ENTERIC ALPHA-SYNUCLEIN INCLUSIONS, IMPAIRED INTESTINAL PERMEABILITY, ENTERIC INFLAMMATION AND COLONIC MOTOR DYSFUNCTIONS PRECEDE CENTRAL NEURODEGENERATION IN A TRANSGENIC MOUSE MODEL OF PARKINSON'S DISEASE

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Introduction: Gastrointestinal (GI) dysfunctions, including dysphagia, altered gastric emptying, infrequent bowel movements, abdominal distension and constipation represent the most common non-motor symptoms of Parkinson's disease (PD). Of note, GI disturbances in PD may occur at all stages of the neurodegenerative process, to such an extent that they are now considered as an integral part of clinical picture of the disease. Several lines of evidence suggest that accumulation of α-synuclein inclusions (a hallmark of PD) in myenteric neurons, altered mucosal permeability and colonic inflammation could contribute to bowel motor dysfunctions since the earliest stages of PD. However, current knowledge does not allow to establish a clear relationship between α-synuclein accumulation, impaired intestinal permeability, enteric inflammation, bowel dysmotility and PD pathology. This study examined concomitantly enteric α-synuclein accumulation, mucosal permeability, enteric inflammation and in vitro colonic motor activity in a transgenic model of PD, before onset of neurodegeneration in the central nervous system (CNS).

Material and methods: PrP human A53T αS transgenic (Tg) mice, Line G2-3develop a progressive PD-like neurological and motor deficiency after 9months of age, accompanied by neuronal degeneration and pathological accumulation of aggregated α-synuclein in the CNS. Animals were sacrificed at the age of 3, 6and 9months, in order to evaluate the presence and timing of enteric α-synuclein inclusions, alterations of intestinal mucosal barrier, enteric inflammation and colonic neuromotor dysfunctions since the very early phases of the disease, before the onset of CNS pathology. In particular, blood samples were collected to evaluate circulating lipopolysaccharide (LPS) levels (an indirect index of intestinal permeability) by ELISA assay; then distal colon was excised and processed for: 1) expression and aggregation of α-synuclein (western blot); 2) expression of zonulin-1and occludin tight junction proteins and toll like receptors-2(TLRs2) (western blot); 3) tissue tumor necrosis factor (TNF) and interleukin-1beta (IL-1β) pro-inflammatory cytokines (ELISA). Other groups of Tg and control mice at the different time points were sacrificed and colonic longitudinal and circular muscle preparations were set up in organ baths with standard Krebs solution, and connected to isometric transducers to record contractions elicited by electrical stimulation (ES, 10 Hz, 0.5ms, 30 mA).

Results: An accumulation of insoluble and aggregated α-synuclein was present in the enteric neurons of the distal colon in Tg mice since 3months of age, as compared with controls. Colonic zonulin-1and TLR-2protein expression were significantly decreased in Tg mice at 3months of age, while they significantly increased at 6and they normalized at 9months. With regard for colonic occludin expression, a significantly decrease were detected in Tg mice at 9months of age. In addition, Tg mice at 3, 6and 9months of age displayed a significant increase in circulating LPS levels along with increased colonic TNF and IL-1β levels. Electrically evoked contractions in longitudinal and circular muscle preparations were significantly decreased in Tg mice since 3months of age, as compared with controls.

Conclusion: The present findings suggest that a concomitance of enteric α-synuclein accumulation, altered intestinal epithelial barrier, increased intestinal permeability, bowel inflammation and impaired colonic motor activity represent early events in PD, occurring before the onset of CNS pathology.

Discussion: These changes could contribute to the pathogenesis of intestinal motor dysfunctions known to be associated with PD in humans.