PLOS ONE

RESEARCH ARTICLE

Lipopolysaccharide-binding protein (LBP) can reverse the amyloid state of fibrin seen or induced in Parkinson's disease

Etheresia Pretorius[1]*, Martin J. Page[1], Sthembile Mbotwe[2], Douglas B. Kell[1,3,4]*

1 Department of Physiological Sciences, Faculty of Science, Stellenbosch University, Stellenbosch, South Africa, **2** Department of Physiology, Faculty of Health Sciences, University of Pretoria, Arcadia, South Africa, **3** School of Chemistry, The University of Manchester, Manchester, Lancs, United Kingdom, **4** The Manchester Institute of Biotechnology, The University of Manchester, Manchester, Lancs, United Kingdom

* resiap@sun.ac.za (EP); dbk@manchester.ac.uk (DBK)

Citation: Pretorius E, Page MJ, Mbotwe S, Kell DB (2018) Lipopolysaccharide-binding protein (LBP) can reverse the amyloid state of fibrin seen or induced in Parkinson's disease. PLoS ONE 13(3): e0192121. https://doi.org/10.1371/journal.pone.0192121

Editor: Mark R. Cookson, National Institutes of Health, UNITED STATES

Received: November 22, 2017

Accepted: January 18, 2018

Published: March 1, 2018

Data Availability Statement: All relevant data (Excel data files, lab SOPs, and the raw data graphpad stats file) to replicate the study's findings are available from figshare: https://doi.org/10.6084/m9.figshare.5844738. Full confocal and electron microscopy micrographs files are available from the corresponding author or at the following link: https://1drv.ms/f/s!AgoCOmY3bkKHmWY8VijKiQ-8_5RY.

Funding: This work was supported by the National Research Foundation grant 91548: Competitive

Abstract

The thrombin-induced polymerisation of fibrinogen to form fibrin is well established as a late stage of blood clotting. It is known that Parkinson's Disease (PD) is accompanied by dysregulation in blood clotting, but it is less widely known as a coagulopathy. In recent work, we showed that the presence of tiny amounts of bacterial lipopolysaccharide (LPS) in healthy individuals could cause clots to adopt an amyloid form, and this could be observed via scanning electron microscopy (SEM) or via the fluorescence of thioflavin-T. This could be prevented by the prior addition of lipopolysaccharide-binding protein (LBP). We had also observed by SEM this unusual clotting in the blood of patients with Parkinson's Disease. We hypothesised, and here show, that this too can be prevented by LBP in the context of PD. This adds further evidence implicating inflammatory microbial cell wall products as an accompaniment to the disease, and may be part of its aetiology. This may lead to novel treatment strategies in PD designed to target microbes and their products.

Introduction

It is widely recognized that that many chronic, inflammatory diseases are accompanied by insoluble amyloid fibril formation [1–7]. Parkinson's Disease (PD) is one such condition, and is accompanied by amyloid forms of α-synuclein in the substantia nigra pars compacta [8–13]. To this end, systems biology approaches [14–18] have made considerable headway in accounting for the known "Parkinson's" genes in biochemical terms, with the additional recognition that dysregulated cytokines, oxidative stress and particularly iron dysregulation are also major contributors to disease progression [19–31].

It is rather less widely recognized that PD may also be accompanied by changes in the normal clotting of blood, i.e. it may be a coagulopathy [32–35], albeit the last of these papers suggested that the changes were more in the complement than the coagulation cascade. Indeed, PD in treated patients has been observed to be accompanied by changes in prothrombin time and D-dimer formation (Sato *et al.*, 2003), as well as by eryptosis [35]. Focusing on the terminal stages of the coagulation cascade, thrombin removes two fibrinopeptides from fibrinogen,

Program and South African Medical Research Council to EP, and the Biotechnology and Biological Sciences Research Council Grant BB/L025752/1 to DBK. Some reagent funds were supplied as a philanthropic gift by an anonymous donor. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors declare the following patent application: Method for treating Parkinson's Disease (ZA2017/05547).

thereby allowing the fibrinogen to self-assemble into insoluble fibrin via a 'knobs and holes' mechanism (e.g. [36–40]). There are not otherwise considered to be any major changes in secondary structure of the protein [36, 41–43]. A final crosslinking step catalyzed by Factor XIII, after it too has been activated by thrombin, [44] increases the stability of the clot.

In healthy individuals, the addition of thrombin to platelet poor plasma (PPP) causes the fibres forming the subsequent clot to appear as loosely collected netlike structures (comparable to a plate of noodles or spaghetti) under the scanning electron microscope [45–49]. However, we and others have observed that fibre diameter and morphology change markedly in a variety of vascular and inflammatory diseases, typically producing 'dense matter deposits' (e.g. [46, 50–59]).

Even though, as mentioned, there are *usually* no major changes in the secondary structure of the fibre protein during polymerization, it *can* undergo structural changes under certain conditions. Although fibrinogen is not considered to be amyloidogenic, nor is fibrin seen as an amyloid protein, it can nonetheless become so in the presence of a rare mutation in the fibrinogen a chain [60–63]), or by extreme mechanical stretching [64–66]. A very specific feature of amyloid proteins is the formation of a cross-β-sheet structure, perpendicular to the fibres with a characteristic spacing (observable in X-ray reflections) of 4.7–4.8Å (e.g. [4, 7, 67, 68]). In contrast to normal structures, thioflavin T (ThT) binds strongly to them, and fluoresces intensely at 480–520 nm when excited at ~440 nm (e.g. [69–74]). Indeed, following many observations in the SEM of anomalous blood clotting (e.g. [27, 48, 49, 58, 59, 75–77]), we have recently established [77, 78] that this anomalous clotting *is* in fact amyloid in nature.

Previously in our laboratory, we have shown that fibrin amyloid formation occurred in the presence of tiny amounts of bacterial lipopolysaccharide (LPS) (0.2 ng·L^{-1}; 1 molecule per 10^8 fibrinogen molecules), but was abolished when this was added together with a two-fold stoichiometric excess of human LBP (lipopolysaccharide binding protein) [77]. We have particularly emphasised that dormant bacteria are widespread in nature [79–83], and we have argued strongly for a role for dormant bacteria in the aetiology of such diseases [84–91]. Indeed, recent work in mice lends strong support to the view that the gut microbiome can play a major role in the aetiology of PD [11]. Iron is also capable of catalysing anomalous blood clotting [75, 76], and as mentioned previously, there are strong indications for both iron dysregulation [16, 21, 25–27, 92–97] and coagulopathies [58] in Parkinson's Disease.

The purpose of the present paper was thus to study whether (and to what extent) fibrin-type amyloid in blood varies between suitably matched controls and individuals with Parkinson's Disease. In addition, we wished to investigate whether LBP affected this in any way, to a potentially therapeutic effect. It became clear that the answers are in the affirmative in both cases.

Materials and methods

Ethical statement

Ethical clearance was obtained from the Health Sciences Ethical Committee of the University of Pretoria and written informed consent was obtained from each of the patients, as well as from control donors (ethical number: 80/2013 and reapproved 2015) (available on request). Exclusion criteria for the PD patients were conditions such as asthma, human immunodeficiency virus (HIV) or tuberculosis, and risk factors associated with metabolic syndrome, smoking, and (if female) being on contraceptive or hormone replacement treatment. Exclusion criteria for the healthy population were known inflammatory conditions such as asthma, human immunodeficiency virus (HIV) or tuberculosis, and risk factors associated with metabolic syndrome, smoking, and if female, being on contraceptive or hormone replacement treatment. This population did not take any anti-inflammatory medication. Whole blood of all participants was obtained in

citrate tubes and platelet poor plasma (PPP) was used for confocal and SEM experiments. The methods were carried out in accordance with the approved guidelines. Blood was collected and methods were carried out in accordance with the relevant guidelines of the ethics committee. We adhered strictly to the Declaration of Helsinki.

Sample population

In this study, 19 healthy, age-controlled individuals, of whom 9 were spouses of some of the Parkinson's Disease (PD) individuals, and 26 individuals diagnosed with PD, were included. The PD patients were diagnosed by a Neurologist and the Unified Parkinson's Disease Rating Scale (UPDRS) was used in this diagnosis. On the day of blood collection, the Hoehn and Yahr scale was used by a clinician to rate the relative level of the PD disability. Margaret M. Hoehn and Melvin D. Yahr developed the Hoehn and Yahr scale to scale practically the severity of PD at the time of treatment, and thereby determine whether the medication or treatment that is used influences the rate of the progression of the disease [98]. Many studies thereafter have used this method in scaling the severity of movement disorders [99–103].

LPS-binding protein

A final LPS-binding protein (LBP) exposure concentration of 2 $ng{\cdot}L^{-1}$ LBP was used [77]. LBP was purchased from Sigma (recombinant product SRP6033; >95% pure). Previously we reported that LBP added to healthy plasma does not change or affect the structure of healthy fibrin viewed with scanning electron microscopy (SEM) [104]. We also previously showed with confocal microscopy that healthy PPP with added LBP (after addition of thrombin) showed little change in fluorescent (amyloid) signal [77].

Airyscan and scanning electron microscopy

PPP was prepared from whole blood collected in citrated tubes, from both healthy and PD individuals. For Airyscan preparation, we added Thioflavin T (ThT) at a final concentration of 5 μM to 200 μL of various prepared PPP samples and incubated it (protected from light) for one minute. This step was followed with the addition of thrombin, added in the ratio 1:2 to create extensive fibrin networks. A coverslip was placed over the prepared clot, and viewed immediately using a Zeiss LSM 510 META confocal microscope with a Plan-Apochromat 63× and 100×/1.4 Oil DIC objective and super-resolution (Airyscan) capabilities. The Airyscan detector increases the resolution by a factor of 1.7, achieving super-resolution of 140 nm. Excitation was at 488 nm and emitted light was measured at 505–550 nm. In addition, PPP from PD individuals was incubated with LBP (final concentration 2 $ng{\cdot}L^{-1}$) for 10 minutes, followed by ThT and clot preparation as for the healthy and naïve PD PPP. Clots were also prepared for SEM analysis, but after addition of thrombin, clots were washed, fixed in 4% formaldehyde and prepared for SEM according to known SEM preparation methods (e.g. Pretorius *et al.* 2013b). Samples were viewed using a Zeiss cross beam electron microscope to study fibrin fibres.

Statistical analysis and data sharing

We used a One-Way ANOVA with Holm-Šídák Multiple Comparison's Test for the SEM analysis (controls vs. PD vs. PD with added LBP), comparing the mean of each column with the mean of every other column (GraphPad 7). The paired t-test analysis was done for the Airyscan analysis (PD with and without LBP). For each picture, we obtained the histogram of intensities (8-bit scale) using the *histogram* function of ImageJ. From this, we calculated the coefficient of variation (CV, as standard deviation/mean) (see [104] for detailed methodology). The

experiments were done blinded and data capturing and analysis of micrographs were performed by different co-authors.

All relevant data (Excel data files, lab SOPs, and the raw data graphpad stats file) to replicate the study's findings are available from figshare: https://doi.org/10.6084/m9.figshare.5844738. Full confocal and electron microscopy micrographs files are available from the corresponding author or at the following link: https://1drv.ms/f/s!AgoCOmY3bkKHmWY8VijKiQ-8_5RY."

Results

Table 1 shows demographics for the healthy and the PD groups. Medication usage may influence the hematological system; however, previously we reviewed literature on PD medication interactions with the haematological system, and no significant interactions were previously reported (see [35] and Table 1). The median of the Hoehn and Yahr scale for the PD individuals were 2.5 (±0.43); suggesting that mostly, individuals participating in this study had mild bilateral PD symptoms at date of blood collection. Airyscan and SEM results are shown in Figs 1–4, for the healthy and PD individuals.

Scanning electron microscopy of PPP clots

Fig 1A and 1B show how fibrin clots created from healthy PPP look like under a 10 000× machine magnification. Fibres have a spaghetti-like appearance, where individual fibres are visible. On the other hand, fibrin clots created from PPP of PD individuals all have a typical matted appearance, where individual fibres are entwined into a matted mass (see Fig 1C), indicative of hypercoagulation.

We recently suggested that this changed fibrin protein structure in inflammatory conditions like T2D, rheumatoid arthritis and others, are due to β-sheet-rich areas forming in the presence of both upregulated inflammatory markers, and particularly the presence or LPS [77]. We also showed that LPS added to healthy PPP created clots lead to hypercoagulation, and that the addition of LBP could reverse this pathological fibrin structure [77]. We additionally demonstrated that the pathological fibrin structure of T2D could be reversed with the addition of LBP (Pretorius *et al*., 2017a). Overall, we suggested that LBP protects the fibres from LPS damage by binding to LPS and, therefore, that LBP could decreaseβ-sheet-rich areas in T2D plasma.

Here we added LBP to PPP from PD individuals (see Fig 1D). We could show that in all our PD samples that a structural reversion to a state similar to clots created from healthy PPP could be obtained.

Table 1. Demographics and medication usage of Parkinson's individuals.

HEALTHY INDIVIDUALS (N = 19)		
	Gender	**Age**
Median; STD; %	74% F; 26% M	All: 67.0 (±9.8) M: 67.0 (± 8.5) F: 67.0 (±10.5)
PARKINSON'S DISEASE INDIVIDUALS (Hoehn and Yahr scale 2.5 (±0.43) (N = 26)		
Median; STD; %	35% F: 65% M	All: 70 (±4.1) M: 70 (± 4.1) F: 71 (±4.2)
PD MEDICATION USAGE		
[Ropinirole (Requip®), SINEMET® (carbidopa-levodopa), Comtan® (entacapone), Pramipexole, Pexola®, Rasagiline, Azilect®,Stalevo® (carbidopa, levodopa, and entacapone), Madopar® (levodopa + benserazide), Carbilev® (carbidopa + levodopa) Amantadine].		

https://doi.org/10.1371/journal.pone.0192121.t001

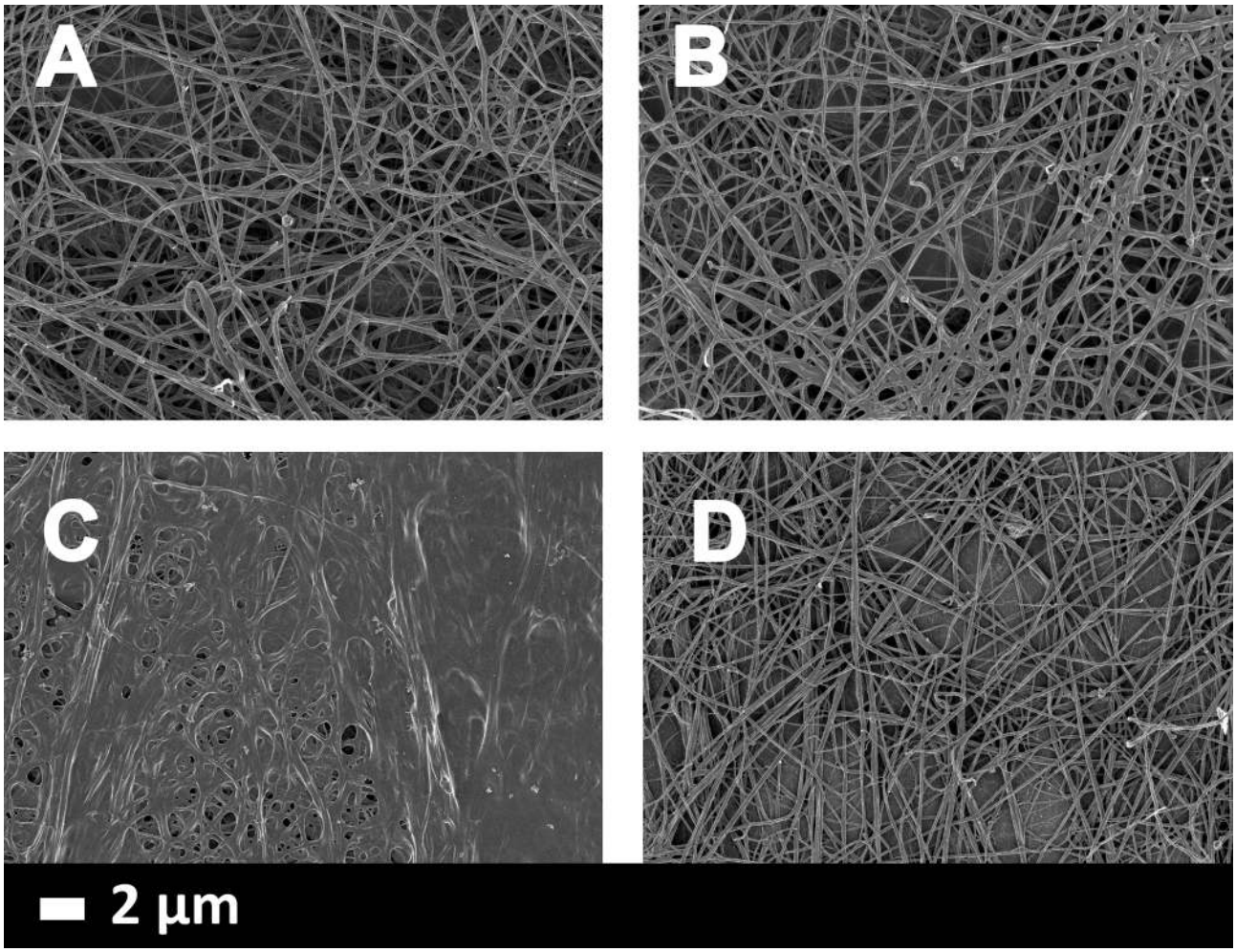

Fig 1. A and B) Clot structure from two healthy individuals, imaged in an SEM and showing a 'normal' 'spaghetti' or 'noodle' type of structure. All clots were created by adding thrombin to PPP. **C)** SEM micrographs of PPP from a Parkinson's disease individuals with added thrombin, showing a 'dense matted deposit'; **D)** PPP from same individual, but exposed to 2ng.L^{-1} LPS-binding protein followed by addition of thrombin; now showing a structure similar to that of healthy controls.

https://doi.org/10.1371/journal.pone.0192121.g001

Airyscan super-resolution microscopy of clots created from PPP

Previously we have noted that in healthy PPP, in the presence of ThT, little fluorescence was present, with only occasional very small patches of fluorescence [77, 78]. This might be because in all individuals (even if they are perceived to be healthy) there might be minor areas of mis-folding of fibrin(ogen). We have also previously shown that when LPS had been incubated in healthy PPP, prior to the addition of thrombin, fluorescence was greatly enhanced, suggesting increased binding of ThT to β-sheet-rich areas on the fibrin(ogen) [77, 78]. From these results, we concluded that LPS binding causes the fibrinogen to polymerise into a form with a greatly increased amount of β-sheet (in the presence of thrombin), reflecting amyloid formation. This causes a strong fluorescence observable (when excited ca 440 nm) in the presence of ThT (see e.g. [70, 71]). In this paper, we also show β-sheet-rich areas in clots created from PPP of PD individuals (Fig 2).

Both Airyscan and SEM techniques are typically used only as qualitative methods. However, due to the increased fluorescence in clots prepared from PPP of PD, and also the more uniform

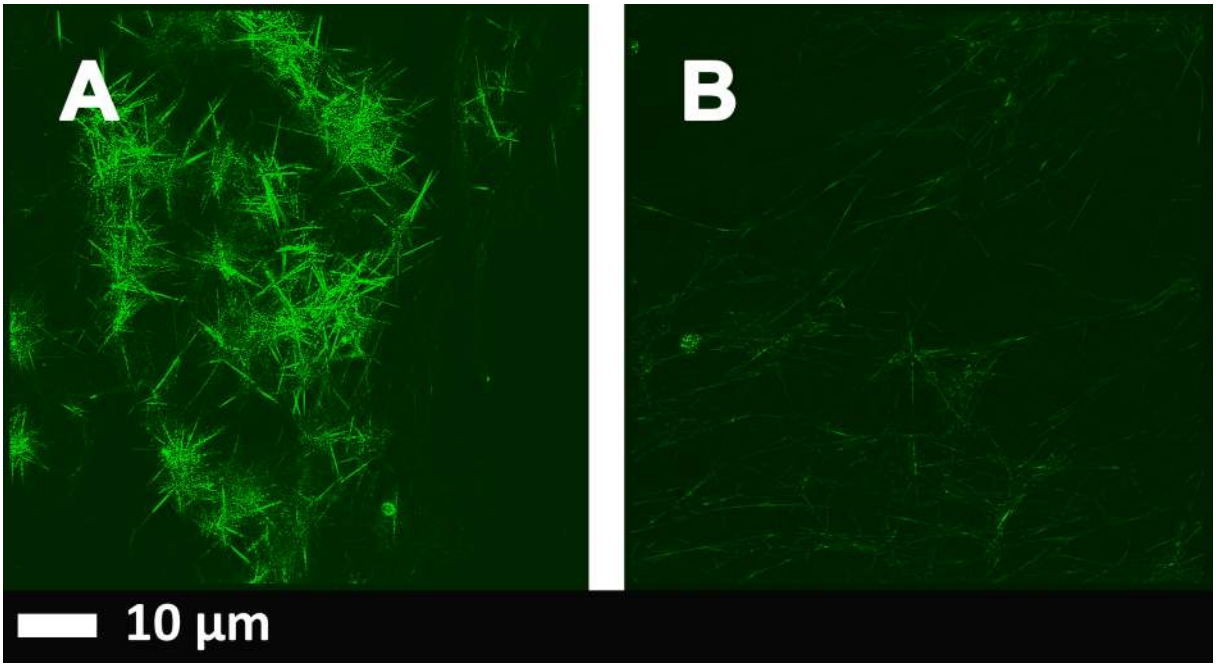

Fig 2. A): Airyscan micrographs of PPP with added thrombin to form extensive fibrin fibres from an individual with Parkinson's disease (PD); **B)** PPP from the same individual, but exposed to 2ng.L^{-1} LPS-binding protein followed by addition of thrombin. Thioflavin T (ThT) (5 μM) was added before thrombin. Micrographs were taken with a Zeiss LSM 510 META confocal microscope with a Plan-Apochromat 63x/1.4 Oil DIC objective. ***LBP dramatically reduced the fluorescence seen in samples from patients with PD.*** Gain settings were kept the same during all data capturing and not changed for statistical analysis, but brightness and contrast was slightly adjusted for fig preparation.

https://doi.org/10.1371/journal.pone.0192121.g002

and matted clot structure shown in SEM analysis of clots prepared from PD PPP, the variance between light and dark pixels are much less than seen in clots prepared from healthy PPP. We therefore propose using the coefficient of variation (CV) as our metric to quantify and discriminate between clots form healthy PPP and clots from PD PPP. We used ImageJ to calculate the mean and standard deviation of the intensity of the pixels in the images of the clot, using the histogram function, followed by the calculation of the coefficient of variation (i.e. SD/mean) of the intensity of the clot structure. Fig 3 shows boxplots of our results and Fig 4A–4D show examples of representative histograms of the 8-bit intensity for a typical SEM and confocal clot with and without LBP of a patient with PD (for the statistical analysis of results see Table 2).

Although LBP added to plasma from PD patients improved the structure of the fibrin(ogen) (see Fig 3, Airyscan boxplot), the PD with added LBP results are still statistically different from the control donors. LBP therefore does not allow the fibrin(ogen) to resemble fully that found in healthy individuals. We previously found in type 2 diabetes (T2D) [104] that when LBP is added to plasma from T2D, it did resemble that of healthy plasma.

We did not, in this paper, repeat Airyscan analysis with clots created with healthy PPP. However, we previously reported that in healthy individuals, little amyloid fluorescence is present when viewing clots with the added fluorescent marker, ThT [104–106].

Discussion

Although we have observed anomalies in the kinds of fibrin fibres produced in the plasma of patients with various inflammatory diseases (e.g. [46, 49, 59, 75, 76, 78, 107–109]), this is the first time that we have observed fibrin amyloid in Parkinson's Disease, as assessed by ThT

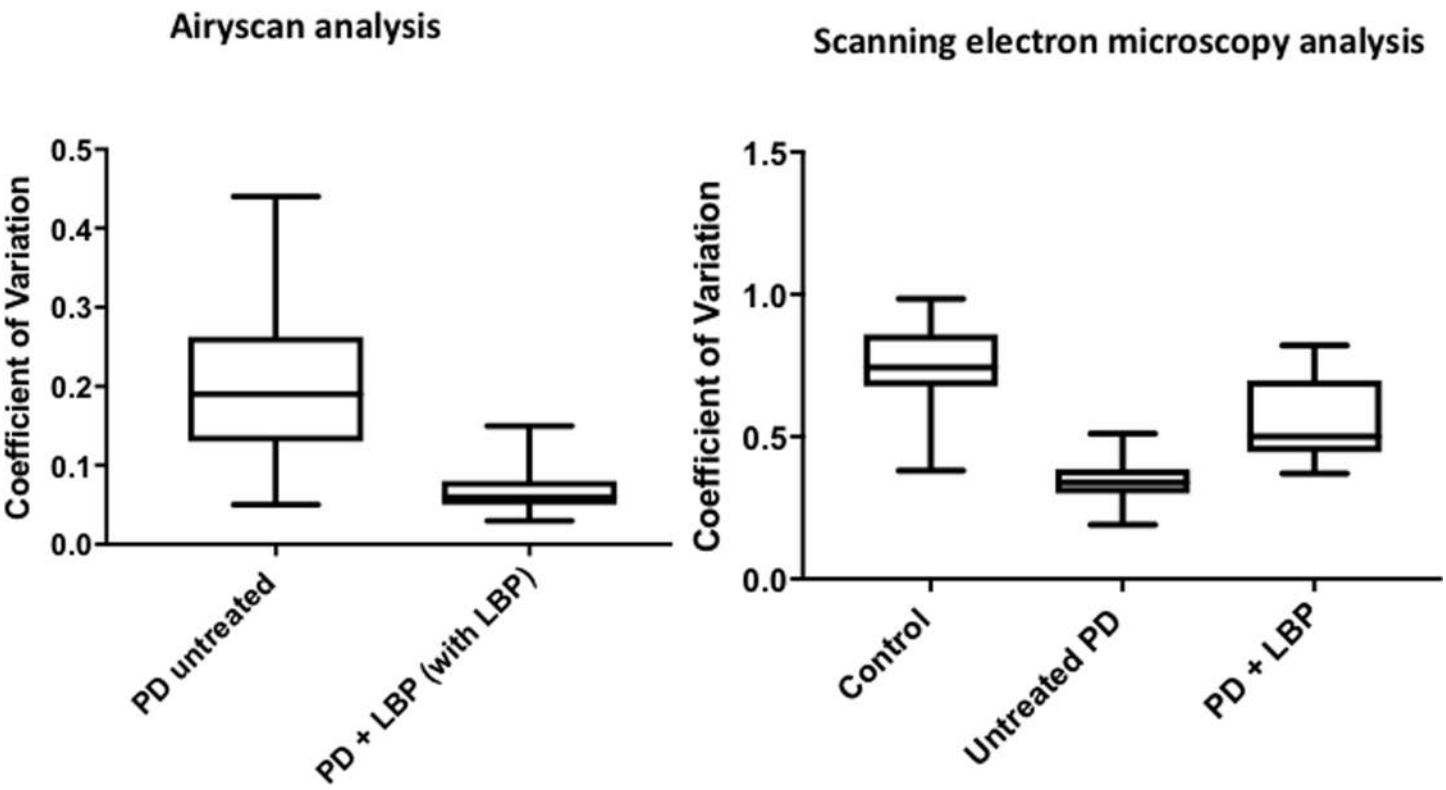

Fig 3. Boxplots of the distribution of the coefficients of variation in the pixel intensities of the SEM and Airyscan clot images.

https://doi.org/10.1371/journal.pone.0192121.g003

staining, and its sensitivity (and that of fibres observed in the SEM) to LBP. While fibrin and α-synuclein can coaggregate [110], it is especially notable that the thrombin-dependence and SEM fibre sizes imply that the fibres we observe are essentially all made of fibrin. Although α-synuclein fibre formation in the substantia nigra is characteristic of PD, it can also occur extracellularly, and its removal may be of therapeutic benefit [111, 112]. The production may be driven by intestinal LPS [113], while gut microbiota-derived short-chain fatty acids may also have a role [11]. Because the addition of LBP to plasma of PD individuals improves the pathological state of the fibrin(ogen), we take it as indirect evidence that LPS has a role in this disease. So far as is known, the only action of LBP, and certainly at these concentrations, is to remove tiny amounts of LPS and related molecules, and only LPS has been shown (by us) to have this massive substoichiometric effect in affecting clotting [77, 104, 105]. Thus, targeting intestinal LPS possibly to not only reduce the production of extracellular α-synuclein, but also to target pathological clotting in PD, is therefore potentially a sensible treatment strategy to investigate further. The discovery that the addition of LBP to plasma influences the structure of fibrin(ogen), furthermore lends credence to the possible role of LPS in the aetiology of PD.

In terms of treatment [114, 115], we have previously discussed the potential role of iron chelators, both to stop the Fenton reaction [25, 26] and to inhibit microbial proliferation (e.g. [84–86, 91]), and iron chelators can definitely also inhibit the formation of dense matted (fibrin(ogen)) deposits (which is a hallmark of coagulopathies) (e.g. [27, 49, 58, 76, 116, 117]). We have also previously reported that the red blood cells (RBCs) of PD individuals are erypto-tic [35], mainly due to the presence of circulating cytokines and increased oxidative stress [118, 119]. The possible removal of amyloid by LBP may also affect other cells of the

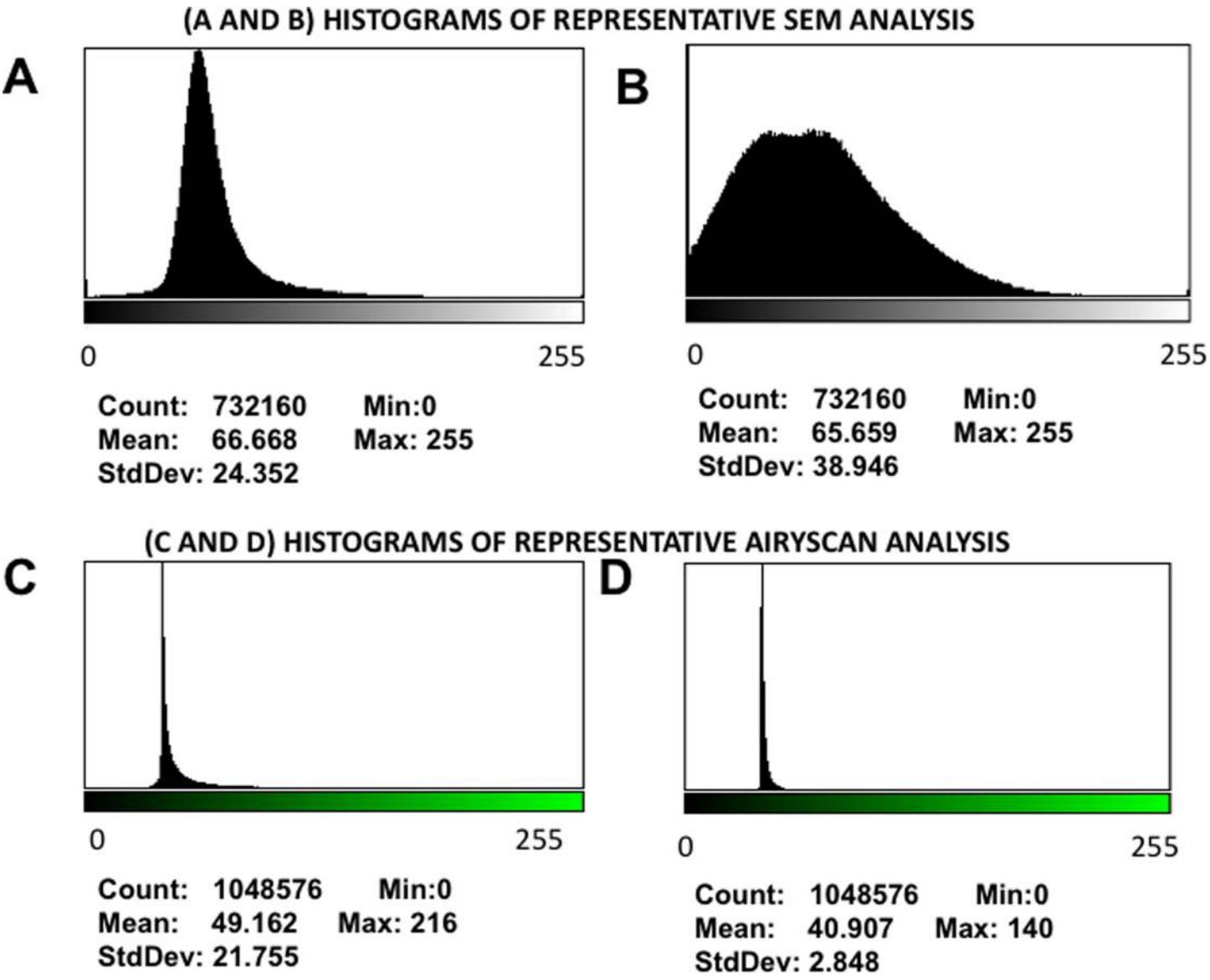

Fig 4. A and B) Representative histograms of the 8-bit intensity for a representative SEM clot from PPP of an individual with Parkinson's disease and after addition of LBP, respectively. **C and D)** Representative histograms of the 8-bit intensity for a typical Airyscan clot from PPP of an individual with Parkinson's disease and after addition of LBP, respectively.

https://doi.org/10.1371/journal.pone.0192121.g004

haematological system, e.g. RBCs. It is now clear that treatment options worth exploring also include anticoagulants; as yet, however, the evidence for any effect of heparin is still awaited, due to Randomised Control Trials not having been done [120].

Concluding remarks

We show here that (1) LBP is a potential treatment for PD-associated coagulopathies and (2) that the normalizing effect of LBP on fibrin(ogen) structure, strongly (albeit indirectly) points to the possibly important role of LPS in the aetiology of PD. Overall, the remarkable reversal of amyloid fibrin formation by LBP addition to the plasma of Parkinson's Disease patients firstly present a novel strategy for treating PD-associated- coagulopathies and

Table 2. Data for Parkinson's disease (PD) and healthy individuals showing P-values of coefficients of variation (CV) of the intensity of the pixels in the clot images using the one-way ANOVA test with Holm-Šídák Multiple Comparison's Test comparing the mean of each column with the mean of every other column for the scanning electron microscopy analysis. The paired t-test was used for the Airyscan analysis (naïve PD vs. PD treated with LBP).

SCANNING ELECTRON MICROSCOPY ANALYSIS (ANOVA)			
	Mean diff. of CVs		**Adjusted P-value**
Control (n = 19) vs PD (n = 26)	0.403		<**0.0001**
Control (n = 19) vs PD + LBP (n = 26)	0.195		<**0.0001**
PD (n = 26) vs PD + LBP (n = 26)	-0.21		<**0.0001**
AIRYSCAN ANALYSIS (PAIRED T-TEST)			
	Naïve PD	**PD treated with LBP**	**P-value**
MEDIAN AND STD	0.19 (± 0.09)	0.06 (± 0.03)	<**0.0001**

https://doi.org/10.1371/journal.pone.0192121.t002

secondly implies strongly that LPS is naturally pre-existing in said plasma. Although almost all the LPS is bound to plasma proteins under normal conditions [86], including presumably to fibrinogen but in concentrations that are consequently hard to determine [86], it is known from experiments where LPS was added exogenously, that LBP molecules can inhibit the LPS-induced formation of the amyloid form of fibrin when thrombin is present [77]. Any endotoxin content of the PPP, ThT, LBP and thrombin is not known; however, the fact that the effect was fully reversed by LBP shows that any such endotoxin content must be negligible and/or irrelevant. So far as is known, the only action of LBP, and certainly at these concentrations, is to remove tiny amounts of LPS and related molecules, and only LPS has been shown (by us) to have this massive substoichiometric effect in affecting clotting. Consequently, the present work lends support to the idea (and evidence [121, 122]) that a dormant blood and tissue microbiome, capable of shedding LPS, is at least part of the aetiology of Parkinson's Disease.

Acknowledgments

National Research Foundation grant 91548: Competitive Program and South African Medical Research Council to EP. Biotechnology and Biological Sciences Research Council Grant BB/L025752/1 to DBK. Some reagent funds were supplied as a philanthropic gift by an anonymous donor. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceptualization: Etheresia Pretorius, Douglas B. Kell.

Data curation: Etheresia Pretorius, Martin J. Page.

Formal analysis: Etheresia Pretorius.

Funding acquisition: Etheresia Pretorius.

Investigation: Etheresia Pretorius.

Methodology: Etheresia Pretorius, Sthembile Mbotwe.

Project administration: Etheresia Pretorius.

Resources: Etheresia Pretorius.

Software: Etheresia Pretorius.

Supervision: Etheresia Pretorius.

Validation: Etheresia Pretorius, Martin J. Page.

Visualization: Etheresia Pretorius.

Writing – original draft: Etheresia Pretorius, Douglas B. Kell.

Writing – review & editing: Etheresia Pretorius, Douglas B. Kell.

References

1. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem. 2006; 75:333–66. https://doi.org/10.1146/annurev.biochem.75.101304.123901 PMID: 16756495.
2. Herczenik E, Gebbink MFBG. Molecular and cellular aspects of protein misfolding and disease. FASEB J. 2008; 22(7):2115–33. https://doi.org/10.1096/fj.07-099671 PMID: 18303094.
3. Rambaran RN, Serpell LC. Amyloid fibrils: abnormal protein assembly. Prion. 2008; 2(3):112–7. PMID: 19158505; PubMed Central PMCID: PMCPMC2634529.
4. Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. Cell. 2012; 148(6):1188–203. https://doi.org/10.1016/j.cell.2012.02.022 PMID: 22424229; PubMed Central PMCID: PMCPMC3353745.
5. Knowles TPJ, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. Nat Rev Mol Cell Biol. 2014; 15(6):384–96. https://doi.org/10.1038/nrm3810 PubMed PMID: WOS:000337245500010. PMID: 24854788
6. Tipping KW, van Oosten-Hawle P, Hewitt EW, Radford SE. Amyloid Fibres: Inert End-Stage Aggregates or Key Players in Disease? Trends Biochem Sci. 2015; 40(12):719–27. https://doi.org/10.1016/j.tibs.2015.10.002 PMID: 26541462.
7. Riek R, Eisenberg DS. The activities of amyloids from a structural perspective. Nature. 2016; 539 (7628):227–35. https://doi.org/10.1038/nature20416 PMID: 27830791.
8. Olanow CW, Brundin P. Parkinson's disease and alpha synuclein: is Parkinson's disease a prion-like disorder? Mov Disord. 2013; 28(1):31–40. https://doi.org/10.1002/mds.25373 PMID: 23390095.
9. Uversky VN, Li J, Fink AL. Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular link between Parkinson's disease and heavy metal exposure. J Biol Chem. 2001; 276(47):44284–96. https://doi.org/10.1074/jbc.M105343200 PMID: 11553618.
10. Vilar M, Chou HT, Luhrs T, Maji SK, Riek-Loher D, Verel R, et al. The fold of alpha-synuclein fibrils. Proc Natl Acad Sci U S A. 2008; 105(25):8637–42. https://doi.org/10.1073/pnas.0712179105 PMID: 18550842; PubMed Central PMCID: PMCPMC2438424.
11. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. Cell. 2016; 167:1469–80. https://doi.org/10.1016/j.cell.2016.11.018 PMID: 27912057
12. Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015; 386(9996):896–912. https://doi.org/10.1016/S0140-6736(14)61393-3 PMID: 25904081.
13. Kalia LV, Kalia SK. alpha-Synuclein and Lewy pathology in Parkinson's disease. Curr Opin Neurol. 2015; 28(4):375–81. https://doi.org/10.1097/WCO.0000000000000215 PMID: 26110807.
14. Antony PMA, Diederich NJ, Krüger R, Balling R. The hallmarks of Parkinson's disease. FEBS J. 2013; 280:5981–93. Epub 2013/05/15. https://doi.org/10.1111/febs.12335 PMID: 23663200.
15. Jones BC, Miller DB, O'Callaghan JP, Lu L, Unger EL, Alam G, et al. Systems analysis of genetic variation in MPTP neurotoxicity in mice. Neurotoxicology. 2013; 37C:26–34. Epub 2013/04/06. https://doi.org/10.1016/j.neuro.2013.03.010 PMID: 23558233.
16. Funke C, Schneider SA, Berg D, Kell DB. Genetics and iron in the systems biology of Parkinson's disease and some related disorders. Neurochem Internat. 2013; 62:637–52.
17. Fujita KA, Ostaszewski M, Matsuoka Y, Ghosh S, Glaab E, Trefois C, et al. Integrating pathways of Parkinson's disease in a molecular interaction map. Mol Neurobiol. 2014; 49(1):88–102. Epub 2013/07/09. https://doi.org/10.1007/s12035-013-8489-4 PMID: 23832570.
18. Krishna A, Biryukov M, Trefois C, Antony PM, Hussong R, Lin J, et al. Systems genomics evaluation of the SH-SY5Y neuroblastoma cell line as a model for Parkinson's disease. BMC Genomics. 2014;

15:1154. https://doi.org/10.1186/1471-2164-15-1154 PMID: 25528190; PubMed Central PMCID: PMCPMC4367834.

19. Altamura S, Muckenthaler MU. Iron toxicity in diseases of aging: Alzheimer's disease, Parkinson's disease and atherosclerosis. J Alzheimers Dis. 2009; 16(4):879–95. Epub 2009/04/24. https://doi.org/10.3233/JAD-2009-1010 PMID: 19387120.

20. Barnham KJ, Bush AI. Metals in Alzheimer's and Parkinson's diseases. Curr Opin Chem Biol. 2008; 12(2):222–8. Epub 2008/03/18. https://doi.org/10.1016/j.cbpa.2008.02.019 PMID: 18342639.

21. Double KL, Gerlach M, Youdim MB, Riederer P. Impaired iron homeostasis in Parkinson's disease. J Neural Transm Suppl. 2000;(60):37–58. Epub 2001/02/24. PMID: 11205155.

22. Gerlach M, Riederer P, Double KL. Neuromelanin-bound ferric iron as an experimental model of dopaminergic neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord. 2008; 14 Suppl 2: S185–8. https://doi.org/10.1016/j.parkreldis.2008.04.028 PMID: 18585086.

23. Jameson GNL. Iron, cysteine and Parkinson's disease. Monatshefte Fur Chemie. 2011; 142(4):325–9. PubMed PMID: ISI:000289798100003.

24. Jones BC, Beard JL, Gibson JN, Unger EL, Allen RP, McCarthy KA, et al. Systems genetic analysis of peripheral iron parameters in the mouse. Am J Physiol Regul Integr Comp Physiol. 2007; 293(1): R116–24. https://doi.org/10.1152/ajpregu.00608.2006 PMID: 17475678.

25. Kell DB. Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. BMC Med Genom. 2009; 2:2

26. Kell DB. Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. Arch Toxicol. 2010; 577:825–89.

27. Levenson CW. Iron and Parkinson's disease: chelators to the rescue? Nutr Rev. 2003; 61(9):311–3. PMID: 14552066.

28. Kell DB, Pretorius E. Serum ferritin is an important disease marker, and is mainly a leakage product from damaged cells. Metallomics. 2014; 6(4):748–73. https://doi.org/10.1039/c3mt00347g PMID: 24549403

29. Oshiro S, Morioka MS, Kikuchi M. Dysregulation of iron metabolism in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Adv Pharmacol Sci. 2011; 2011:378278. https://doi.org/10.1155/2011/378278 PMID: 22013437.

30. Perez CA, Tong Y, Guo M. Iron chelators as potential therapeutic agents for Parkinson's disease. Current Bioactive Compounds. 2008; 4(3):150–8. https://doi.org/10.2174/157340708786305952 PMID: 19809592

31. Weinreb O, Mandel S, Youdim MBH, Amit T. Targeting dysregulation of brain iron homeostasis in Parkinson disease by iron chelators. Free Radic Biol Med. 2013; 62:52–64. Epub 2013/02/05. https://doi.org/10.1016/j.freeradbiomed.2013.01.017 PMID: 23376471.

32. Sato Y, Kaji M, Metoki N, Yoshida H, Satoh K. Coagulation-fibrinolysis abnormalities in patients receiving antiparkinsonian agents. J Neurol Sci. 2003; 212(1–2):55–8. https://doi.org/10.1016/S0022-510x(03)00101-1 PubMed PMID: ISI:000183659500008. PMID: 12809999

33. Rosenbaum H, Aharon-Peretz J, Brenner B. Hypercoagulability, parkinsonism, and Gaucher disease. Semin Thromb Hemost. 2013; 39(8):928–34. https://doi.org/10.1055/s-0033-1357485 PMID: 24129683.

34. Infante J, Prieto C, Sierra M, Sánchez-Juan P, González-Aramburu I, Sanchez-Quintana C, et al. Comparative blood transcriptome analysis in idiopathic and LRRK2 G2019S-associated Parkinson's disease. Neurobiol Aging. 2016; 38:214 e1–5. https://doi.org/10.1016/j.neurobiolaging.2015.10.026 PMID: 26675812.

35. Pretorius E, Swanepoel AC, Buys AV, Vermeulen N, Duim W, Kell DB. Eryptosis as a marker of Parkinson's disease. Aging. 2014; 6(10):788–819. https://doi.org/10.18632/aging.100695 PMID: 25411230

36. Weisel JW. Fibrinogen and fibrin. Adv Protein Chem. 2005; 70:247–99. Epub 2005/04/20. https://doi.org/10.1016/S0065-3233(05)70008-5 PMID: 15837518.

37. Wolberg AS. Thrombin generation and fibrin clot structure. Blood Rev. 2007; 21(3):131–42. Epub 2007/01/09. https://doi.org/10.1016/j.blre.2006.11.001 PMID: 17208341.

38. Cilia La Corte AL, Philippou H, Ariëns RAS. Role of fibrin structure in thrombosis and vascular disease. Adv Protein Chem Struct Biol. 2011; 83:75–127. Epub 2011/05/17. https://doi.org/10.1016/B978-0-12-381262-9.00003-3 PMID: 21570666.

39. Undas A, Ariëns RAS. Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. Arterioscler Thromb Vasc Biol. 2011; 31(12):e88–99. Epub 2011/08/13. https://doi.org/10.1161/ATVBAHA.111.230631 PMID: 21836064.

40. Wolberg AS. Determinants of fibrin formation, structure, and function. Curr Opin Hematol. 2012; 19 (5):349–56. Epub 2012/07/05. https://doi.org/10.1097/MOH.0b013e32835673c2 PMID: 22759629.

41. Averett LE, Geer CB, Fuierer RR, Akhremitchev BB, Gorkun OV, Schoenfisch MH. Complexity of "A-a" knob-hole fibrin interaction revealed by atomic force spectroscopy. Langmuir. 2008; 24 (9):4979–88. https://doi.org/10.1021/la703264x PMID: 18351791.

42. Yermolenko IS, Lishko VK, Ugarova TP, Magonov SN. High-resolution visualization of fibrinogen molecules and fibrin fibers with atomic force microscopy. Biomacromolecules. 2011; 12(2):370–9. Epub 2011/01/05. https://doi.org/10.1021/bm101122g PMID: 21192636.

43. Protopopova AD, Barinov NA, Zavyalova EG, Kopylov AM, Sergienko VI, Klinov DV. Visualization of fibrinogen alphaC regions and their arrangement during fibrin network formation by high-resolution AFM. J Thromb Haemost. 2015; 13(4):570–9. https://doi.org/10.1111/jth.12785 PMID: 25393591.

44. Dickneite G, Herwald H, Korte W, Allanore Y, Denton CP, Matucci Cerinic M. Coagulation factor XIII: a multifunctional transglutaminase with clinical potential in a range of conditions. Thromb Haemost. 2015; 113(4):686–97. https://doi.org/10.1160/TH14-07-0625 PMID: 25652913.

45. Campbell RA, Aleman M, Gray LD, Falvo MR, Wolberg AS. Flow profoundly influences fibrin network structure: implications for fibrin formation and clot stability in haemostasis. Thromb Haemost. 2010; 104(6):1281–4. Epub 2010/10/05. https://doi.org/10.1160/TH10-07-0442 PMID: 20886193; PubMed Central PMCID: PMC3236083.

46. Pretorius E, Steyn H, Engelbrecht M, Swanepoel AC, Oberholzer HM. Differences in fibrin fiber diameters in healthy individuals and thromboembolic ischemic stroke patients. Blood Coagul Fibrinolysis. 2011; 22(8):696–700. Epub 2011/10/18. https://doi.org/10.1097/MBC.0b013e32834bdb32 PMID: 22001525.

47. Weigandt KM, White N, Chung D, Ellingson E, Wang Y, Fu XY, et al. Fibrin clot structure and mechanics associated with specific oxidation of methionine residues in fibrinogen. Biophys J. 2012; 103(11):2399–407. https://doi.org/10.1016/j.bpj.2012.10.036 PubMed PMID: ISI:000311963300019. PMID: 23283239

48. Bester J, Soma P, Kell DB, Pretorius E. Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). Oncotarget Gerontology. 2015; 6(34):35284–303.

49. Kell DB, Pretorius E. The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen). Integr Biol. 2015; 7:24–52. https://doi.org/10.1039/c4ib00173g PMID: 25335120

50. Jörneskog G, Egberg N, Fagrell B, Fatah K, Hessel B, Johnsson H, et al. Altered properties of the fibrin gel structure in patients with IDDM. Diabetologia. 1996; 39(12):1519–23. Epub 1996/12/01. PMID: 8960835.

51. Dunn EJ, Ariëns RAS. Fibrinogen and fibrin clot structure in diabetes. Herz. 2004; 29(5):470–9. Epub 2004/09/02. https://doi.org/10.1007/s00059-004-2607-z PMID: 15340732.

52. Dunn EJ, Ariëns RAS, Grant PJ. The influence of type 2 diabetes on fibrin structure and function. Diabetologia. 2005; 48(6):1198–206. Epub 2005/05/03. https://doi.org/10.1007/s00125-005-1742-2 PMID: 15864538.

53. Dunn EJ, Philippou H, Ariëns RAS, Grant PJ. Molecular mechanisms involved in the resistance of fibrin to clot lysis by plasmin in subjects with type 2 diabetes mellitus. Diabetologia. 2006; 49(5):1071–80. Epub 2006/03/16. https://doi.org/10.1007/s00125-006-0197-4 PMID: 16538489.

54. Pieters M, Covic N, Loots du T, van der Westhuizen FH, van Zyl DG, Rheeder P, et al. The effect of glycaemic control on fibrin network structure of type 2 diabetic subjects. Thromb Haemost. 2006; 96 (5):623–9. Epub 2006/11/03. PMID: 17080220.

55. Alzahrani SH, Ajjan RA. Coagulation and fibrinolysis in diabetes. Diabet Vasc Dis Res. 2010; 7 (4):260–73. Epub 2010/09/18. https://doi.org/10.1177/1479164110383723 PMID: 20847109.

56. Pretorius E, Oberholzer HM, van der Spuy WJ, Swanepoel AC, Soma P. Qualitative scanning electron microscopy analysis of fibrin networks and platelet abnormalities in diabetes. Blood Coagul Fibrinol. 2011; 22(6):463–7. Epub 2011/04/07. https://doi.org/10.1097/MBC.0b013e3283468a0d PMID: 21467917.

57. Alzahrani SH, Hess K, Price JF, Strachan M, Baxter PD, Cubbon R, et al. Gender-specific alterations in fibrin structure function in type 2 diabetes: associations with cardiometabolic and vascular markers. J Clin Endocrinol Metab. 2012; 97(12):E2282–7. Epub 2012/09/22. https://doi.org/10.1210/jc.2012-2128 PMID: 22996148.

58. Pretorius E, Kell DB. Diagnostic morphology: biophysical indicators for iron-driven inflammatory diseases. Integrative Biol. 2014; 6(5):486–510.

59. Pretorius E, Bester J, Vermeulen N, Alummoottil S, Soma P, Buys AV, et al. Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics. Cardiovasc Diabetol. 2015; 13:30.

60. Serpell LC, Benson M, Liepnieks JJ, Fraser PE. Structural analyses of fibrinogen amyloid fibrils. Amyloid. 2007; 14(3):199–203. https://doi.org/10.1080/13506120701461111 PMID: 17701467.

61. Picken MM. Fibrinogen amyloidosis: the clot thickens! Blood. 2010; 115(15):2985–6. https://doi.org/10.1182/blood-2009-12-236810 PMID: 20395420.

62. Stangou AJ, Banner NR, Hendry BM, Rela M, Portmann B, Wendon J, et al. Hereditary fibrinogen A alpha-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. Blood. 2010; 115(15):2998–3007. https://doi.org/10.1182/blood-2009-06-223792 PMID: 19633201.

63. Haidinger M, Werzowa J, Kain R, Antlanger M, Hecking M, Pfaffenberger S, et al. Hereditary amyloidosis caused by R554L fibrinogen Aalpha-chain mutation in a Spanish family and review of the literature. Amyloid. 2013; 20(2):72–9. https://doi.org/10.3109/13506129.2013.781998 PMID: 23551149.

64. Zhmurov A, Brown AE, Litvinov RI, Dima RI, Weisel JW, Barsegov V. Mechanism of fibrin(ogen) forced unfolding. Structure. 2011; 19(11):1615–24. https://doi.org/10.1016/j.str.2011.08.013 PMID: 22078561; PubMed Central PMCID: PMCPMC3337780.

65. Litvinov RI, Faizullin DA, Zuev YF, Weisel JW. The alpha-helix to beta-sheet transition in stretched and compressed hydrated fibrin clots. Biophys J. 2012; 103(5):1020–7. https://doi.org/10.1016/j.bpj.2012.07.046 PMID: 23009851; PubMed Central PMCID: PMCPMC3433599.

66. Zhmurov A, Kononova O, Litvinov RI, Dima RI, Barsegov V, Weisel JW. Mechanical transition from alpha-helical coiled coils to beta-sheets in fibrin(ogen). J Am Chem Soc. 2012; 134(50):20396–402. Epub 2012/09/08. https://doi.org/10.1021/ja3076428 PMID: 22953986; PubMed Central PMCID: PMC3526676.

67. Maji SK, Wang L, Greenwald J, Riek R. Structure-activity relationship of amyloid fibrils. FEBS Lett. 2009; 583(16):2610–7. https://doi.org/10.1016/j.febslet.2009.07.003 PMID: 19596006.

68. Tycko R, Wickner RB. Molecular structures of amyloid and prion fibrils: consensus versus controversy. Acc Chem Res. 2013; 46(7):1487–96. https://doi.org/10.1021/ar300282r PMID: 23294335; PubMed Central PMCID: PMCPMC3632659.

69. LeVine H, 3rd. Quantification of beta-sheet amyloid fibril structures with thioflavin T. Methods Enzymol. 1999; 309:274–84. PMID: 10507030.

70. Biancalana M, Makabe K, Koide A, Koide S. Molecular mechanism of thioflavin-T binding to the surface of beta-rich peptide self-assemblies. J Mol Biol. 2009; 385(4):1052–63. https://doi.org/10.1016/j.jmb.2008.11.006 PMID: 19038267; PubMed Central PMCID: PMCPMC2664162.

71. Biancalana M, Koide S. Molecular mechanism of Thioflavin-T binding to amyloid fibrils. Biochim Biophys Acta. 2010; 1804(7):1405–12. Epub 2010/04/20. https://doi.org/10.1016/j.bbapap.2010.04.001 PMID: 20399286; PubMed Central PMCID: PMCPmc2880406.

72. Groenning M. Binding mode of Thioflavin T and other molecular probes in the context of amyloid fibrils-current status. J Chem Biol. 2010; 3(1):1–18. https://doi.org/10.1007/s12154-009-0027-5 PMID: 19693614; PubMed Central PMCID: PMCPMC2816742.

73. Sulatskaya AI, Kuznetsova IM, Turoverov KK. Interaction of thioflavin T with amyloid fibrils: stoichiometry and affinity of dye binding, absorption spectra of bound dye. J Phys Chem B. 2011; 115(39):11519–24. https://doi.org/10.1021/jp207118x PMID: 21863870.

74. Freire S, de Araujo MH, Al-Soufi W, Novo M. Photophysical study of Thioflavin T as fluorescence marker of amyloid fibrils. Dyes and Pigments. 2014; 110:97–105. https://doi.org/10.1016/j.dyepig.2014.05.004 PubMed PMID: WOS:000340333900010.

75. Pretorius E, Vermeulen N, Bester J, Lipinski B, Kell DB. A novel method for assessing the role of iron and its functional chelation in fibrin fibril formation: the use of scanning electron microscopy. Toxicol Mech Methods. 2013; 23(5):352–9. Epub 2013/01/03. https://doi.org/10.3109/15376516.2012.762082 PMID: 23278212.

76. Pretorius E, Bester J, Vermeulen N, Lipinski B, Gericke GS, Kell DB. Profound morphological changes in the erythrocytes and fibrin networks of patients with hemochromatosis or with hyperferritinemia, and their normalization by iron chelators and other agents. PLoS One. 2014; 9(1):e85271. https://doi.org/10.1371/journal.pone.0085271 PMID: 24416376

77. Pretorius E, Mbotwe S, Bester J, Robinson CJ, Kell DB. Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. J R Soc Interface. 2016; 123(13):20160539. https://doi.org/10.1098/rsif.2016.0539.

78. Kell DB, Pretorius E. Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. Progr Biophys Mol Biol. 2017; 123:16–41. https://doi.org/10.1016/j.pbiomolbio.2016.08.006.

79. Kaprelyants AS, Gottschal JC, Kell DB. Dormancy in non-sporulating bacteria. FEMS Microbiol Rev. 1993; 10:271–86. PMID: 8318260

80. Domingue GJ, Woody HB. Bacterial persistence and expression of disease. Clin Microbiol Rev. 1997; 10(2):320–44. PMID: 9105757

81. Kell DB, Kaprelyants AS, Weichart DH, Harwood CL, Barer MR. Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. Antonie van Leeuwenhoek. 1998; 73:169–87. PMID: 9717575

82. Mattman L. Cell wall deficient forms: stealth pathogens, 3rd Ed. Boca Raton: CRC Press; 2001.

83. Domingue GJ. Demystifying pleomorphic forms in persistence and expression of disease: Are they bacteria, and is peptidoglycan the solution? Discov Med. 2010; 10(52):234–46. Epub 2010/09/30. PMID: 20875345.

84. Potgieter M, Bester J, Kell DB, Pretorius E. The dormant blood microbiome in chronic, inflammatory diseases. FEMS Microbiol Rev. 2015; 39:567–91. https://doi.org/10.1093/femsre/fuv013 PMID: 25940667

85. Kell DB, Potgieter M, Pretorius E. Individuality, phenotypic differentiation, dormancy and 'persistence' in culturable bacterial systems: commonalities shared by environmental, laboratory, and clinical microbiology. F1000Research. 2015; 4:179. https://doi.org/10.12688/f1000research.6709.2 PMID: 26629334

86. Kell DB, Pretorius E. On the translocation of bacteria and their lipopolysaccharides between blood and peripheral locations in chronic, inflammatory diseases: the central roles of LPS and LPS-induced cell death Integr Biol. 2015; 7:1339–77. https://doi.org/10.1039/C5IB00158G PMID: 26345428

87. Itzhaki RF, Lathe R, Balin BJ, Ball MJ, Braak H, Bearer EL, et al. Microbes and Alzheimer's Disease. J Alzheimers Dis. 2016; 51(4):979–84. https://doi.org/10.3233/JAD-160152 PMID: 26967229.

88. Kell DB, Pretorius E. To what extent are the terminal stages of sepsis, septic shock, SIRS, and multiple organ dysfunction syndrome actually driven by a toxic prion/amyloid form of fibrin?. Seminars in Thrombosis and Hemostasis 2017;(eprint) https://doi.org/10.1055/s-0037-1604108 PMID: 28778104

89. Pretorius E, Akeredolu O-O, Soma P, Kell DB. Major involvement of bacterial components in rheumatoid arthritis and its accompanying oxidative stress, systemic inflammation and hypercoagulability. Exp Biol Med. 2017; 242(4):355–73.

90. Pretorius E, Bester J, Kell DB. A bacterial component to Alzheimer-type dementia seen via a systems biology approach that links iron dysregulation and inflammagen shedding to disease J Alzheimers Dis. 2016; 53(4):1237–56. https://doi.org/10.3233/JAD-160318 PMID: 27340854

91. Kell DB, Kenny LC. A dormant microbial component in the development of pre-eclampsia. Front Med Obs Gynecol. 2016; 3:60. https://doi.org/10.3389/fmed.2016.00060 PMID: 27965958

92. Kaur D, Yantiri F, Rajagopalan S, Kumar J, Mo JO, Boonplueang R, et al. Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity in vivo: A novel therapy for Parkinson's disease. Neuron. 2003; 37(6):899–909. PubMed PMID: ISI:000181899600005. PMID: 12670420

93. Valko M, Morris H, Cronin MTD. Metals, toxicity and oxidative stress. Curr Med Chem. 2005; 12 (10):1161–208. PubMed PMID: ISI:000228824700003. PMID: 15892631

94. Berg D, Riederer P, Gerlach M. Contribution of disturbed iron metabolism to the pathogenesis of Parkinson's disease. Future Med. 2008; 3(4):447–61.

95. Friedman A, Galazka-Friedman J. The history of the research of iron in parkinsonian substantia nigra. J Neural Transm. 2012; 119(12):1507–10. https://doi.org/10.1007/s00702-012-0894-8 PMID: 22941506.

96. Hare DJ, Lei P, Ayton S, Roberts BR, Grimm R, George JL, et al. An iron–dopamine index predicts risk of parkinsonian neurodegeneration in the substantia nigra pars compacta. Chem Sci. 2014: https://doi.org/10.1039/c3sc53461h

97. McDowall JS, Brown DR. Alpha-synuclein: relating metals to structure, function and inhibition. Metallomics. 2016; 8(4):385–97. https://doi.org/10.1039/c6mt00026f PMID: 26864076.

98. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology. 1967; 17(5):427–42. Epub 1967/05/01. PMID: 6067254.

99. Stocchi F, Carbone A, Inghilleri M, Monge A, Ruggieri S, Berardelli A, et al. Urodynamic and neurophysiological evaluation in Parkinson's disease and multiple system atrophy. J Neurol Neurosurg Psychiatry. 1997; 62(5):507–11. Epub 1997/05/01. PMID: 9153611; PubMed Central PMCID: PMC486869.

100. Karlsen KH, Tandberg E, Arsland D, Larsen JP. Health related quality of life in Parkinson's disease: a prospective longitudinal study. J Neurol Neurosurg Psychiatry. 2000; 69(5):584–9. Epub 2000/10/14. https://doi.org/10.1136/jnnp.69.5.584 PMID: 11032608; PubMed Central PMCID: PMC1763406.

101. Schrag A, Jahanshahi M, Quinn N. How does Parkinson's disease affect quality of life? A comparison with quality of life in the general population. Mov Disord. 2000; 15(6):1112–8. PMID: 11104193.

102. Schrag A, Jahanshahi M, Quinn N. What contributes to quality of life in patients with Parkinson's disease? J Neurol Neurosurg Psychiatry. 2000; 69(3):308–12. Epub 2000/08/17. https://doi.org/10.1136/jnnp.69.3.308 PMID: 10945804; PubMed Central PMCID: PMC1737100.

103. Rodríguez-Violante M, Camacho-Ordoñez A, Cervantes-Arriaga A, González-Latapí P, Velázquez-Osuna S. Factors associated with the quality of life of subjects with Parkinson's disease and burden on their caregivers. Neurologia. 2015; 30(5):257–63. https://doi.org/10.1016/j.nrl.2014.01.008 PMID: 24704248.

104. Pretorius E, Mbotwe S, Kell DB. Lipopolysaccharide-binding protein (LBP) reverses the amyloid state of fibrin seen in plasma of type 2 diabetics with cardiovascular co-morbidities. Sci Rep. 2017; 7 (1):9680. Epub 2017/08/31. https://doi.org/10.1038/s41598-017-09860-4 PMID: 28851981; PubMed Central PMCID: PMCPMC5574907.

105. Pretorius E, Page MJ, Engelbrecht L, Ellis GC, Kell DB. Substantial fibrin amyloidogenesis in type 2 diabetes assessed using amyloid-selective fluorescent stains. Cardiovasc Diabetol. 2017; 16(1):141. Epub 2017/11/04. https://doi.org/10.1186/s12933-017-0624-5 PMID: 29096623; PubMed Central PMCID: PMCPMC5668975.

106. Pretorius E, Page MJ, Bester J, Kell DB. Reversal of amyloid formation in the plasma fibrin of individuals with Alzheimer-type dementia using LPS-binding protein. Front Aging Neurosci. 2018;(Commissioned paper).

107. Pretorius E, Swanepoel AC, Oberholzer HM, van der Spuy WJ, Duim W, Wessels PF. A descriptive investigation of the ultrastructure of fibrin networks in thrombo-embolic ischemic stroke. J Thromb Thrombolysis. 2011; 31(4):507–13. Epub 2011/01/12. https://doi.org/10.1007/s11239-010-0538-5 PMID: 21221717.

108. Pretorius E, Oberholzer HM, van der Spuy WJ, Swanepoel AC, Soma P. Scanning electron microscopy of fibrin networks in rheumatoid arthritis: a qualitative analysis. Rheumatol Int. 2012; 32(6):1611–5. Epub 2011/02/19. https://doi.org/10.1007/s00296-011-1805-2 PMID: 21331577.

109. Pretorius E, du Plooy J, Soma P, Gasparyan AY. An ultrastructural analysis of platelets, erythrocytes, white blood cells, and fibrin network in systemic lupus erythematosus. Rheumatol Int. 2014; 34:1005–9. Epub 2013/07/09. https://doi.org/10.1007/s00296-013-2817-x PMID: 23832292.

110. Bhattacharjee P, Bhattacharyya D. An Insight into the Abnormal Fibrin Clots—Its Pathophysiological Roles. In: Kolev K, editor. Fibrinolysis and Thrombolysis: InTechOpen; 2014. p. 1–29.

111. Kim KS, Choi YR, Park JY, Lee JH, Kim DK, Lee SJ, et al. Proteolytic cleavage of extracellular alpha-synuclein by plasmin: implications for Parkinson disease. J Biol Chem. 2012; 287(30):24862–72. https://doi.org/10.1074/jbc.M112.348128 PMID: 22619171; PubMed Central PMCID: PMCPMC3408156.

112. Park SM, Kim KS. Proteolytic clearance of extracellular alpha-synuclein as a new therapeutic approach against Parkinson disease. Prion. 2013; 7(2):121–6. https://doi.org/10.4161/pri.22850 PMID: 23154633; PubMed Central PMCID: PMCPMC3609117.

113. Kelly LP, Carvey PM, Keshavarzian A, Shannon KM, Shaikh M, Bakay RA, et al. Progression of intestinal permeability changes and alpha-synuclein expression in a mouse model of Parkinson's disease. Mov Disord. 2014; 29(8):999–1009. Epub 2014/06/06. https://doi.org/10.1002/mds.25736 PMID: 24898698; PubMed Central PMCID: PMCPmc4050039.

114. Kalia LV, Kalia SK, Lang AE. Disease-modifying strategies for Parkinson's disease. Mov Disord. 2015; 30(11):1442–50. https://doi.org/10.1002/mds.26354 PMID: 26208210.

115. Kakkar AK, Dahiya N. Management of Parkinsons disease: Current and future pharmacotherapy. Eur J Pharmacol. 2015; 750:74–81. https://doi.org/10.1016/j.ejphar.2015.01.030 PMID: 25637088.

116. Nielsen VG, Pretorius E. Iron-enhanced coagulation is attenuated by chelation: thrombelastographic and ultrastructural analysis. Blood Coagul Fibrinolysis. 2014; 25(8):845–50. https://doi.org/10.1097/MBC.0000000000000160 PMID: 24991945.

117. Pretorius E, Vermeulen N, Bester J, Lipinski B. Novel use of scanning electron microscopy for detection of iron-induced morphological changes in human blood. Microsc Res Tech. 2013; 76:268–71. https://doi.org/10.1002/jemt.22163 PMID: 23280783

118. Pretorius E, Olumuyiwa-Akeredolu OO, Mbotwe S, Bester J. Erythrocytes and their role as health indicator: Using structure in a patient-orientated precision medicine approach. Blood Reviews. 2016; 30 (4):263–74. Epub 2016/02/18. https://doi.org/10.1016/j.blre.2016.01.001 PMID: 26878812.

119. Pretorius E, du Plooy JN, Bester J. A Comprehensive Review on Eryptosis. Cell Physiology and Biochemistry. 2016; 39(5):1977–2000. Epub 2016/10/25. https://doi.org/10.1159/000447895 PMID: 27771701.

120. Li J, Wu HM, Zhang L, Zhu B, Dong BR. Heparin and related substances for preventing diabetic kidney disease. Cochrane Database Syst Rev. 2010;(9):CD005631. https://doi.org/10.1002/14651858.CD005631.pub2 PMID: 20824845.

121. Tlaskalová-Hogenová H, Štěpánková R, Kozáková H, Hudcovic T, Vannucci L, Tučková L, et al. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cell Mol Immunol. 2011; 8(2):110–20. https://doi.org/10.1038/cmi.2010.67 PMID: 21278760; PubMed Central PMCID: PMCPMC4003137.

122. Gabrielli M, Bonazzi P, Scarpellini E, Bendia E, Lauritano EC, Fasano A, et al. Prevalence of small intestinal bacterial overgrowth in Parkinson's disease. Mov Disord. 2011; 26(5):889–92. https://doi.org/10.1002/mds.23566 PMID: 21520278.