, among these studies, two also investigated blood or sigmoid mucosa microbiota. All the studies were relatively small in size (6 to 40 subjects). The majority used 16S ribosomal RNA gene amplicon sequencing, while only one employed metagenomic sequencing, allowing for a more precise identification of bacterial presence (at the species taxonomic level). Although 5 studies reported microbial differences between subjects with MSA and controls, the majority of the results were not cross-comparable (detailed results are presented in Table 1). Additionally, Qian et al. [146] identified a signature of 25 gut microbial gene markers discriminating *subjects with PD* from normal controls using shotgun metagenomics sequencing of feces. Subsequently, this signature also showed a good capacity to discriminate between subjects with PD and MSA. On the other hand, metabolomic studies investigating fecal or plasma concentrations of short-chain fatty acids may indirectly argue for a different composition of the gut microbiota in patients with MSA since these metabolites are mainly produced by the gut microbiota. He et al. [147] highlighted a decrease in plasma acetic acid levels in 25 patients with MSA compared to 46 healthy controls, while Tan et al. [144] reported a decrease in fecal acetic acid, propionic acid and butyric acid levels in 17 patients with MSA compared to 17 controls. However, the significance of all of these results remains uncertain, as they might be a consequence rather than cause of the disease.

Studies not focused on a particular pathogen

Using a more global approach, Hasan et al. [148] compared the frequencies of hospitalization-required infections or sepsis between 459 patients with clinically diagnosed alpha-synucleinopathies (307 with PD, 80 with DLB, 56 with PD dementia and 16 with MSA) and 459 age- and sex-matched controls. After adjusting for several confounding factors, they found no significant association between histories of severe infections (preceding clinical motor symptom onset) and the occurrence of alpha-synucleinopathies, whether they considered the type of synucleinopathies or the type of infections (focusing on pneumonia, urinary tract infection, cellulitis, influenza or *Helicobacter pylori*) separately or as a whole. In another cross-sectional study including 37 patients with DLB and 14 with PD dementia, a history of systemic infection treated with antibiotics was significantly associated with an older age at dementia onset [149].

Results from studies of changes in epigenetic or microRNA expression profiles

Using postmortem brain samples, Bettencourt et al. [150] highlighted numerous DNA methylation modifications (i.e., epigenetic changes) between subjects with MSA and controls. They subsequently performed a comethylation network analysis that identified "molecular signatures" significantly associated with MSA and, using Gene Ontology and pathway enrichment analysis, investigated the underlying pathophysiological mechanisms. Interestingly, while the molecular signature most strongly correlated with the MSA status was associated with the SNCA gene, the second pointed to pathways related to infections (HTLV-1 and toxoplasmosis). In addition, using sera from patients with MSA and controls, Pérez et al. [151] identified several changes in the microRNA expression profile (i.e., presence of small noncoding RNAs that are capable of preventing the translation of certain messenger RNAs and thus controlling gene expression). A biological enrichment analysis of genes targeted by differentially expressed microRNAs involved fatty acid metabolism, prion disease, Notch signaling and senescence pathways, as well as pathways related to hepatitis B and viral carcinogenesis. Similarly, two other studies reported an upregulation of miR-223 [152,153], a microRNA that appears to be involved in the response to infections [154], in the serum of patients with MSA compared to controls.

V- Are certain susceptibility factors necessary for an infectious agent to trigger the disease?

When proposing an effect of infectious agents on the onset of alpha-synucleinopathies, several other factors should also be considered, including the host immune response and a plethora of additional possible susceptibility factors that might explain why some infected subjects remain healthy carriers while others develop neurological symptoms.

The host immune system response is an essential element that must be considered when examining the "infectious hypothesis" [40]: an inadequate response of the immune system, whether altered or excessive, might be a factor determining the appearance of lesions in the nervous system. Indeed, some studies have suggested specific immunological profiles of subjects with PD [40,155], taking into account both innate and adaptive immunity. An altered immune response, secondary to the onset of immunosenescence, might facilitate infections with new pathogens and the reactivation/worsening of infections acquired earlier in life - both resulting in a potential increase in aS production. Immunosenescence also exerts a strong effect on microglial cells [156], responsible for the clearance of aS aggregates in the CNS [36], and might therefore lead to an accumulation of aS deposits, which are recognized as neurotoxic. Such an implication of the immune system's progressive alteration upon aging may explain the slow onset of the disease at a relatively advanced age. Moreover, damage to the nervous system may also result from excessive activation of the immune system, particularly microglial cells, subsequently leading to a state of neuroinflammation that is deleterious to the brain [36]. Notably, recent evidence shows that microglial cells respond to environmental challenge, including microbiota challenge [157], which, therefore, might trigger an altered microglial response to specific insults in the aged brain. In addition to microglia, peripheral immune cells, such as lymphocytes, are involved in the pathogenesis of PD [158]. An underlying infection present in the organism has the potential to alter the blood–brain barrier in numerous ways [159], increasing the infiltration of peripheral immune cells into the CNS and therefore potentially contributing to disease development.

The additional susceptibility factors are divided into genetic and environmental factors. On the one hand, some of the (suspected or confirmed) genetic risk factors for PD, MSA or DLB [71,160–164] seem to be related to the host's susceptibility to infections, including the LRRK2 gene [165–171], the PRKN gene [172–177], the VPS35 gene [178], the GBA gene [179], the E4 allele of the apolipoprotein gene [180] and finally the CTSB gene [181]. Similar associations were also found for the COQ2, EDN1, SHC2 and MAPT genes, but with p values not reaching the threshold usually used in GWAS [182].

On the other hand, several links might exist between a potential implication of infectious agents and suspected environmental risk factors for alpha-synucleinopathies [6,183] (including neurotoxins such as MPTP, pesticides, herbicides, xenobiotics, heavy metals or nutrition). Interestingly, in the context of AD, Robinson et al. proposed that, in addition to its antimicrobial role, the aβ peptide might also be a bioflocculant [184], which is a molecule that binds neurotoxic substances, including infectious agents, neurotoxins and metal ions, to facilitate their clearance by the immune system. As aS also interacts with different neurotoxins and metal ions [6,11], this hypothesis may also apply to aS. Moreover, in some animal models, there seems to be a synergy between exposure to pesticides and certain infections: pesticides worsening the severity of the infection [185,186]. However, to our knowledge, this process has yet to be studied in animal models of alpha-synucleinopathies or in humans. Some researchers also suggest that dietary factors (including some polyphenols derived from gut microbiota metabolism) also modulate the risk of disease onset [187]. Finally, the geographical distribution of these susceptibility factors (whether genetic or environmental) or that of different infectious strains might explain the differences in terms of the prevalence of certain alpha-synucleinopathies which seem to be found between certain regions of the world [2,188].

VI- Conclusions and future directions

In summary, arguments favoring a potential implication of aS in the antimicrobial defense and its propagation from entry points of infectious agents to the CNS seem to provide an appealing explanation for the onset and pathophysiological heterogeneity of alpha-synucleinopathies. Nevertheless, clear results from human studies are still lacking (in particular for DLB and MSA), unabling full support for such a hypothesis.

Further studies should consider the pathological peculiarities of individual synucleinopathies when studying potential entry points and cellular tropisms of infectious agents suspected to promote their onset (and in particular differentiate MSA from PD and DLB). The presence of possible susceptibility factors modulating the effects of these infections on the CNS must also be considered to better understand in whom and when a specific disease develops (Figure 1). New studies are also needed to confirm or deny the antimicrobial role of the various deposits responsible for neurodegenerative diseases and possibly to identify whether some are specific to particular types of pathogens. Moreover, the absence of studies on the gut microbiota of patients with DLB contrasts with the large number of studies on subjects with PD and MSA,

and new studies using postmortem brain samples (assessing the presence of a specific microorganism or using a more agnostic approach) would be very interesting to verify the proposed effects of neurotropic infectious agents. These additional investigations may have important implications in developing more suitable treatment options, whether through the potential development of vaccines, anti-infective or microbiome therapies (including drugs or diet), or by influencing current clinical trials testing immunotherapies against aS.

Acknowledgement:

We would like to thank the inter-university diploma "Diagnosis and Management of Alzheimer's Disease and Related Diseases" (DIU MA², University of Lille, France) under the aegis of the "Federation of Memory Centers" which made possible carrying out this work. Thank you also to the Fondation Vaincre Alzheimer for facilitating access to this excellent training. Figures created with BioRender.com.

Authors' roles:

ML, AR, LM, AFS, FB and CH participated in the design of the research project. ML, AR and LM performed the systematic literature review on the association between infectious agents and the onset of DLB or MSA. ML wrote the first draft of the article, ALB and IG brought their expertise in animal models and associated literature and all authors critically reviewed the manuscript. IG generated the figures in Biorender.

Financial disclosure of all authors (for the preceding 12 months):

ML received a doctoral grant from the French Ministry of Higher Education and Research from 2017 to 2021. AR, LM, IG, ALB, AFS, CH have nothing to disclose.

FB was the national coordinator for France for the Eisai Delphia (E2027) and Axovant Headway-DLB therapeutic trials. He is currently the national coordinator for France of the Roche Graduate therapeutic trial.

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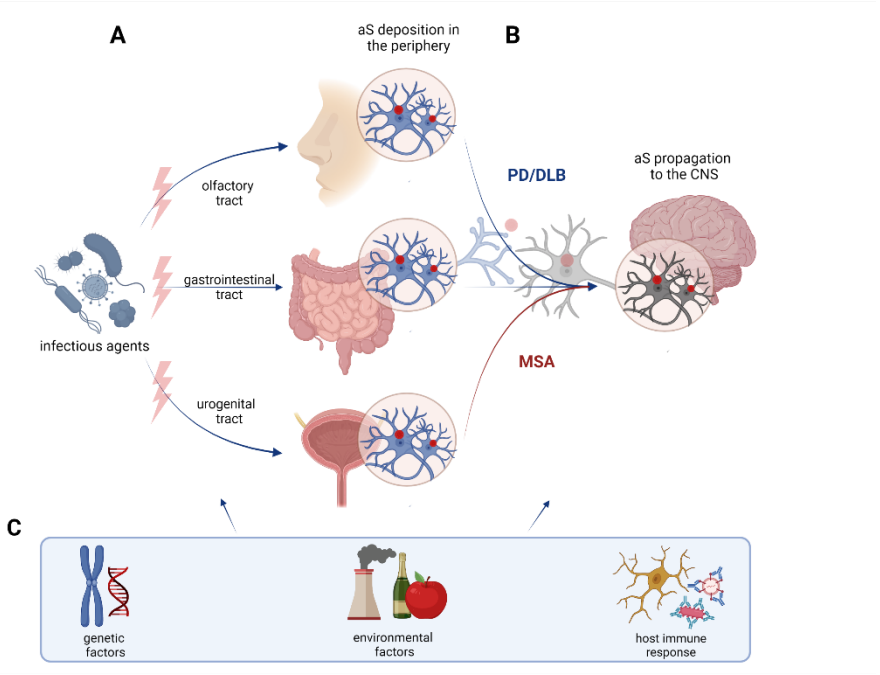
Table 1: Studies assessing the potential effect of the gut microbiota on the onset of MSA.

Articles	Subjects and methods	Comparison of the microbiota of patients with MSA and controls
Engen 2017 USA	- 6 patients with MSA and 11 healthy controls - Exploration of i) fecal microbiota and ii) microbiota present in the mucous membrane of the sigmoid colon using 16S ribosomal RNA gene amplicon sequencing	**Fecal microbiota:** - **At the phylum level**: higher relative abundance of *Bacteroidetes*, lower relative abundance of *Firmicutes* - **At the family level**: higher relative abundance of *Clostridiaceae* and *Rikenellaceae* and lower relative abundance of *Lachnospiraceae* and *Ruminococcaceae* - **At the genus level**: lower relative abundance of *Blautia* and *Doera* **Sigmoid mucosa microbiota:** - **At the family level**: higher relative abundance of *Oxalobacteraceae* and *Porphyromonadaceae* - **At the genus level**: higher relative abundance of *Ralstonia*
Tan 2017 Malaysia	- 17 patients with MSA and 17 age-matched healthy controls living in the same community (avoiding environmental confounding factors) - Exploration of the fecal microbiota using16S ribosomal RNA gene amplicon sequencing	**Fecal microbiota** - **At the phylum level**: no significant difference - **At the genus level**: higher abundance in *Bacteroides* and lower abundance in *Paraprevotella*
Barichella 2019 Italy	- 22 patients with MSA, 193 patients with idiopathic PD, 22 patients with progressive supranuclear palsy and 113 healthy controls matched for age, body mass index and geographical area. - Exploration of the fecal microbiota using16S ribosomal RNA gene amplicon sequencing	**Fecal microbiota** - **At the family level:** higher abundance of *Verrucomicrobiaceae*, lower abundance of *Prevotellaceae* - **At the genus level:** higher abundance of *Akkermansia*, *Parabacteroides*, lower abundance of *Faecalibacterium*
Du 2019 China	- 40 patients with MSA and 40 healthy controls (spouses) - Exploration of i) fecal and ii) blood microbiota using16S ribosomal RNA gene amplicon sequencing	**Fecal microbiota** - **At the genus level**: higher relative abundance of *Lactobacillus*, *Gordonibacter*, and *Phascolarctobacterium* and lower relative abundance of *Haemophilus* **Blood microbiota** - **At the genus level**: higher relative abundance of *Bacteroides* and lower relative abundance of *Leucobacter*
Wan 2019 China	- 15 patients with MSA and 15 healthy controls - Exploration of the fecal microbiota using metagenomic sequencing (sequencing of the entire DNA and not just the hypervariable loci in the 16S rDNA gene)	**Fecal microbiota** - **At the phylum level:** higher abundance of *Verrucomicrobia* and lower abundance of *Actinobacteria* - **At the genus level:** higher abundance of *Akkermansia a*nd lower abundance of *Megamonas*, *Bifidobacterium*, *Blautia*, and *Aggregatibacter* - **At the species level:** higher abundance of *Roseburia hominis*, *Akkermansia muciniphila*, *Alistipes onderdonkii*, *Streptococcus parasanguinis*, and *Staphylococcus xylosus* and lower abundance of *Bacteroides coprocola*, *Megamonas funiformis*, *Bifidobacterium pseudocatenulatum*, *Clostridium nexile*, *Bacteroides plebeius*, and *Granulicatella adiacens*.

Figure 1: The “infectious hypothesis”

Alpha-synuclein, a potential antimicrobial peptide, may accumulate in the periphery due to infectious agents present in mucous membranes (A). Its secondary spread through neuronal networks (B), in concomitance with susceptibility factors (C), may damage the central nervous system.



Appendix 1: Systematic review methodology

We searched the PubMed and Scopus databases for articles published up to June 2021 without any time or geographic limitations and identified 1113 articles on our topic of interest.

The following search algorithms were used:

- In PubMed:

- (((((((((((((((Multiple system atrophy[Title/Abstract]) OR Multiple system atrophies[Title/Abstract]) OR Multisystem atrophy[Title/Abstract]) OR Multisystem atrophies[Title/Abstract]) OR Multisystemic atrophy[Title/Abstract]) OR Multisystemic atrophies[Title/Abstract]) OR Lewy Body Disease[Title/Abstract]) OR Lewy Body Diseases[Title/Abstract]) OR Lewy Body Dementia[Title/Abstract]) OR Lewy Body Dementias[Title/Abstract]) OR Dementia with Lewy bodies[Title/Abstract]) OR Dementias with Lewy bodies[Title/Abstract]) OR Disease with Lewy bodies[Title/Abstract]) OR Diseases with Lewy bodies[Title/Abstract]))
- AND
- (((infect*[Title/Abstract]) OR bacteria*[Title/Abstract]) OR bacillus[Title/Abstract]) OR fungal[Title/Abstract]) OR fungus[Title/Abstract]) OR fungi[Title/Abstract]) OR mycos*[Title/Abstract]) OR parasite*[Title/Abstract]) OR virus[Title/Abstract]) OR viruses[Title/Abstract]) OR viral[Title/Abstract]) OR microbiotas[Title/Abstract]) OR microbiota[Title/Abstract]) OR microbial[Title/Abstract]) OR microbiome[Title/Abstract]) OR microbiomes[Title/Abstract]) OR microbes[Title/Abstract]) OR microbe[Title/Abstract]) OR flora[Title/Abstract]) OR microflora[Title/Abstract]) OR microorganism[Title/Abstract]) OR microorganisms[Title/Abstract]) OR micro-organism[Title/Abstract]) OR micro-organisms[Title/Abstract]) OR pathogen[Title/Abstract]) OR pathogens[Title/Abstract]) OR prion[Title/Abstract]) OR prions[Title/Abstract]) OR anti-infective*[Title/Abstract]) OR anti infective*[Title/Abstract]) OR antiinfective*[Title/Abstract]) OR antimicrobial*[Title/Abstract]) OR anti-microbial*[Title/Abstract]) OR anti-bacterial*[Title/Abstract]) OR antibacterial*[Title/Abstract]) OR anti bacterial*[Title/Abstract]) OR bactericidal[Title/Abstract]) OR bacteriocide*[Title/Abstract]) OR antibiotic[Title/Abstract]) OR

antibiotics[Title/Abstract]) OR anti-biotic[Title/Abstract]) OR anti-biotics[Title/Abstract]) OR antifungal*[Title/Abstract]) OR anti-fungal*[Title/Abstract]) OR fungicide*[Title/Abstract]) OR antiparasitic*[Title/Abstract]) OR anti-parasitic*[Title/Abstract]) OR parasiticide*[Title/Abstract]) OR antiviral*[Title/Abstract]) OR anti-viral*[Title/Abstract]) OR anti viral*[Title/Abstract]) OR anti-retroviral*[Title/Abstract]) OR antiretroviral*[Title/Abstract]) OR anti retroviral*[Title/Abstract])

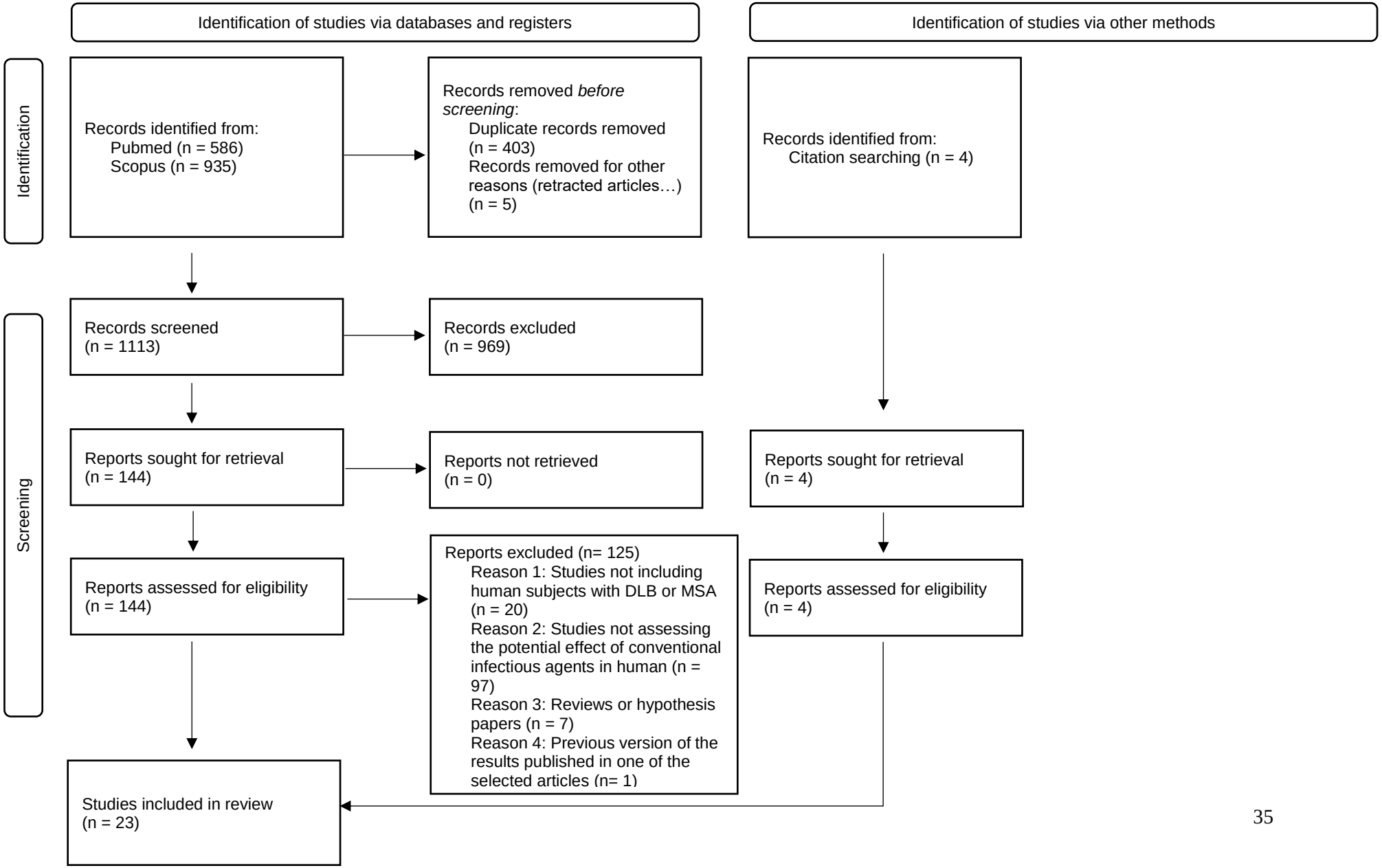
- In Scopus:

TITLE-ABS-KEY ("Multiple system atrophy" OR "Multiple system atrophies" OR "Multisystem atrophy" OR "Multisystem atrophies" OR "Multisystemic atrophy" OR "Multisystemic atrophies" OR "Lewy Body Disease" OR "Lewy Body Diseases" OR "Lewy Body Dementia" OR "Lewy Body Dementias" OR "Dementia with Lewy bodies" OR "Dementias with Lewy bodies" OR "Disease with Lewy bodies" OR "Diseases with Lewy bodies")
AND
TITLE-ABS-KEY (infect* OR bacteria* OR bacillus OR fungal OR fungus OR fungi OR mycos* OR parasite* OR virus OR viruses OR viral OR microbiotas OR microbiota OR microbial OR microbiome OR microbiomes OR microbes OR microbe OR flora OR microflora OR microorganism OR microorganisms OR “micro-organism” OR “micro-organisms” OR pathogen OR pathogens OR prion OR prions OR “anti-infective*” OR “anti infective*” OR antiinfective* OR antimicrobial* OR “anti-microbial*” OR “anti-bacterial*” OR antibacterial* OR “anti bacterial*” OR bactericidal OR bacteriocide* OR antibiotic OR antibiotics OR “anti-biotic” OR “anti-biotics” OR antifungal* OR “anti-fungal*” OR fungicide* OR antiparasitic* OR “anti-parasitic*” OR parasiticide* OR antiviral* OR “anti-viral*” OR “anti viral*” OR “anti-retroviral*” OR antiretroviral* OR “anti retroviral*”)

After removing duplicates, three investigators (AR, ML and LM) independently screened the titles and abstracts of the 1113 records identified (2/3 each). Discrepancies were resolved through discussion between the investigators. 969 articles were thus excluded based on the

title or on the abstract, and 125 articles were excluded based on the full text. We also reviewed the reference lists from the selected articles and identified four additional articles that were not captured by our research algorithm. Twenty-three articles were included in our review.

Supplemental figure 1: Flow chart



Supplemental table 1: Articles included in the systematic review

	First author	Year	Country	Pathology	Infectious agent	Type of study
1	Chapman	2003	USA	DLB	*Nocardia Asteroides*	Examination of post-mortem brain samples
2	Lu	2005	USA	DLB	*Nocardia Asteroides*	Examination of post-mortem brain samples
3	Woulfe	2000	Canada	DLB and MSA	Epstein Barr virus	Examination of post-mortem brain samples
4	Segers	2021	Belgium	DLB	West Nile virus	Case report
5	Gadila	2021	USA	DLB	Lyme disease	Case report
6	Blanc	2014	France	DLB	Lyme disease	Assessment of the presence of intrathecal synthesis of anti-*Borrelia* antibodies
7	Cassarino	2003	USA	MSA	Lyme disease	Case report
8	Kano	1989	Japan	MSA	HTLV-1	Case report
9	Yokota	1994	Japan	MSA	HLTV-1	Assessment of the presence of HTLV-1 plasma antibodies
10	Hjelle	1992	USA	MSA	HLTV-2	Case report
11	Engen	2017	USA	MSA	Gut microbiota	Exploration of i) fecal microbiota and ii) microbiota present in the mucous membrane of the sigmoid colon using16S rRNA gene amplicon sequencing
12	Tan	2017	Malaysia	MSA	Gut microbiota	Exploration of the fecal microbiota using16S rRNA gene amplicon sequencing + Exploration of fecal concentrations of short-chain fatty acids
13	Barichella	2019	Italy	MSA	Gut microbiota	Exploration of the fecal microbiota using16S rRNA gene amplicon sequencing
14	Du	2019	China	MSA	Gut microbiota	Exploration of i) fecal and ii) blood microbiota using16S rRNA gene amplicon sequencing
15	Wan	2019	China	MSA	Gut microbiota	Exploration of the fecal microbiota using metagenomic sequencing
16	Qian	2020	China	PD (and MSA)	Gut microbiota	Exploration of the fecal microbiota using metagenomic sequencing
17	He	2021	China	MSA	Gut microbiota	Exploration of plasma concentrations of short-chain fatty acids

18	Hasan	2020	USA	DLB and MSA	None in particular	Case-control study assessing the association between clinically diagnosed alpha-synucleinopathies and histories of hospitalization-required infections or sepsis
19	De Oliveira	2020	Brazil	DLB	None in particular	Cross-sectional study assessing if history of systemic infection treated with antibiotic (among other risk factors) modify age at dementia onset
20	Bettencourt	2019	UK	MSA	None in particular	Epigenetic changes in post-mortem brain tissues
21	Pérez	2020	Spain	MSA	None in particular	MicroRNA changes in the serum
22	Kume	2018	Japan	MSA	None in particular	MicroRNA changes in the serum
23	Vallelunga	2014	USA	MSA	None in particular	MicroRNA changes in the serum

Abbreviations: DLB: Dementia with Lewy Bodies, MSA: Multiple system atrophy, PD: Parkinson's disease, rRNA: ribosomal RNA