

# Exploiting *Caenorhabditis elegans* to discover human gut microbiota-mediated intervention strategies in protein conformational diseases

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Age-dependent protein-conformational diseases (PCDs), such as Alzheimer’s disease (AD), Parkinson’s disease (PD), or amyotrophic lateral sclerosis (ALS), are characterized by misfolding and aggregation of metastable proteins present within the proteome of the affected individual. Recent evidence supports the notion that bacteria and bacterial products may be affecting the stability of these culprit host proteins and therefore influence disease progression and perhaps even its onset. Although specific culprit proteins are associated with each disease (e.g., Aβ in AD, α-synuclein in PD, and TDP-43 in ALS), bacteria found to affect these diseases do not seem to differentiate between these specific proteins but likely affect host proteostasis in general, leading to misfolding of metastable proteins encoded within the proteome. The evidence that supports this hypothesis comes from studies where a single bacterial genus was linked to multiple PCDs. For example, a decrease in *Prevotella* spp. is linked to constipation, a condition that precedes PD motor symptoms by as much as 20 or more years (Savica et al., 2009; Zhu et al., 2014). Additionally, the abundance of *Prevotella* found in PD patients negatively correlates with the severity of the disease (Jin et al., 2019). *Prevotella* abundance was also lower in an AD mouse model (Shen et al., 2017) and ALS patients (Hertzberg et al., 2021), suggesting that this genus may provide protection against proteotoxicity. However, more research has to concentrate on *Prevotella* to understand its role in PCDs, as other studies have seen increased abundance in affected patients (Guo et al., 2021). Such discrepancy could be attributed to the large number of *Prevotella* species that may play diverse roles in disease pathogenesis, and since most studies looked at the genus level, the effect of individual species may provide additional clues. A recent study found two *Prevotella* species (i.e., *disiens* and *corporis*) that significantly suppressed polyglutamine protein aggregation in the *Caenorhabditis elegans* (*C. elegans*) model (Walker et al., 2021), suggesting that these specific species may enhance host proteostasis and protect the host against protein misfolding and aggregation. What is most promising is that the effect of *Prevotella* spp. on host proteostasis is observed across organisms, including worms, mice, and humans; therefore, less expensive, and manageable models, such as *C. elegans*, can be used as a discovery tool. The example of *Prevotella* and its potential role in the suppression of various PCDs demonstrates that bacteria affect host proteostasis and may be capable of influencing disease pathogenicity mediated by aggregation-prone proteins.

Butyrogenic bacteria are among other beneficial microbes whose abundance has negatively correlated with the pathogenicity of PCDs across many models. In the study by Walker et al. (2021), it was also noted that supplementation of exogenous butyrate, a

short-chain fatty acid, protects *C. elegans* against bacteria-mediated polyglutamine aggregation and the associated toxicity. Additionally, butyrogenic bacteria had a similar protective effect. The results obtained in the *C. elegans* model seem to be conserved across all higher organisms, including humans, providing more traction in the exploration of butyrate as a potential therapeutic. These findings demonstrate that bacterial products can affect disease progression, which opens new avenues to target the microbial composition of the human gut as a promising therapeutic strategy. Further studies need to identify the mechanism by which butyrate suppresses bacteria-mediated disruption of host proteostasis.

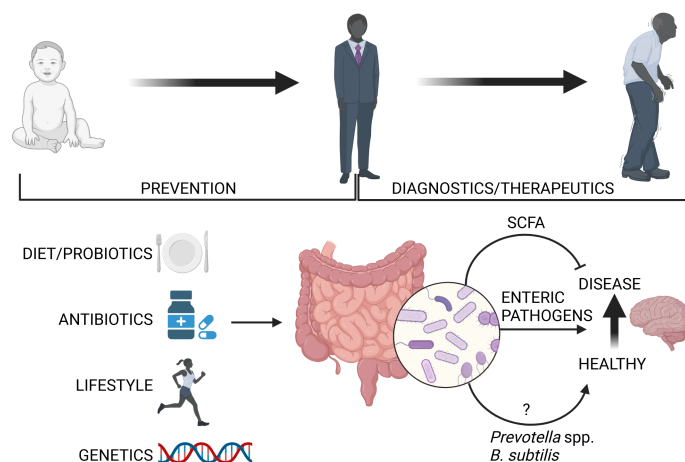
Collectively the protective effect of butyrate and butyrogenic bacteria, *Prevotella* spp., and possibly other microbes, such as *Bacillus subtilis* that was recently found to suppress aggregation of α-synuclein in a PD *C. elegans* model (Goya et al., 2020), will likely open new opportunities for prevention, detection, and treatment of PCDs.

**Prevention:** Nearly all cases of PCDs have a late-onset and are sporadic, meaning individuals develop the disease without prior family history and only a small fraction of the affected individuals develop the early-onset familial type, which is inherited and is linked to specific genetic mutations. Individuals affected by the familial type are at the highest risk of developing the disease and therefore, any preventative measures early in life may offer opportunities to delay the onset or prevent disease pathogenesis (Figure 1); though, gut-targeted preventative

approaches could be equally beneficial to both types of diseases since no differences in clinical symptoms were noted between familial and sporadic PD patients, suggesting a common etiology (Papapetropoulos et al., 2007). If bacteria indeed contribute to the pathogenesis of sporadic and familial cases, then early strategies that influence gut microbiota by targeting factors such as genetics, lifestyle, antibiotics, and diet may play a critical role in disease onset and progression. Among these various factors, antibiotics are the major contributor to gut dysbiosis – not surprisingly, the use of antibiotics, especially with broad-spectrum activity, was recently linked with increased risk of PD and ALS (Sun et al., 2019; Mertsalmi et al., 2020); however, the exact effect of antibiotics on PCDs is not well understood because of the lack of knowledge of the bacteria that contribute to these diseases. Walker et al. (2021) found that *Enterobacteriaceae* and other enteric gram-negative bacteria, namely *Pseudomonas aeruginosa*, disrupt host proteostasis and lead to toxic protein aggregation. In support of the idea that antibiotics contribute to PCDs, all of these bacterial species are well known for their resistance to antibiotics. As such, antibiotics could potentially select for resistant culprit bacteria that contribute to the disease pathogenesis while at the same time eliminate the population of the protective commensal bacteria.

Thus, bacteria-targeted prevention strategies would concentrate on eliminating harmful species and enriching the beneficial ones. Such a strategy will only be possible if we fully understand the contribution that each bacterial resident of the human microbiome has on host proteostasis. Ongoing research aims at deciphering the role of the human microbiome on host proteostasis and consequently PCDs.

**Diagnostics:** The accumulation of aggregate deposits of misfolded proteins leads to toxicity that manifests in disease onset and early symptoms. Diagnosing the disease or identifying the pre-disease stage early enough to apply intervention strategies is critical in mitigating PCDs. However, current methods of diagnostics rely on tests that often fail to capture the disease in the pre-symptom stage. The composition of the gut microbiota may offer a potential diagnostic tool (Figure 1).



**Figure 1 | Targeting the human gut microbiota as a potential preventative, diagnostic, and therapeutic tool.** Factors such as diet/probiotics, drugs/antibiotics, lifestyle, or genetics influence the human gut microbiota. Targeting these factors early in life could provide effective prevention strategies against PCDs. Furthermore, understanding the contribution of individual microbes and their products in disease pathogenesis will open new opportunities for disease diagnostics and therapeutics. SCFA: Short-chain fatty acids. Created with BioRender.com.

For instance, as stated earlier, changes in the gut microbiota can precede PD diagnosis as early as 20 years (Savica et al., 2009). Gut dysbiosis has been associated with other PCDs as well; however, how the presence and abundance of individual bacterial species affect disease onset remains unknown. A recent meta-analysis study that looked at the relationship between gut microbiome alpha-diversity and neurodegenerative diseases, including PD, found no correlation between the two (Plassais et al., 2021). These results further emphasize the complexity of the gut-brain axis and the need to decipher the roles of individual microbes. Understanding the contribution of each microbe in the disease pathogenesis could offer new preventative and therapeutic approaches.

Numerous companies that perform direct-to-consumer microbiome sequencing emerged over the past years. Such personalized genomic services have a high potential to identify individuals predisposed to PCDs whose gut microbiome composition could be assessed based on the abundance of bacteria that play detrimental or beneficial roles in disease pathogenesis. While such an approach offers a promising strategy, more work needs to be done to decipher the role of bacteria on PCDs. Walker et al. (2021) used *C. elegans* to identify microbes that affect host proteostasis and revealed gram-negative enteric bacteria that disrupt protein stability in a tissue non-autonomous manner. While these results offer only a snapshot of bacterial contribution to protein stability, ongoing research is identifying additional bacterial modulators of host proteostasis. Collectively, these results could be leveraged to develop a diagnostic strategy.

**Therapeutics:** The utilization of data on bacterial contribution to PCDs combined with the advances in personalized genomic services will provide novel opportunities for therapeutic interventions. Such treatment avenue would encompass two strategies: enrichment in beneficial bacteria and elimination of detrimental ones. Additional approaches could encompass direct administration of bacterial metabolites, such as butyrate, which was shown to attenuate bacteria-mediated protein aggregation (Walker et al., 2021). The enrichment of potentially beneficial microbes, such as butyrogenic bacteria, *B. subtilis*, *Prevotella*, or other species, may face some challenges as, for example, *Prevotella* is categorized as a biological safety level-2 (BSL-2) bacterium and is known to be associated with human infections (Goya et al., 2020; Walker et al., 2021). However, administration of BSL-2 organisms as probiotics could still be possible as fecal matter transfer was not only shown to be beneficial in AD, but in general, it is regarded as a safe procedure (Hazan, 2020). Enrichment of specific gut microbes is also possible with diet, pre-, and probiotics. For example, the Mediterranean and the ketogenic diet has been shown to decrease the risk for and alleviate symptoms of AD (Scarmeas et al., 2006; Rusek et al., 2019). Additionally, the administration of probiotics provides broad health benefits to AD patients (Arora et al., 2020). Such gut-targeted therapeutic interventions offer a promising approach in the management of PCDs. However, such therapeutic strategies will likely be more feasible and effective once we understand the influence of individual bacterial species on disease pathogenesis and determine their respective growth and nutritional requirements.

Walker et al. (2021) found bacteria that disrupt protein folding upon intestinal colonization of the *C. elegans* polyglutamine model. While all of the identified microbes are detrimental and known to cause infections, they also colonize asymptomatic individuals; as such, identification and elimination of these bacteria, especially from genetically predisposed individuals who are at risk of developing the familial disease type, could potentially affect disease onset and progression. One approach to target these detrimental species is by employing phages, viruses that kill bacteria with superb efficacy. Unlike antibiotics, phages have superior specificity towards bacteria which would allow for their utility in modifying the gut microbiome at the species level.

As the knowledge on bacterial contribution to PCDs increases, it is becoming more evident that healthcare will soon expand into personalized medicine to provide Intervention strategies that modify individual gut microbiota. An increase in the abundance of beneficial bacteria and eliminating microbes that contribute to PCDs could offer an effective strategy to target these debilitating ailments (**Figure 1**).

**Where do we go from here?** While the protective effect of butyrogenic bacteria and *Prevotella* species described by Walker et al. provides only a glimpse of gut microbiota, the next obvious step is to decipher the effect of all residents of the human microbiome on PCDs. Such studies will be challenging and likely not feasible in rodents or humans; however, the *C. elegans* model offers scalability, affordability, and as emphasized above, the results seem to be conserved across higher organisms, including humans. A comprehensive understanding of the individual microbial contribution to host proteostasis is critical in the development of effective intervention strategies for PCDs. Further research will also have to explore the influence of disease stage and sexual dimorphism on the efficacy of microbiome-targeted therapeutic interventions.

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