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Case Report

Application of Metagenomics Sequencing in a Patient with Dementia: A New Case Report

Maria Minelli ^{1,2}, Federico Anaclerio ^{1,3,*}, Dario Calisi ^{1,3}, Marco Onofri ³, Ivana Antonucci ^{1,4},
Valentina Gatta ^{1,4} and Liborio Stuppia ^{1,3}

¹ Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy.; mariaminelli60@gmail.com (M.M.); dariocalisi95@outlook.it (D.C.); i.antonucci@unich.it (I.A.); v.gatta@unich.it (V.G.); stuppia@unich.it (L.S.)

² Department of Medical Genetics, "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy

³ Department of Neurosciences, Imaging and Clinical Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy.; onofri@unich.it

⁴ Department of Psychological, Health and Territorial Sciences, "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy.

* Correspondence: federico.anaclerio@unich.it

Abstract: (1) Background: The study of microbiome is being crucial for his role in major systemic diseases, in particular the oral and gut microbiota. In the last years the study of microorganism correlated for example with neurodegenerative disease is increasing the prospective of a possible link between gut microbiota and brain. Here we report a new case report about a patient evaluated genetically firstly for dementia and late-onset dyskinesia, and then tested with 16S metagenomics sequencing. (2) Methods: Starting from buccal swab, we extracted bacterial DNA and then we performed a NGS metagenomics sequencing based on the amplification of hypervariable regions of the 16S in bacteria. (3) Results: The sequencing revealed the presence of *Spirochaetes* Phylum, a pathogenic bacterium generally known to be able to migrate to the Central Nervous System and not only. (4) Conclusions: Oral infections as our results shows could be possible contributing factors to various neurodegenerative conditions.

Keywords: microbiome; metagenomics; NGS; dementia; neurodegenerative disease; 16S

1. Introduction

The human microbiome is known to play a role in the development of major systemic diseases. In particular, experimental and clinical studies suggest a possible link between these biomarkers, oral health and cognitive decline. The mechanism by which the oral microbiota, the second most diverse and populous one in the mammalian body (as shown in Figure 1 by Orr, Miranda E et al, 2020), affects cognition is likely to be mediated by modifications in the oral microenvironment that select for pathogens and facilitate the transmission of bacteria outside the mouth [1].

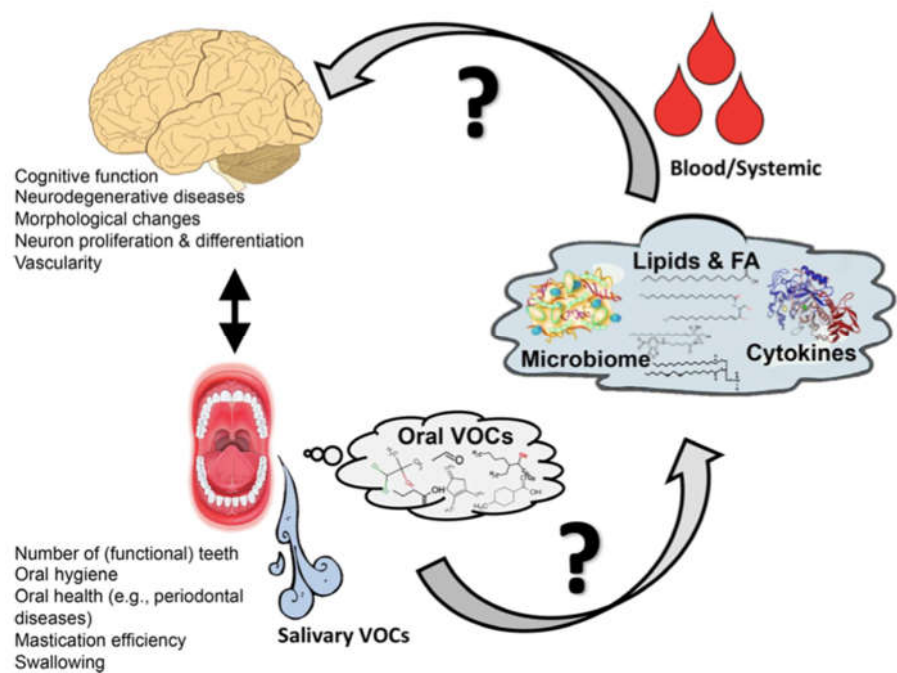


Figure 1. The possible mechanism and interaction between oral microbiota, brain and health function.

Different species of Spirochaetes were correlated to various neurodegenerative diseases and other pathologies, particularly the genus *Treponema*, a microaerophilic bacterium that survives for short time outside the infected organism. It is closely related (>99% DNA homology) with other species that were falling in the Spirochaete Phylum. Pathogenic *Treponemes* are not culturable in the laboratory, unlike nonpathogenic *Treponemes*; in fact, we can distinguish: the *Treponema pallidum*, the etiologic agent of syphilis, the *Treponema carateum* that causes pinta, the *Treponema pertenuis*, the etiologic agent of framboesia and the *Treponema endemicum* that causes bejel or "endemic syphilis." [2]. Previous reports have also documented amplification of *T. pallidum* nucleic acid from oral or pharyngeal swabs [3,4], or in one instance, saliva [5], in individuals with syphilis.

Actually, Spirochaetes are highly neurotropic and can spread along nerve fibers and the lymphatic system. They have been identified in the trigeminal nerve and ganglia, which might be the preferential access to brain. It has also been shown that typical oral species of the phylum Spirochaetes (including multiple species of the genus *Treponema*) often comprise amyloid plaques (as shown in Figure 2 by Bacali C et al, 2022) [6].

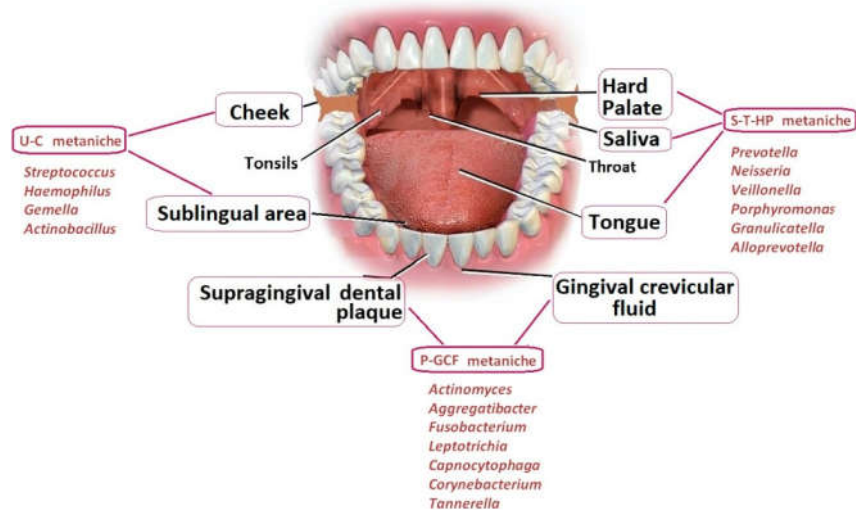


Figure 2. The different areas and the typical bacteria that could potentially invade the oral microbiota.

In 2021, Su et al. confirmed that oral *Treponema denticola* can induce β -amyloid accumulation in the hippocampus of C57BL/6 mice [7].

Here we present a patient with dementia tested for Huntington disease, which resulted with a normal number of CAG repeat in HTT gene but presented, at oral microbiome analysis from buccal swabs, the presence of a new Phylum that we had never encountered, the Spirochaetes Phylum, of which 20% represented by the species *denticola*. Our goal is to understand if beyond the genetic diagnostic test, it was a particular condition linked to dementia that was proinflammaging for the patient.

2. Case Presentation

We describe the case of an 83-year-old woman, evaluated genetically due to dementia and late-onset dyskinesia. At first the neurologist made the diagnosis of Lewy Body Dementia but then she had been started to present akathisia, and somatic dyskinesia involving even the diaphragm and the abdomen. Moreover, she was suffering of sleep disorder, complex mood disorder, psychosis including unintelligible confabulations, and parkinsonism. Last head CT scan revealed signs of marked cerebral atrophy and slight pallidal calcifications. Her medical history included previous deep venous thrombosis, pulmonary embolism and femoral fracture. She's being treated with Pradaxa, Xenazine, Madopar, Astazin, Quietapine and Tavor. She was an elementary school teacher and there was no clear family history of neurodegenerative disorders. She is a widow and has two sons. Genetic testing for Huntington disease revealed a normal number of CAG repeats. However, when we processed the extraction of DNA to perform the analysis we noticed an unparalleled reaction, in the filter column we usually use for the buccal swab with the buffer reaction. Generally, when we perform the extraction of the DNA, we obtain a transparent colour of the elution of DNA. In this case, after centrifugation and before to proceed for the automatic extraction of DNA, we observed a black volume of elution. This led us to consider whether any medications the patient was taking might have interfered with the reaction. After a careful literature review, we came to no conclusion and consequently decided to proceed with an analysis of the oral microbiota to assess the presence of possible pathogens.

3. Materials and Methods

Starting from buccal swab, bacterial DNA was extracted using the MagPurix instrument and the Bacterial DNA Extraction Kit (Zinexts Life Science Corp, Taipei, Taiwan-CatZP02006), according to the manufacturer's protocol. The bacterial DNA was quantified with Qubit 3.0 fluorometer (ThermoFisher). NGS was performed with the Ion 16S™ Metagenomics Kit, designed for analyses of mixed microbial populations using the Ion Torrent™ semiconductor sequencing workflow. The kit permits PCR amplification of hypervariable regions of the 16S rDNA gene from bacteria. The kit includes two primer sets that selectively amplify the corresponding hypervariable regions of the 16S region in bacteria:

- Primer set V2-4-8
- Primer set V3-6, 7-9

After quantification of libraries with the Real-Time Step One PCR System (Thermo Fisher Scientific, Waltham, MA, USA), the amplified fragments can then be sequenced using the Ion S5™ (ThermoFisher) platform, loaded onto an Ion 520™ chip and analyzed using the Ion 16S™ metagenomics analyses module within the Ion Reporter™ software, enabling a rapid and semi-quantitative assessment of complex microbial samples. These comprehensive primer sets allow for accurate detection and identification of a broad range of bacteria down to the genus or species level. The primer sets are paired with Environmental Master Mix v2.0 that is optimized to tolerate high levels of PCR inhibitors and amplify targets from complex samples such as environmental, food,

tissue and other challenging samples. The combination of the two primer pools allows for sequence-based identification of a broad range of bacteria within a mixed population.

4. Results

The sequencing performed with 16S Metagenomics Kit revealed an important result. Using the Ion Reporter Software, with the Krona software we analyzed all the phylogenetic classes, starting from the major Phyla. In particular, the Phylum Firmicutes revealed the 43% of the entire microbial population, while the Phylum Bacteroidetes the 26%, the Phylum Proteobacteria 19%, the Phylum Fusobacteria 6% and Actinobacteria the 4% (Figure 3).

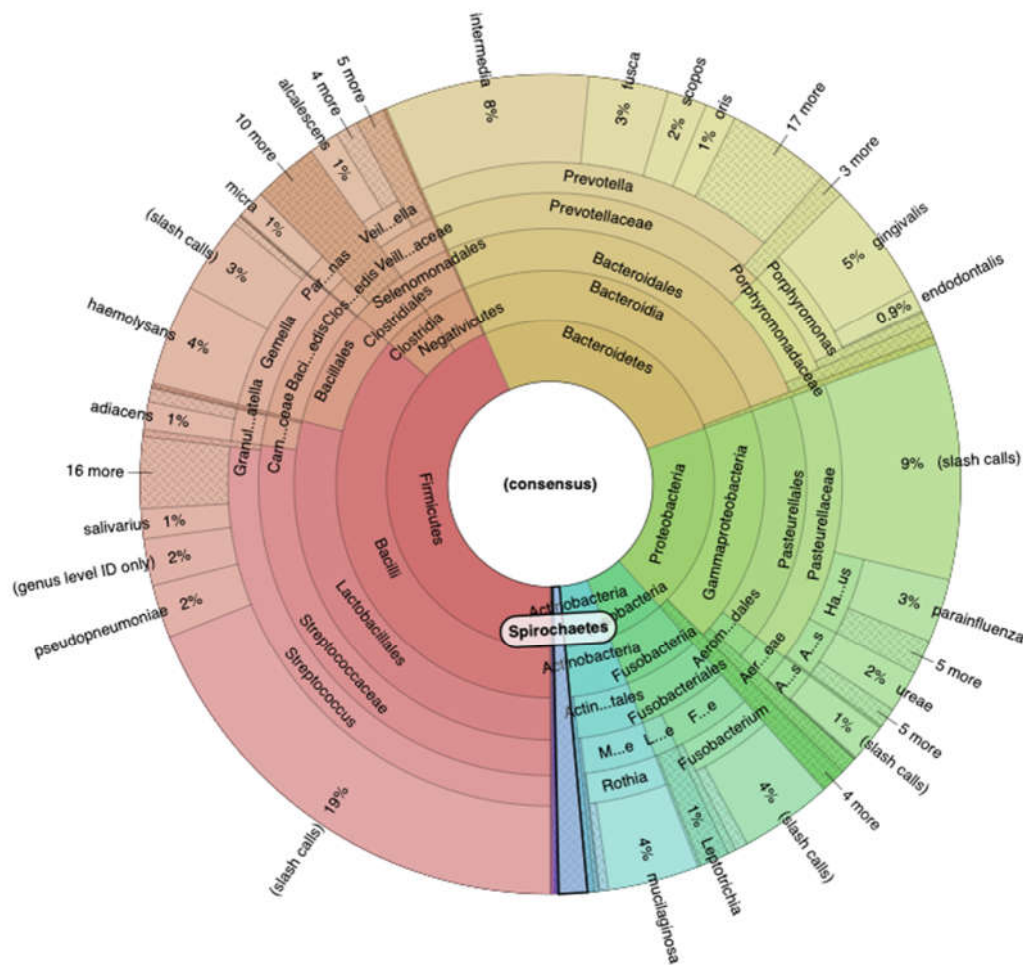


Figure 3. The result of 16S Metagenomics sequencing that revealed the presence of *Spirochaetes* Phylum.

Due to the characterization of all the bacteria community based on hypervariable regions, we discover and detect with Primer V2, V3, V6, V7 and V8 the presence of a new Phylum that we had never encountered, the Spirochaetes Phylum. In particular, the Genre Treponema was over the 90% of the entire Phylum, and continuing the Phylogenetic analysis, the more abundant species are Medium with 42%, sp. with 31% and Denticola with 20% (Figure 4)

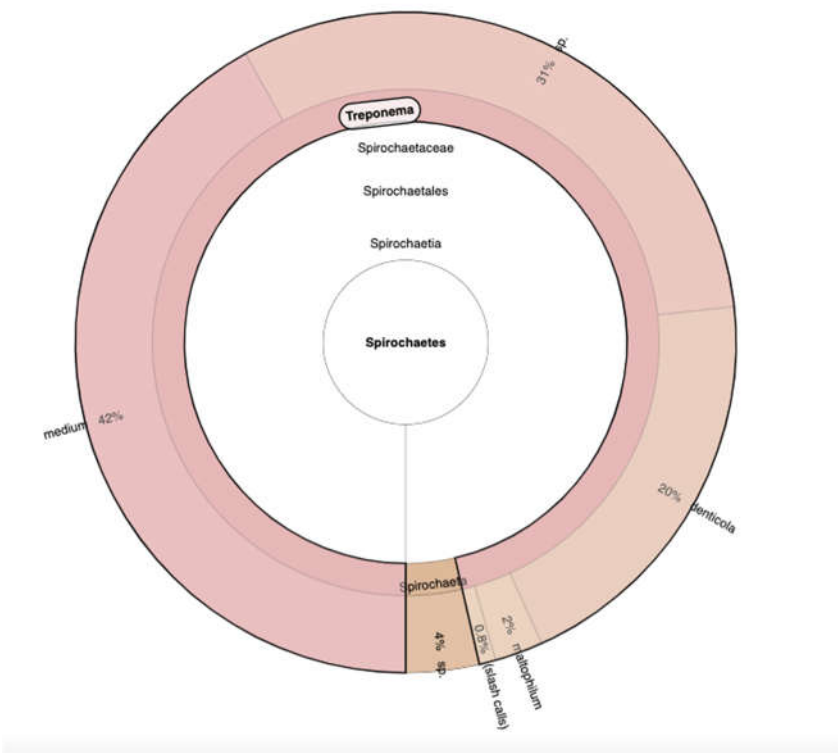


Figure 4. The analysis of Spirochaetes Phylum with the Krona software to comprehend the different genre and species.

5. Discussion

Different microorganisms belonging to the oral and intestinal microbiota have been linked to various diseases. Within the Spirochaetes phylum, *Treponema denticola* is known to be able to migrate to the Central Nervous System (CNS) along the peripheral and central nerves, like the trigeminal pathway which is out of the brain blood barrier control, or lymphatic vessels proven by the finding of the spirochetal chemokine CXCL13 in high concentrations in the cerebrospinal fluid but not in the serum. It is the larger bacterium upon which other pathogens, like *P. gingivalis*, could easily enter the CNS thanks to the marked neurotrophism displayed by all Spirochaetes. The above-mentioned pathway is likely to evade the initial immune recognition of these microorganisms because of their ability to hide within the ganglia, perhaps for many years, then invading the neighboring areas of the brain, like the locus coeruleus, affecting neurotransmitter release in the host [8]. Recent research indicates that also oral infections could be possible contributing factors to various neurodegenerative conditions [9].

More studies are required to establish the causal link between oral infections and neurodegeneration. The research relies on fairly small samples and cross-sectional surveys, which only allow for hypotheses regarding the presence of an oral-brain connection. Therefore, most findings still seem preliminary, as their authors generally acknowledge. To elucidate the nature of the connection between the oral microbiome and the CNS, it is crucial to create and validate a methodological approach grounded in a precise definition of oral dysbiosis and the mental disorders analyzed. The identification of specific oral dysbiosis signatures associated with various mental disorders raises the question of whether the CNS modulate the oral microbiome. This would constitute the complementary element of a bidirectional relationship suggesting the existence of an oral cerebral axis equivalent to the gastrointestinal axis. If the bidirectional connection between the oral microbiome and the CNS were confirmed, rebalancing microbial homeostasis with probiotics could offer a potential therapeutic pathway [10]. Our study, even if, at the moment, we cannot state with certainty the causal role of our findings, suggests correlations between oral microbiota and cognitive disorders or dementia and supports the hypothesis that analyzing oral other than the fecal

microbiota could be potentially useful in diagnostic practice of various diseases. The patient's microbial profile might serve as biomarker for evaluating disease risk, early treatment, assessing therapy response, or guiding new treatments and preventive measures [6].

6. Conclusions

Future microbiome analysis could become a novel approach for preventing systemic diseases and for applying possible therapies in individuals with dysbiosis or the presence of pathogenic microorganisms, especially in the era of precision medicine. This field is still underexplored, and further research with larger sample sizes and involving various types of mental diseases is necessary to clarify the biological connection or interactions between oral microbiota and all mental health disorders, and to develop consistent patterns to generate more definitive data.

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