

PARKINSON'S DISEASE: A COMPREHENSIVE ANALYSIS OF FUNGI AND  
BACTERIA IN BRAIN TISSUE

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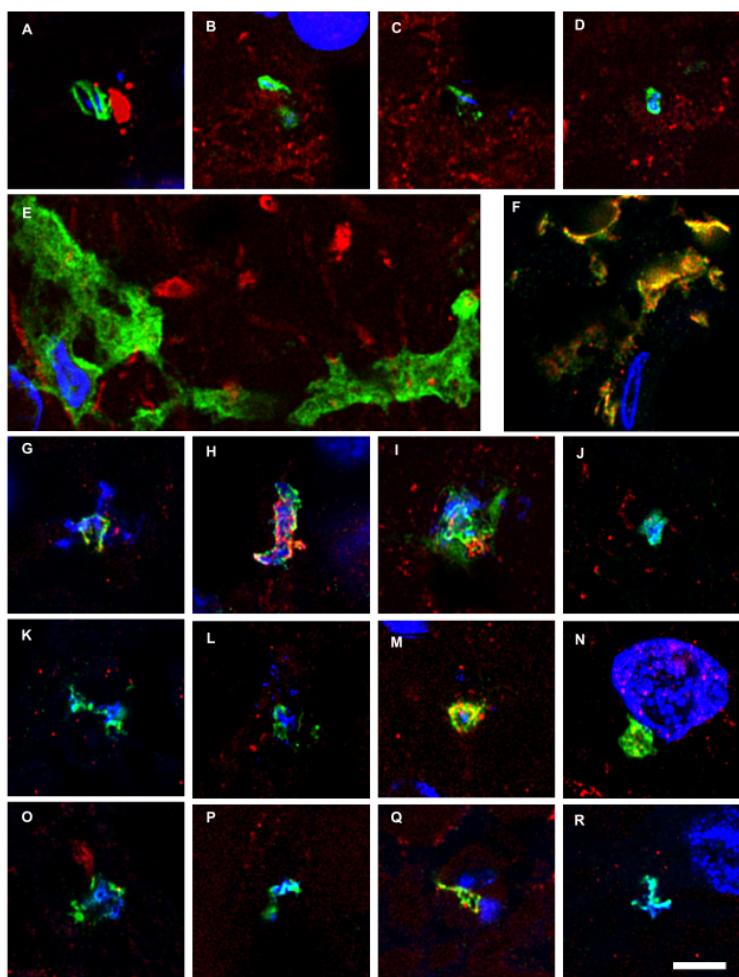
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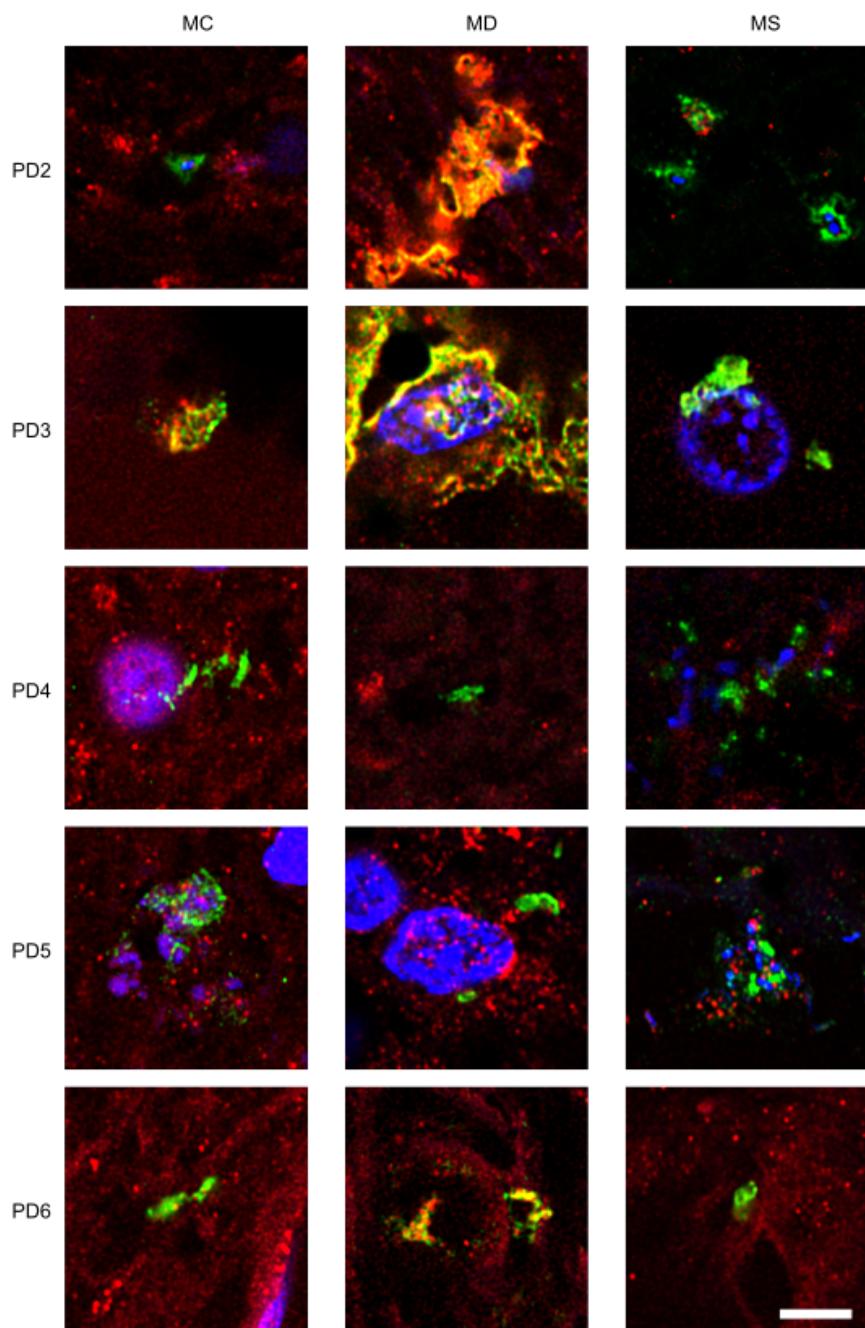
Running title: Parkinson's disease and microbial infection

Key words: Parkinson's disease; neurodegenerative diseases; polymicrobial infections;  
fungal infection; next-generation sequencing

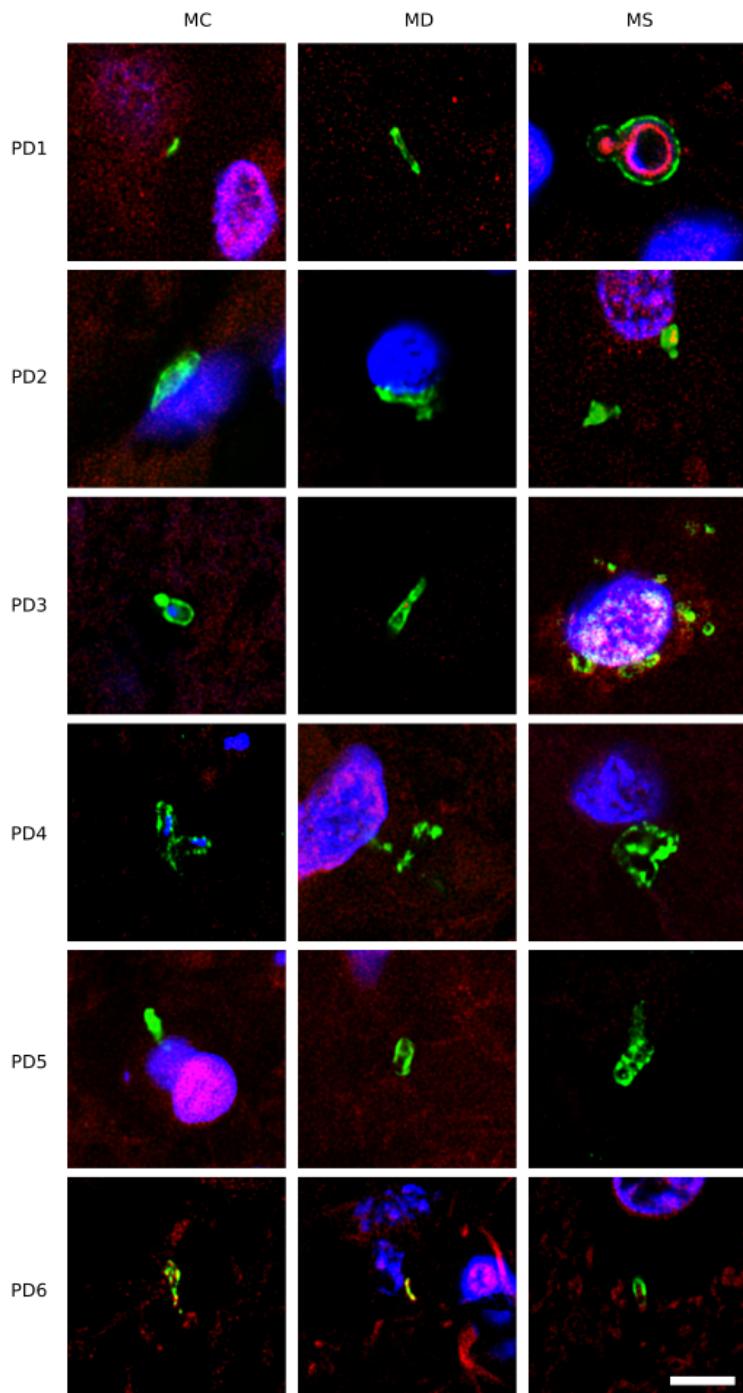
**Supplementary Figure 1. Fungal structures of CNS sections from PD1 revealed with anti-fungal enolase and anti-fungal tubulin antibodies.** Immunohistochemistry analysis of CNS sections from patient PD1 was performed by confocal microscopy as detailed in Materials and Methods. Sections were immunostained with rabbit polyclonal antibodies against fungal  $\beta$ -tubulin and enolase (green) and human neurofilaments or  $\alpha$ -tubulin (red). Panels A–E: anti-fungal  $\beta$ -tubulin and anti-human neurofilaments antibodies. Panels F–R: anti-fungal enolase and anti-human  $\alpha$ -tubulin antibodies. Panels A–D and R: caudate and lenticular nuclei. Panels E and P–Q: callosal body. Panels F and O: pons. Panels G–J: medulla. Panels K and L: mesencephalon. Panels M and N: hypothalamus. DAPI staining appears in blue. Scale bar: 5  $\mu$ m.



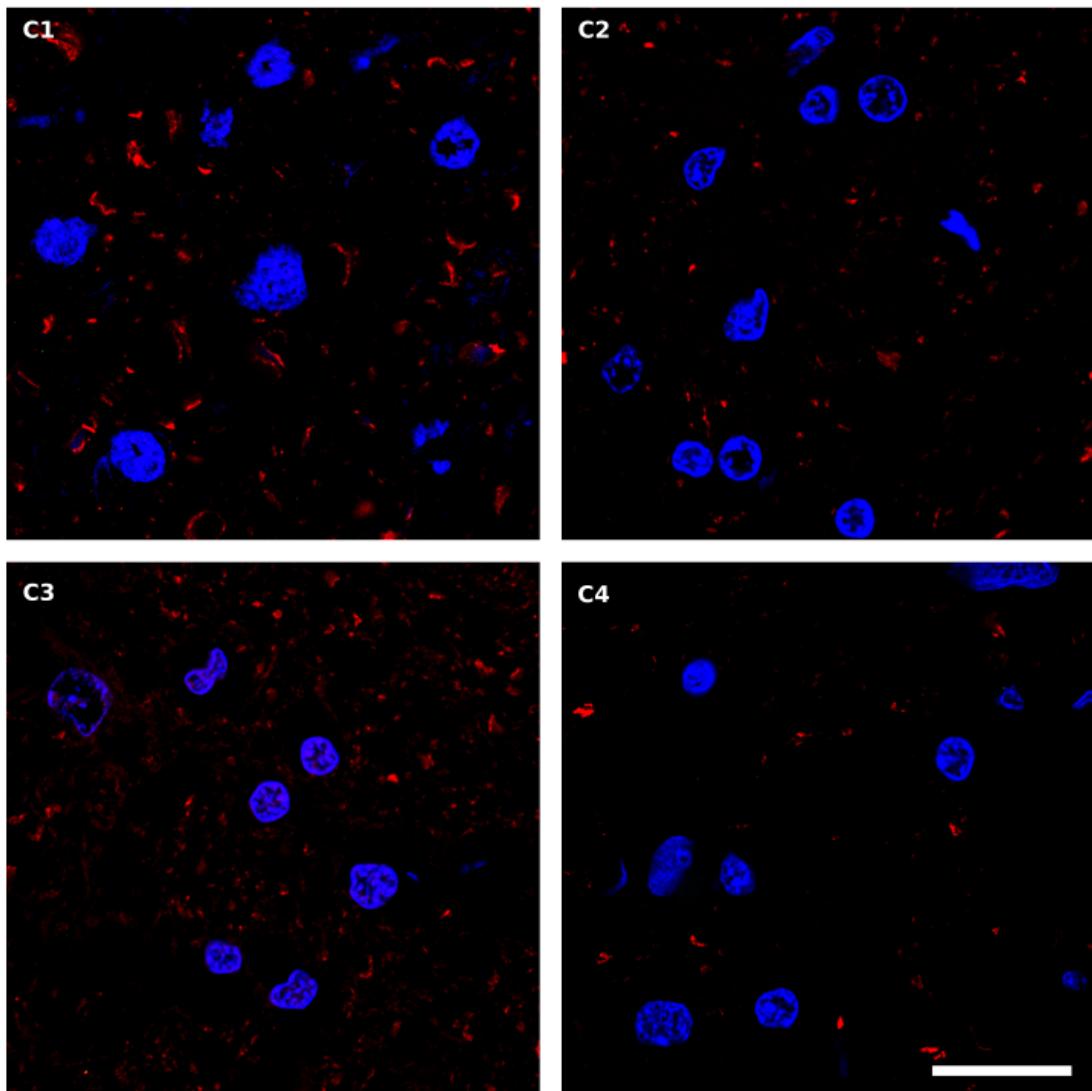
**Supplementary Figure 2. Immunohistochemistry with an anti-*P. betae* antibody of CNS sections from five PD patients.** Three CNS regions (motor cortex, MC; medulla, MD; and mesencephalon, MS) from five PD patients (PD2–PD6) were immunostained with a rabbit polyclonal antibody against *P. betae* (green) and a mouse monoclonal antibody against human  $\alpha$ -tubulin (red). Nuclei were stained with DAPI (blue). Scale bar: 5  $\mu$ m.



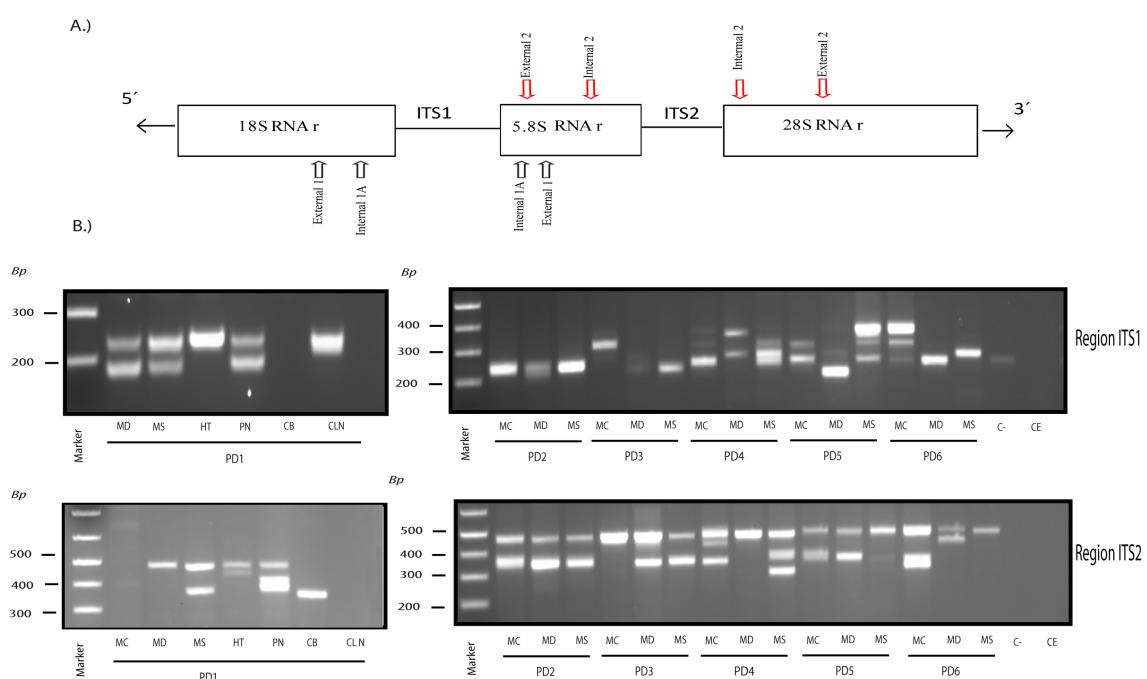
**Supplementary Figure 3. Immunohistochemistry of CNS sections from six PD patients using an anti-chitin antibody.** Different regions of six PD patients were incubated using anti-chitin (green) and anti-human  $\alpha$ -tubulin (red) antibodies. Nuclei appear in blue. Scale bar: 5  $\mu$ m.



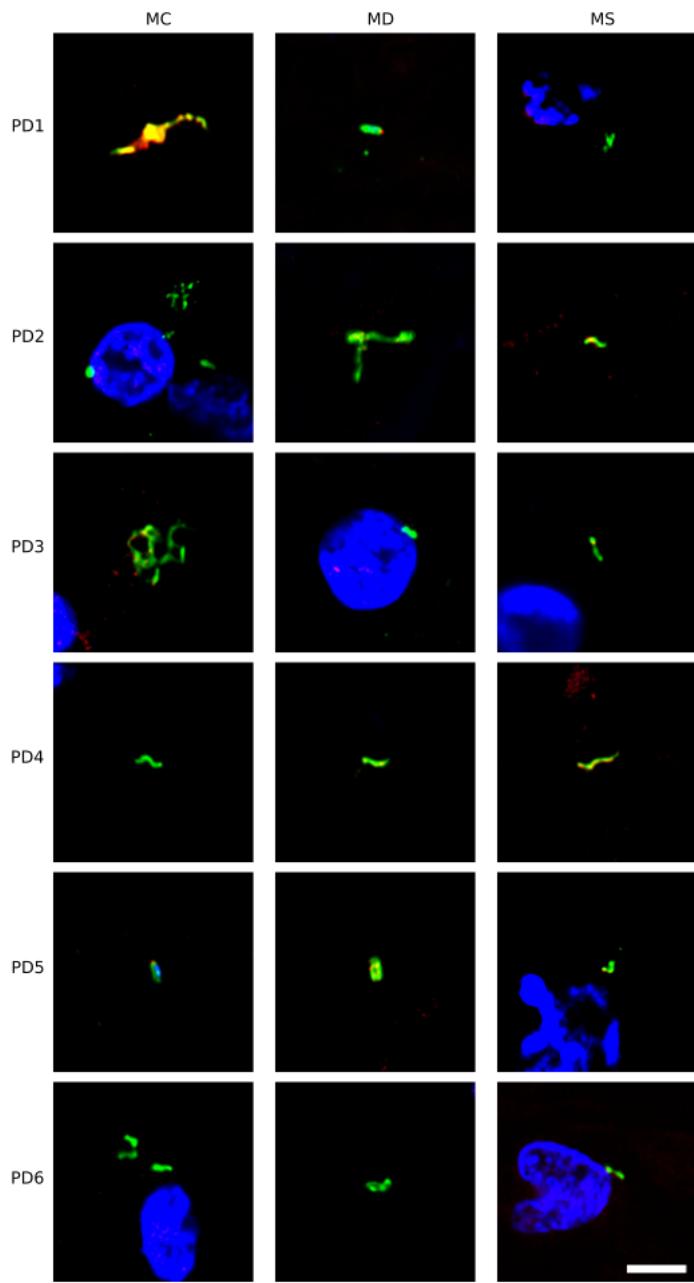
**Supplementary Figure 4. Immunohistochemistry of CNS samples from control subjects using an anti-chitin antibody.** Different regions of four control subjects were incubated using anti-chitin (green) and anti-human  $\alpha$ -tubulin (red) antibodies. Nuclei appear in blue. C1 and C2: spinal cord. C3 and C4: medulla. Scale bar: 20  $\mu$ m.



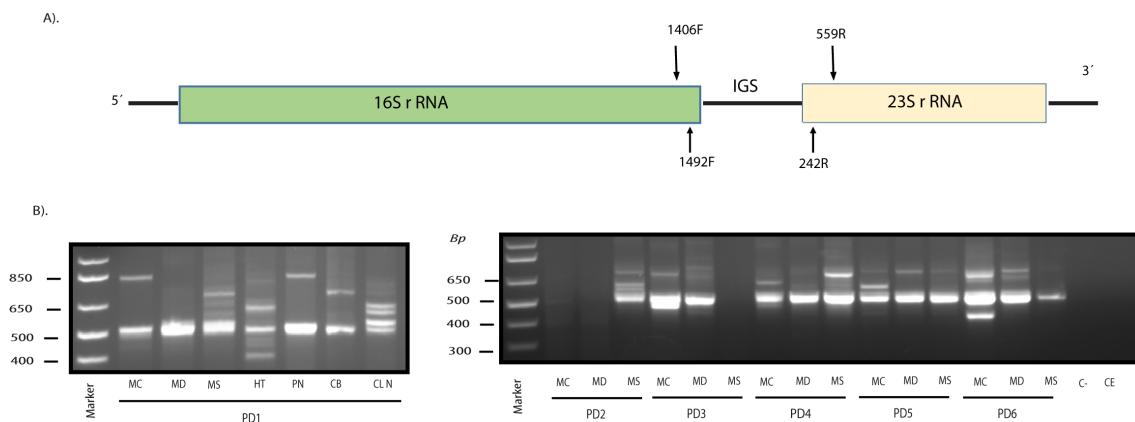
**Supplementary Figure 5. Nested PCR analysis of fungal ITS1 and ITS2 in DNA extracted from frozen CNS tissue.** Nested PCR of fungal DNA from PD patients. PCR analysis was carried out as described (see Materials and Methods). Agarose gel electrophoresis of the DNA fragments amplified by nested PCR. Panel A shows a schematic representation of fungal rRNA genes (18S, 5.8S and 28S rRNA) and the ITS1 and ITS2 regions, including location of the primers employed for the different nested PCRs; primers External 1 employed in the first PCR, primers Internal 1 employed in the second PCR to amplify ITS1, primers Internal 2 employed in the second PCR to amplify ITS2. Panel B shows nested PCR of ITS1, ITS2 in six PD patients. Upper panel, agarose gel electrophoresis of the DNA fragments amplified by nested PCR using DNA extracted from different regions of six PD patients using primers Internal 1 to amplify the ITS1 region. Bottom panel, agarose gel electrophoresis of the ITS2 region DNA fragments amplified from different CNS regions of six PD patients using primers Internal 2. C: Control PCR without DNA. CE: Control of DNA extraction without DNA. MC: motor cortex; MD: medulla; MS: mesencephalon; HT: hypothalamus; PN: pons; CB: callosal body; CLN: caudate and lenticular nuclei



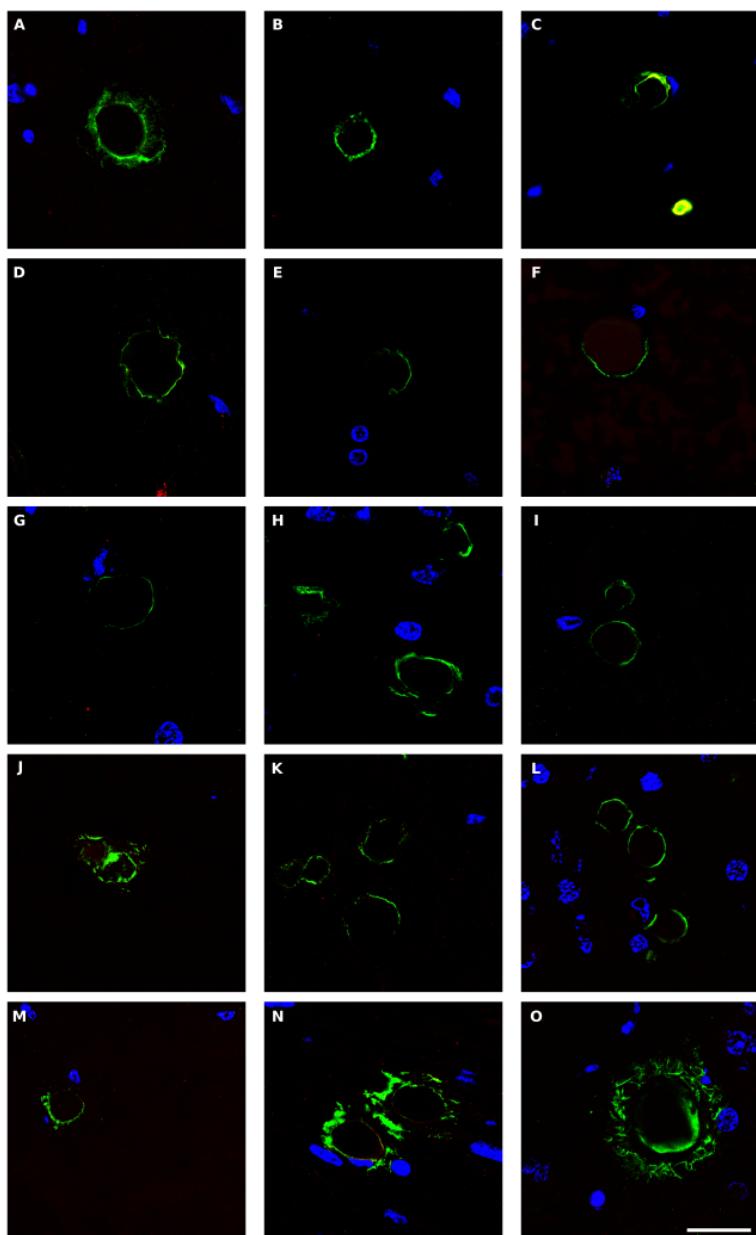
**Supplementary Figure 6. Prokaryotic structures revealed by immunohistochemistry using an anti-*C. pneumoniae* antibody in CNS sections from PD patients.** Three CNS regions (motor cortex, MC; medulla, MD; and mesencephalon, MS) of six PD patients were immunostained with a rabbit polyclonal antibody against *C. pneumoniae* (green) and a rat polyclonal antibody against *T. viride* (red). Nuclei were stained with DAPI (blue). Scale bar: 5  $\mu$ m.



**Supplementary Figure 7. Nested PCR analysis of the bacterial intergenic sequence region in DNA samples from frozen CNS tissue.** PCR analysis was carried out as described in Materials and Methods. Panel A shows a schematic representation of the intergenic sequence (IGS) region, including the location of the primers employed for the different reactions. Panel B shows agarose gel electrophoresis of the DNA fragments amplified by nested PCR; analysis of different regions from six patients amplifying the IGS region. Primers 1406 (F)-559 (R) and 1492 (F)-242 (R) were used in the first and second round PCR, respectively: C: control PCR without DNA; CE: control of DNA extraction; MC: motor cortex; MD: medulla; MS: mesencephalon; HT: hypothalamus; PN: pons; CB: callosal body; CLN: caudate and lenticular nuclei.



**Supplementary Figure 8. Bacterial antigens in *corpora amylacea* observed in CNS sections from PD patients.** Different sections from PD patients were incubated with a mouse monoclonal antibody against bacterial peptidoglycan (green), and a rabbit polyclonal antibody against *C. albicans* (red). Panels A–C: patient PD1; panels D and E: PD2; panels F and G: PD3; panels H and I: PD4; panels J–L: PD5; and panels M–O: PD6. Panels A, D, H, K and N: medulla; panels B, E, G, I, L and O: mesencephalon; panel C: callosal body; and panels F, J and M: motor cortex. DAPI appears in blue. Scale bar: 20  $\mu\text{m}$ .



**Supplementary Table I. Age and gender of PD patients studied in this work**

PD patients	AGE	GENDER	CONTROLS	AGE	GENDER
PD1	65	MALE	C1	53	MALE
PD2	77	MALE	C2	78	MALE
PD3	84	MALE	C3	74	FEMALE
PD4	79	FEMALE	C4	83	FEMALE
PD5	82	MALE			
PD6	89	MALE			

**Supplementary Table II. Fungal species identified by NGS**

PD1 MC	PD1 MD	PD1 MS	PD1 HT		PD1 PN	PD1 CB	PD1 CLN						
Original paired reads	809499	Original paired reads Joined sequences (%)	779383	Original paired reads : Joined sequences (%):	760401 68,8	Original paired reads : Joined sequences (%):	756460 63,9	Original paired reads : Joined sequences (%):	757500 55,6	Original paired reads : Joined sequences (%):	745017 66,33	Original paired reads : Joined sequences (%):	696133 65,2
Uncultured candida	18,7	<i>Uncultured candida</i> <i>Uncultured fungus clone S24T</i>	20,7	<i>Uncultured fungus clone S24T</i> <i>Candida deformans</i> <i>Uncultured fusarium</i>	4,4 2,5	<i>Candida_sp</i> <i>Xylaria_curtia</i>	40,7 16,6	<i>Xylaria_curtia</i> <i>Uncultured Trichosporon</i>	21,9 14,9034661	<i>Ascomycota</i>	47,5	<i>Uncultured trichosporon</i>	27,4
Penicillium_digitatum	7	<i>Uncultured fung</i> <i>clone S24T</i>	4,5	<i>Candida deformans</i> <i>Uncultured</i>				<i>Cryptococcus_curvatus</i>	26,2	<i>Candida_sp</i>	21,7		
Xylaria_curtia	3,3	<i>Xylaria_curtia</i> <i>fusarium</i> <i>Uncultured</i>	2,6	<i>malassezia</i> <i>Uncultured fungus clone S346</i>	2,5 1,8	<i>Cryptococcus_magnus</i>	11,1	<i>Uncultured candida</i> <i>Uncultured basidiomycota</i>	12,9	<i>Cryptococcus_magnus</i>	12,6	<i>Xylaria_curtia</i> <i>Uncultured basidiomycota</i>	19,2
Candida deformans	2,3	<i>Candida deformans</i> <i>malassezia</i> <i>Uncultured fungus clone S346</i>	2,4	<i>Botrytis cinerea</i> <i>Malassezia globosa</i>	1,9 1,5	<i>Botrytis cinerea</i> <i>Malassezia globosa</i>	1,8	<i>Cryptococcus_curvatus</i> <i>Uncultured fung</i> <i>clone S24T</i>	7	<i>Xylaria_curtia</i>	9,8	<i>Uncultured candida</i>	3,5
Uncultured fusarium	2,3	<i>Uncultured malassezia</i>		<i>Botrytis cinerea</i> <i>Malassezia globosa</i>	1,9	<i>Candida_sp</i> <i>Uncultured fung</i> <i>clone S24T</i>	1,8	<i>Candida_sp</i> <i>Uncultured fung</i> <i>clone S24T</i>	1,5	3,2		<i>unculturedCladosporium</i>	2,2
Botrytis cinerea	1,7	<i>Botrytis cinerea</i> <i>Uncultured fung</i> <i>clone S346</i>	1,7	<i>Ascomycota sp</i> <i>Uncultured fung</i> <i>clone S44T_79</i>	1,6		1,3	<i>Candida deformans</i>	1,6			<i>Cryptococcus_curvatus</i>	1,1
Ascomycota sp	1,2	<i>Ascomycota sp</i> <i>Uncultured fung</i> <i>clone S44T_79</i>	1,2	<i>Uncultured fung</i> <i>isolate F-RISA</i>	1,1	<i>Uncultured fung</i> <i>isolate F-RISA</i>	1	<i>Uncultured fusarium</i> <i>Uncultured malassezia</i>	1,6 1,5				
								<i>Botrytis cinerea</i> <i>Uncultured fung</i> <i>clone S346</i>	1,2				
								<i>Malassezia globosa</i>	1				



<i>PD4 MC</i>	<i>PD4 MD *</i>	<i>PD4 MS</i>	<i>PD5 MC</i>	<i>PD5 MD</i>	<i>PD5 MS</i>
Original paired reads : 614956 Joined sequences (%): 71,9	Original paired reads : 640520 Joined sequences (%): 54,6	Original paired reads : 735236 Joined sequences (%): 63,2	Original paired reads : 683167 Joined sequences (%): 58,3	Original paired reads : 861501 Joined sequences (%): 67,4	Original paired reads : 681001 Joined sequences (%): 61,1
<i>Uncultured candida</i> <i>Uncultured basidiomycota</i> <i>Uncultured fungus isolate F-RISA</i> <i>Uncultured fungus clone S24T</i> <i>Davidiella_tassiana</i> <i>Candida deformans clone S44T_79</i> <i>Uncultured fusarium</i> <i>Botrytis cinerea Uncultured fungus clone S346</i> <i>Malassezia globosa</i> <i>Ascomycota sp</i> <i>Uncultured malassezia</i> <i>Uncultured fungus clone S44T_79</i> <i>Uncultured fungus isolate F-RISA</i>	20,2 9,2 5,6 4,4 3,2 2,5 2,5 1,9 1,8 1,5 1,3 1,3 1 1	<i>Uncultured candida basidiomycota</i> <i>Uncultured fungus clone S24T</i> <i>Uncultured fusarium</i> <i>Candida deformans</i> <i>Uncultured fungus clone S346</i> <i>Candida deformans clone S44T_79</i> <i>Ascomycota sp</i> <i>Malassezia globosa</i> <i>Uncultured malassezia</i> <i>Botrytis cinerea</i> <i>Uncultured fungus isolate F-RISA</i> <i>Ustilago_hordei</i> <i>Ascomycota sp</i>	<i>Uncultured candida</i> <i>Uncultured candida</i> <i>Penicillium_digitatum</i> <i>Emericella_nidulans</i> <i>Uncultured fungus clone S24T</i> <i>Candida deformans</i> <i>Uncultured fusarium</i> <i>Malassezia globosa</i> <i>Uncultured malassezia</i> <i>Botrytis cinerea</i> <i>Ustilago_hordei</i> <i>Ascomycota sp</i>	<i>Uncultured Trichosporon</i> <i>Uncultured basidiomycota</i> <i>Uncultured Trichosporon</i> <i>Uncultured fungus clone S24T</i> <i>Xylaria_curta</i> <i>Rhodotorula_mucilaginosa</i> <i>Ustilago_bullata</i> <i>Candida deformans</i> <i>Uncultured fusarium</i> <i>Exophiala_sp_NH1238</i> <i>Uncultured basidiomycota</i> <i>Candida deformans</i> <i>Uncultured fusarium</i> <i>Botrytis cinerea</i> <i>Xylaria_cubensis</i> <i>Malassezia globosa</i> <i>trichoderma_sp</i> <i>Ascomycota sp</i>	<i>Trichosporon</i> <i>Cryptococcus_magnus</i> <i>Uncultured candida</i> <i>trichoderma_sp</i> <i>Xylaria_curta</i> <i>Ustilago_bullata</i> <i>Uncultured fungus clone S24T</i> <i>Penicillium_aurantiogriseum</i> <i>Exophiala_sp_NH1238</i> <i>Uncultured basidiomycota</i> <i>Candida deformans</i> <i>Uncultured fusarium</i> <i>Botrytis cinerea</i> <i>Ascomycota sp</i> <i>Uncultured malassezia</i> <i>Ascomycota sp</i>

<i>PD6 MC</i>	<i>PD6 MD</i>	<i>PD6 MS</i>
Original paired reads : 744266	Original paired reads : 667716	Original paired reads : 667716
Joined sequences (%): 69,6	Joined sequences (%): 43,4	Joined sequences (%): 43,8
<i>Uncultured basidiomycota</i> 13,3	<i>Uncultured candida</i> <i>Uncultured fungus clone</i> S24T 2,9	<i>Penicillium_digitatum</i> 10,7 <i>Uncultured candida</i> 8,3 <i>Candida deformans</i> 1,1 <i>Uncultured fusarium</i> 1,1 <i>Uncultured malassezia</i> 1 <i>unculturedzygomycete</i> <i>Uncultured fungus clone</i> S24T 1,8 <i>Uncultured malassezia</i> 1 <i>Candida deformans</i> 1 <i>Uncultured fusarium</i> 1
<i>Candida deformans</i> 1,6		
<i>Uncultured fusarium</i> 1,6		
<i>Uncultured malassezia</i> 1,5		
<i>Botrytis cinerea</i> <i>Uncultured fungus clone</i> S346 1,2		
<i>Malassezia globosa</i> 1		

**Supplementary Table III. Bacterial species identified by NGS**

PD1 MC	PD1 MD	PD1 MS	PD1 PN	PD1 HT	PD1 CB	PD1 CLN							
Streptococcus	33	Pseudomonas viridiflava	63,7	Propionibacterium acnes	21,2	Lentzea albidocapillata	75	Actinomycetospora	21	Streptophyta	31,3	Megamonas	77
Gaiellaceae	13	Elizabethkingia meningoseptica	8,69	Corynebacterium	19,4	Sphingomonadales	18	Roseococcus	19	Acinetobacter schindleri	26,7	Scardovia	11
Virgosporangium ochraceum	11	Brevundimonas diminuta	6,78	Peptoniphilus	19,3	Lentzea;Other	2,3	Sphingomonas;s__	11	Arthrobacter;Other	15,4	Propionibacterium acnes	3,7
Arthrobacter	8,7	Pseudomonas	5,6	Aerococcaceae	8,8	Lentzea violacea	1,3	Megamonas	6	Acinetobacter lwoffii	7,81	Veillonellaceae	3,6
Actinomycetales	7,8	Pseudomonas pseudoalcaligenes	2,39	OD1	3,7			Pseudanabaenales	5,2	Chloroflexi	5,07	Firmicutes	1,4
Streptococcus	5	Escherichia coli	1,38	Methylobacterium;s__	2,63	Lysinibacillus		TM7	4,4	Lactobacillus;Other	3,93		
Myxococcales	3,7	Enterobacter cloacae	1,15	boronitolerans	2,6			Hymenobacter;s__	4,1	mitochondria;Other	1,68		
Spirobaillales	3,2			Actinobacteria	2,1			Corynebacterium;s__kroppenstedtii	2,5	Leptotrichia;s__	1,49		
Actinobacteria;Other	2,2			Neisseriaceae	1,89			Intrasporangiaceae	2,4				
Actinobacteria;Other	1,9			Propionibacterium	1,27			Sphingomonas;Other	2,4				
Thermoleophilia;Other	1,8			Paucibacter	1,24			Xenococcaceae;Other	2,4				
Streptococcus minor	1,5							Arthrobacter;Other	2,3				
Solirubrobacterales	1,3							Comamonadaceae;Other	1,1				

PD2 MC	PD2 MD	PD2 MS	PD3MC		PD3 MD		PD3 MS				
Streptococcus	34	Ralstonia	26,1	Porphyromonas	43,3	Marinilabiaceae	17	Corynebacterium;s__kroppenstedtii	19,25	Veillonella dispar	19,35
Leptotrichia	6,9	Pseudomonas viridiflava	20,5	Actinomycetales	23,6	Corynebacterium;s__kroppenstedtii	13	Rhizobiales	11,6	Atopococcus abaci	18,32
Haemophilus parainfluenzae	6,3	Pseudomonas pseudoalcaligenes	20,4	Actinobacteria	9,88	Microbacteriaceae	6,9	OD1	6,618	OD1	12,68
Streptococcus	5,1	Streptococcus	9,25	Lentzea albidoapillata	6,9	Microbacterium	6,5	Flectobacillus	6,179	Actinomyces	8,815
Corynebacterium	5	Atopococcus abaci	5,99	TM7	3,81	Staphylococcus	4,8	Atopococcus abaci	5,164	Aerococcaceae	8,217
Veillonella dispar	4,5	Pseudomonas	4,21	Streptophyta	3,34	Comamonadaceae	4,6	Cytophagaceae	5,043	Porphyromonas	6,196
Actinobacteria	3,2	Sphingomonas	4,11	Streptococcus	2,95	Propionibacterium acnes	4,3	Bacillus foraminis	2,636	Corynebacterium	5,037
Fusobacterium	3,1	Kaistobacter	2,74	Bacteroidales	1,24	Flavobacterium	4,1	Staphylococcus	2,408	Comamonadaceae	4,932
Porphyromonas	3					Sulfuricurvum kuijense	4	Flavobacterium	1,85	C39	4,17
Gemmellaceae	2,7					Sulfurospirillum	3,8	Arthrobacter psychrolactophilus	1,308	Actinobacteria;Other	1,717
Corynebacterium	2,5					Neisseriaceae	3,1	Actinobacteria;Other	1,137	Legionellales	1,57
Corynebacterium;s__kroppenstedtii	2,3					Geobacillus	2,9				
Ralstonia	1,8					ACK-M1	2,8				
Nesterenkonia	1,7					Bacteriovoracaceae	2				
Prevotella	1,3					OD1	1,9				
Streptococcus minor	1,3					Candidatus Aquiluna rubra	1,9				
Granulicatella	1					Oxalobacteraceae	1,6				
						Asticcacaulis	1,5				
						Actinobacteria;Other	1,4				
						Rothia mucilaginosa	1,2				

PD4 MC	PD4 MD	PD4 MS	PD5 MC	PD5 MD	PD5 MS		
Corynebacterium;s__	2 2	Arthrobacter;Other 11,	OD1 8	Butyricimonas;s__ 10, 1	Acinetobacter schindleri KD8-87 Nocardioidace	53, 73 26,	
Streptococcus;s__	5	Peptostreptococcus;s__ 9,2	Staphylococcus Bdellovibrio	OD1 6,1 7	Acinetobacter schindleri ae	Acinetobacter schindleri Corynebacterium;s_kro ppenstedtii	49 9,9 38
Prevotella	7	Staphylococcus Corynebacterium;s_kro	1 6,4	bacteriovorus Corynebacterium;s_kro	4,6 4	Staphylococcus Acinetobacter lwoffii	1,7 1,2
Propionibacterium acnes	8 3, 8	ppenstedtii Rothia mucilaginosa	6,4 5,5 2,5	ppenstedtii Hyphomicrobium;s__ 3,9	Acinetobacter lwoffii Varibaculum;s__ 7	Ellin517 Acinetobacter;Other	5 08
Prevotella	6 3,	Selenomonas;s__	3	Acinetobacter schindleri 3,8	OD1 6, 1	Acinetobacter johnsonii	1,1 85
Proteobacteria	5	Corynebacterium;s_kro	2,3	Exiguobacteraceae 1	Bacteria m;s__	Flavobacteriu	1,2
Corynebacterium;s_kro	3, 4	ppenstedtii Flavobacterium;s__	2,1		3, 3,	Flectobacillus;	
Nocardioides	2, 3	Flavobacterium;Other	4	GN02 Parvimonas;s__	Veillonella dispar 3,2 3,0	Sphingobacter	1,653
Actinobacteria;Other	2, 3	Propionibacterium acnes	2	Microbacterium;s__ 2,9	3 2, 2,	Propionibacte	1,564
Bacillus foraminis_	2, 2	Arthrobacter psychrolactophilus	1,4 5	Candidatus Aquiluna Proteobacteria	5 rubra 2,7	rium acnes	1,362
Corynebacterium;Other	2	Streptophyta	4 0,7	Microbacteriaceae;Other Sphingomonas;s__	5 4	Pseudonocard	
Nocardioidaceae	2 1,	Microbacteriaceae;Other	1	Leptotrichiaceae Bdellovibrio;s__	2,7 2,6 2, 2,1	ia;s__ Actinomyceto spora Acinetobacter ;Other	1,318 1,292 1,21
Streptococcus;Other	9 1,			Aerococcaceae MIZ46	9 2,1 2,0	Microbacteriu	
Veillonella dispar	7			OD1 Peptoniphilus;s__	1 5	1 m;s__	1,145
Haemophilus	1, 6			Corynebacterium;s_kro ppenstedtii	2, 1		
parainfluenzae							
Candidatus Aquiluna	1, 6						
rubra	1, 5						
Microbacterium;s__	1, 1,			Kaistobacter;s__	1, 3		
Leptotrichia;s__	4			Bacteria	1, 1,8 1	Flavobacterium;s__	1

Microbacteriaceae;Other	1, 2	Corynebacterium;s__	1,7 3
Actinomyces;s__	1, 1	Sphingomonadaceae	1,5 9
OD1	1, 1	Fusobacterium;s__	1,4 2
Sphingomonas;s__	1	Hydrogenophilus;s__	1,1
		Arthrobacter;Other	1,1
		OD1	1,1
		Staphylococcus;Other	1,0 9
		Prevotella	1,0 5

PD6 MC		PD6 MD		PD6 MS	
OD1	48	<i>Acinetobacter schindleri</i>	28	OD1	22,2
<i>Faecalibacterium prausnitzii</i>	24	<i>Propionibacterium acnes</i>	23	<i>Arthrobacter;Other</i>	14,7
Megamonas	13	<i>Sphingomonas;s_</i>	11	<i>Corynebacterium;s_</i>	11,7
<i>Stenotrophomonas;s_</i>	3,6	<i>Arthrobacter;Other</i>	11	<i>Acinetobacter lwoffii</i>	11
EW055	1,7	<i>Gaiellaceae</i>	6,9	<i>Flavobacterium;s_</i>	10,2
<i>Flavobacterium;s_</i>	1,3	<i>Corynebacterium;s_</i>	6,6	<i>Propionibacterium acnes</i>	4,66
Bacteria	1,2	<i>Actinobacteria;Other</i>	2,4	<i>Actinobacteria;Other</i>	2,85
<i>Faecalibacterium;s_</i>	1	<i>Streptococcus;s_</i>	1,1	<i>Nocardioidaceae</i>	2,61
		<i>Nocardioidaceae</i>	0,4	<i>Chitinophagaceae;Other</i>	2,25
				<i>Streptococcus;s_</i>	1,95
				ACK-M1	1,91
				Bacteria	1,81
				OD1	1,67