Mechanisms for Gut Microbiome Dysbiosis Following Stroke

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Introduction

- Over 795,000 people in the US suffer a stroke every year.
- Survivors are often left with severe cognitive, functional, and emotional impairments.
- Gut dysbiosis and increased gut permeability have been shown to occur following stroke, leading to a systemic flood of neuro- and immuno-modulatory substances.
- Evidence from animal model studies suggests that gut microbes modulate the bidirectional gut brain axis.
- It is unknown how post-stroke dysbiosis correlates with gut permeability.

Aims

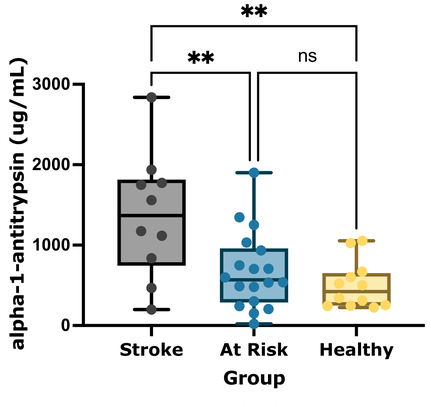
- Correlate post-stroke gut dysbiosis with gut permeability in humans.
- Explore mechanisms utilized by microbes contributing to dysbiosis to identify potential interventional targets to promote stroke rehabilitation.

Methods

- We recruited 12 participants after a first-time ischemic stroke, 18 at risk participants who had not had a stroke but had 1+ cardiovascular risk factors, and 12 healthy participants.
- Participants were between the ages of 55-85 years old and had no clinically significant chronic conditions or history of major gastrointestinal surgery in the past 5 years.
- Stool samples were collected from each participant to measure leaky gut markers and the gut microbiome with shotgun metagenomics sequencing.
- Differential analyses of bacterial taxa were performed using the software package edgeR on raw sequence counts. Data were normalized and then fit using a negative binomial generalized linear model using experimental covariates, and statistical tests were performed using a likelihood ratio test. Adjusted p values (q values) were calculated based on a false discovery rate threshold of 5% (0.05).
- Differential analyses for functional gene counts were performed using the same methods as for bacterial taxa.

Results

Stroke Patients had Higher Leaky Gut Markers



The stroke group had a significant increase in the leaky gut marker alpha-1-antitrypsin compared to the at-risk and healthy groups.

**p < 0.05

Bacterial Taxa in Stroke vs. Control Groups

Taxonomy	Healthy/Acute Stroke : logFC	Healthy/Acute Stroke : QValue	At Risk/Acute Stroke : logFC	At Risk/Acute Stroke : QValue
Roseburia	1.67	0.04	1.84	1.88E-03
Anaerostipes	1.89	0.02	1.67	0.01
Coprococcus	2.10	9.48E-03	2.35	5.52E-04
Ruminococcus	1.96	4.49E-03	2.38	6.42E-04
Eggerthella	-2.04	0.01	-2.33	6.57E-05
Acidaminococcus	-4.20	1.13E-05	-2.50	0.02
Butyribacter	6.01	0.03	6.64	0.01
Limosilactobacillus	-6.23	1.67E-06	-6.27	2.39E-12
Lacticaseibacillus	-4.64	3.26E-03	-4.00	2.79E-04
Lactobacillus	-4.57	3.47E-03	-5.43	9.44E-09
Acutalibacter	-1.01	3.47E-03	-0.75	0.02
Klebsiella	-2.74	3.47E-03	0.68	0.64

Increased in Stroke

Decreased in Stroke

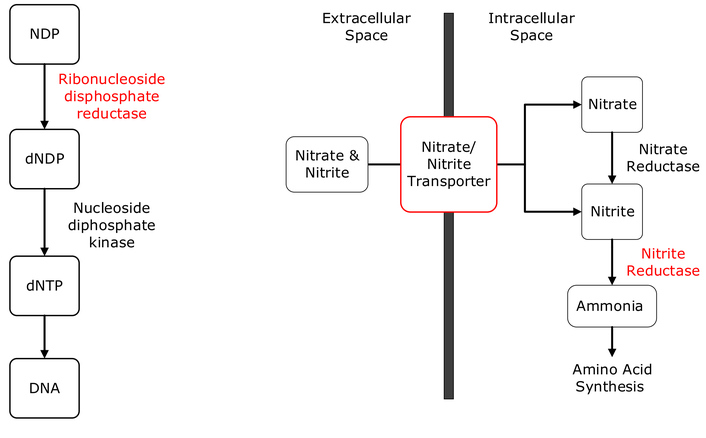
Taxa negatively associated with alpha-1-antitrypsin were decreased in stroke, including Ruminococcus, Coprococcus, Anaerostipes, and Roseburia.

Taxa positively associated with alpha-1-antitrypsin were increased in stroke including Eggerthella.

Metabolic Changes in Stroke Patients

Purine Synthesis Pathway	Healthy/Acute Stroke : logFC	Healthy/Acute Stroke : QValue
ribonucleoside-diphosphate reductase alpha chain	-0.74	3.39E-04
ribonucleoside-diphosphate reductase beta chain	-0.93	3.05E-03
Nitrogen Metabolism Pathway	**Healthy/Acute Stroke : logFC**	**Healthy/Acute Stroke : QValue**
MFS transporter, NNP family, nitrate/nitrite transporter	-2.51	0.03
nitrite reductase (NAD(P)H)	-2.26	0.04
nitrite reductase (NADH) large subunit	-1.58	0.04

Functional analysis uncovered a significant increase in KEGG orthologs implicated in purine synthesis and nitrogen metabolism pathways present in the stroke group compared to the healthy group.



Conclusion

- Stroke leads to an inflammatory response and increased permeability in the gut.
- Leaky gut alters the microbial environment by increasing oxygen and nitrate availability in the lumen.
- One potential mechanism of dysbiosis includes oxygen utilization by aerobic and facultative anaerobic bacteria.
- The stroke group had increased levels of oxygen-dependent ribonucleoside reductase, an enzyme that synthesizes DNA precursors.
- Increased nitrate utilization is a second potential mechanism for dysbiosis.
- The stroke group had increased levels of nitrate/nitrite transporter and nitrite reductase.
- There was in increase in aerotolerant bacteria from the Lactobacillaceae family.
- There was a decrease in butyrate producing bacteria in the stroke group.
- Analysis showed a decrease in butyrate producers including Roseburia, Anaerostipes, and Butyribacter in the stroke group compared to the at risk and healthy groups. Butyrate plays a role in inflammatory homeostasis and inhibiting pro-inflammatory cytokines.
- These findings may shed light on future intervention developments targeting on gut dysbiosis and permeability to promote stroke rehabilitation.

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