ALS and the Gut-Brain Axis: A protocol for a systematic review and meta-analysis assessing the relationship between amyotrophic lateral sclerosis, the gut and its microbiome.

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ABSTRACT

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Aim: The aim of this review is to perform a comprehensive analysis of the literature to identify potential pre-symptomatic pathological hallmarks of ALS in the gut, including, but not limited to, changes in gut microbiota and protein aggregation.

Methods: This protocol details the strategy for the proposed systematic review and meta-analysis to identify and compare studies assessing gut-related pathological changes in ALS separated in to two analyses assessing (i) gut microbiota and (ii) non-microbiota related pathways, with the ultimate aim of identifying opportunities for early disease detection before neurological symptom onset.

ALS and the Gut-Brain Axis: A protocol for a systematic review and meta-analysis assessing the relationship between amyotrophic lateral sclerosis, the gut and its microbiome.

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Abstract

Background: ALS is a devastating neurodegenerative disease characterized by progressive loss of motor function. At the time of symptom manifestation, effective treatment options are extremely limited, due in part to the irreversible loss of motor neurons at this late stage of the disease. Recent research has highlighted the involvement of both gut pathology and gut microbiota as important factors in early disease pathogenesis, which may represent an opportunity for early diagnosis and intervention, prior to neurological symptom onset and thus prior to significant motor neuron loss.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a rare but fatal neurodegenerative disease characterized by progressive degeneration of both the upper and lower motor neurons in the brain and spinal cord. The clinical phenotype of ALS classically manifests as focal muscle weakness and wasting as well as upper and lower motor neuron clinical signs. Typically, patients develop progressive muscle weakness with mortality related to respiratory failure ~2-5 years following the onset of symptom, however there is significant heterogeneity in individual disease manifestation and outcome (Hardiman et al. 2011). Over the last decade, many associated gene defects have been identified including mutations in *C9orf72*, *FUS*, *SOD1*and *TDP-43* (Gregory et. al 2020). These monogenic aberrances have only been attributed to around 15% of sporadic ALS cases and 60-70% of familial cases, with many studies pointing towards a multifactorial pathogenesis (Roberts et al. 2016; Wang et al. 2017). Though ALS was originally defined as a purely motor neuron disease, it has since been recognised as having multi-systemic involvement, with frontotemporal, sensory and autonomic impairment now attributed to ALS disease pathogenesis in many cases (Silani et al. 2017). However, despite this genetic and phenotypic heterogeneity, the majority of ALS cases show the same pathological hallmark at *post-mortem*, the pathological accumulation of

protein aggregates composed of phosphorylated TDP-43 (pTDP-43).

The gut-brain axis describes the bidirectional connection between the gut microbiota and the brain. This axis does not exist as a single connective strand but rather a range of communication pathways. Such pathways include neuroanatomical connections, neuroendocrine signalling, and chemical mechanisms (Morais, Schreiber & Mazmanian, 2021). Neuroanatomical connections classically describe the enteric nervous system (ENS) and vagus nerve which represent a clear physical link between the gut and brain. Vagal afferents originate in the myenteric plexus, longitudinal and circular muscle layers, and the intestinal mucosa before terminating in the nucleus tractus solitaris of the medulla. Among other communicative pathways, these afferents are able to receive signals from enteroendocrine cells (EEC). EECs detect products of the microbiota secretome (e.g. lipopolysaccharides) and release over 30 different peptides including ghrelin and GLP-1, activating vagal afferent receptors. (Han et al., 2022). Neuroendocrine signalling in the gut-brain axis is best described through the hypothalamic-pituitary axis (HPA), which has an established relationship with the gut microbiota. Germ-free mouse models demonstrate exaggerated increases in plasma ACTH and corticosteroid in response to stress, suggesting an important role of the gut microbiome in regulation of the HPA (Sudo et al., 2004). Chemical pathways of the gut-brain axis involve the products of the microbiota secretome such as short chain fatty acids (SCFAs) which have a range of vital functions in the CNS: upregulation of tight junction proteins to maintain the blood-brain barrier, modulation of neurotrophic factors, and regulation of gut neurotransmitter production (Chidambaram et al., 2022). In addition to the gut-brain axis pathways, there is increasing evidence of the importance of non-microbiota gut changes in neurological conditions. In other neurodegenerative conditions such as Parkinson's disease, there is evidence of alpha synuclein protein aggregation in the ENS of the gut that is thought to manifest peripherally before spreading to the CNS. Indeed, GI symptoms can often occur before the onset of Parkinsonian motor symptoms (Schaeffer et al., 2020).

The connection between gut and brain is especially important given that ALS is not simply a neurological condition. There is increasing evidence of a link between the gut and manifestation of the disease. A study performed in Sweden established that individuals exhibiting gastrointestinal symptoms but with normal colonoscopy and histologically normal biopsy findings had an increased risk of developing ALS later in life (Sun et al., 2021). This evidence was supported by a study which established the manifestation of neurotoxic pTDP-43 protein aggregates in the GI tract before diagnosis of ALS and, in some cases, even before appearance of CNS symptoms (Pattle et al., 2022). In addition to the role of gut pathology in ALS, the literature also provides evidence for the influence of gut microbiota. A recent systematic review established a consistent pattern of microbiome changes in ALS mouse models, though drew inconclusive results from human studies (Sun et al., 2021). This review drew a number of conclusions from animal studies: (i) pre-symptomatic changes to the gut microbiome in ALS mouse models exist, (ii) the gut microbiome undergoes progressive degeneration simultaneous to ALS progression, and (iii) microbiome-targeted intervention can successfully modify disease progression. Although the systematic review drew no conclusive results from human studies, a later literature review on the subject (Martin, Battistini and Sun, 2022) supports the involvement of the gut microbiome in ALS in humans, as well as highlighting a potential role for gut microbiota-targeted interventions in ALS management. Furthermore, this review illustrates the importance of these early gut changes in the context of early disease identification, as GI symptoms are often ignored in the initial stages of the disease.

The influence of gut pathology, including the influence of gut microbiota, on disease is a rapidly developing field of research which, in combination with the inconclusive results of previous systematic analysis, provides scope for a

new systematic review into the topic. Furthermore, this systematic review will examine evidence for microbiota-related and non-microbiota-related gut pathology in the disease, combining both fields to produce a comprehensive analysis of the relationship between gut and CNS in the context of ALS. We will perform a systematic review of the involvement of the gut microbiota in ALS as well as the impact of ALS on gut pathology, including histological changes to the ENS. We also detail a structure for quality assessment of each paper to provide insight for future studies. As many of these microbiota and non-microbiota changes occur before onset of neurological symptoms, the gut may represent an opportunity for a novel mechanism of early disease recognition which this review will endeavour to identify.

Approach

A systematic review will be performed to assess studies reporting gut-related pathology in ALS. These studies will be split into (1) microbiota studies and (2) non-microbiota studies.

Microbiota studies will undergo quantitative analysis to assess impact of intervention or microbiota change (e.g. species composition/diversity, etc) on ALS pathology. These studies will be further divided into (1a) interventional and (1b) non-interventional. Interventional studies will be further subdivided based on intervention type: (1a)i) faecal transplantation studies, (1a)ii) dietary intervention studies, and (1a)iii) drug treatment studies. Non-interventional studies will be subdivided based on changes in the microbiota correlating with ALS phenotyping: (1b)i) changes to microbe species diversity and (1b)ii) changes to species ratio.

Non-microbiota studies will undergo both qualitative and quantitative analysis. Qualitative analysis will be performed to assess depth of information currently present, aiming to inform future mechanistic research of potential areas of interest. Quantitative analysis will be performed on variables consistent across 3 or more studies, with an aim to determine the nature of the relationship between gut pathology and ALS disease pathology.

Objectives (PICOS framework)

Population: All studies including preclinical animal studies and human studies.

Intervention: All interventional and non-interventional studies assessing the gastrointestinal system in the context of ALS

Comparison: Control or vehicle groups, where appropriate.

Outcome measures: Outcome measures will include, but will not be limited to:

- Intervention studies:
  o Treatment efficacy as: Time to mortality, disease features, disease progression

- Non-intervention studies:
  o Microbe species diversity
  o Species ratio

o Relevant numerical outcome measure in >3 studies

- Non-microbial studies:
  o Clinical metadata (ALS-FRS/disease duration)
  o Pathological outcomes (e.g. aggregation burden, cell loss etc)

Study design: All study types where outcomes in animals and humans assess microbiota or non-microbiota related pathways in ALS patients or ALS animal models, compared to appropriate control populations. Specifically, (i) all Microbial interventional studies where ALS patients exposed to an intervention are compared with patients not exposed to the intervention. (ii) All Microbial non-interventional studies where ALS patients are compared to a control group (without ALS). (iii) All Non-microbial studies where Gastrointestinal pathology, inflammation, protein aggregation or alternative proposed disease marker is explored in the context of concurrent ALS

Methods

Search terms

Source databases: (1) PubMed (2) Medline (3) Embase. No restrictions were implemented for publication date or language. Searches were performed on 20th January 2023.

PubMed

((((((("enteric nervous system") OR ("microbiome")) OR ("microbiota")) OR ("GI tract")) OR ("gastrointestinal tract")) OR (gut)) AND ((("motor neurone disease") OR ("amyotrophic lateral sclerosis")) OR ("motor neuron disease"))
•Limit “English” = 196

Ovid (Medline (1946- 01/2023) and Embase (1974-01/2023))

((ALS or MND or Motor Neuron Disease or Motor Neurone Disease or Amyotrophic Lateral Sclerosis) and (gut or gastrointestinal tract or GI tract or colon or microbiome or microbiota or enteric nervous system)).af. not Review.pt
•Limit “English” = 1014

Other sources

Studies identified within included articles that were not captured by the original search terms and/or inclusion criteria.

Screening

This review will make use of the Systematic Review Facility screening tool found at app.syrf.org.uk. Using this tool, we will screen the title and abstract of each paper found in the database search. Papers with relevant titles and/or abstracts will be selected and imported to Endnote, discarding duplicates in the process. Each paper will be assessed by two independent reviewers utilising our inclusion and exclusion criteria, producing a quality score for each paper. Data will be extracted from relevant, quality papers as described below. Any paper featuring reviewer concordance <0.66 will be assessed by a third reviewer.

Inclusion and Exclusion Criteria

Inclusion criteria:

- All studies where ALS and the GI tract are mentioned

Exclusion criteria:

- Narrative reviews
- Systematic reviews
- Letters
- Conference abstracts
- Commentaries

Quality Checklist

Quality checklist adapted from the NHLBI study quality assessment tools and CAMARADES checklist.

- Publication in a peer reviewed journal
- Blinded group allocation if appropriate
- Blinded outcome assessment
- Identification of confounding variables
- Appropriate control group selected (if relevant)
- Declaration of any conflict of interest
- Animal welfare regulation compliance/ Appropriate ethics approvals for human studies
- Sample size calculation
- Appropriate statistical methods employed (if relevant)

Study characteristics to be extracted

- Study ID: (1) Authors (2) Year
- Intervention type: (1) Dietary supplementation (2) Dietary intervention (3) Probiotic (4) Antibiotic (5) Faecal transplantation (6) None (7) Other
- Animal outcome data: (1) Microbe species diversity (2) Microbe species ratio (3) ALS phenotype (survival/motor function)
- Clinical outcome metadata: (1) ALS-FRS (2) disease duration (3) cognitive score
- Pathological outcome data: protein aggregation, cell counts etc
- Mode of intervention delivery (oral, intravenous, intramuscular, rectal administration, intrathecal, subcutaneous, intraperitoneal)
- Type of model: (1) Animal type (2) Presence of mutation (3) Mated or non-mated or N/A (4) Familial or sporadic disease
- Type of ALS (for human studies): (1) ALS (2) ALS-FTD (3) FTD (4) C9orf72 (5) SOD1 (6) other mutation (7) sporadic
- Sample size
- Intervention duration: (1) Single (2) Multiple (3) Continuous
- Timing of intervention: (1) pre-symptomatic (2) at symptom onset (3) post-symptom onset
- 9-point quality checklist score as detailed above

Statistical Analysis

Outcome measures will be plotted for microbial studies subdivided into intervention and non-intervention groups as detailed above. Each of the studies identified will be included on a forest plot. Given the possibility of variability in methodologies used for these studies a random effects model will be used for analysis. Animal and human data will be treated separately. Where possible continuous variables will be compared as standardized mean differences (SMD) and survival as odds ratios. SMD will be compared using Hedges g statistic, to account for bias from small sample sizes. Survival summary measures and SMDs will be reported as odds ratios with 95%

confidence intervals. Non-microbial studies will be coded into similar groups using qualitative methodologies. Where three or more studies have assessed the same quantitative variable, a quantitative analysis as described above will be conducted. Heterogeneity will be assessed for all quantitative outcome measures using I^2 values, and a funnel plot and Egger's regression test will be used to assess publicationbias. Quality checklist items will be discussed qualitatively, and total 9-point checklist scores will be included on a frequency distribution for comparison and visualization purposes. All data will be made available through open access at publication.

Conflicts of interest

The authors declare no conflicts of interest.

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