

Statistical Considerations Of Microbiome Studies

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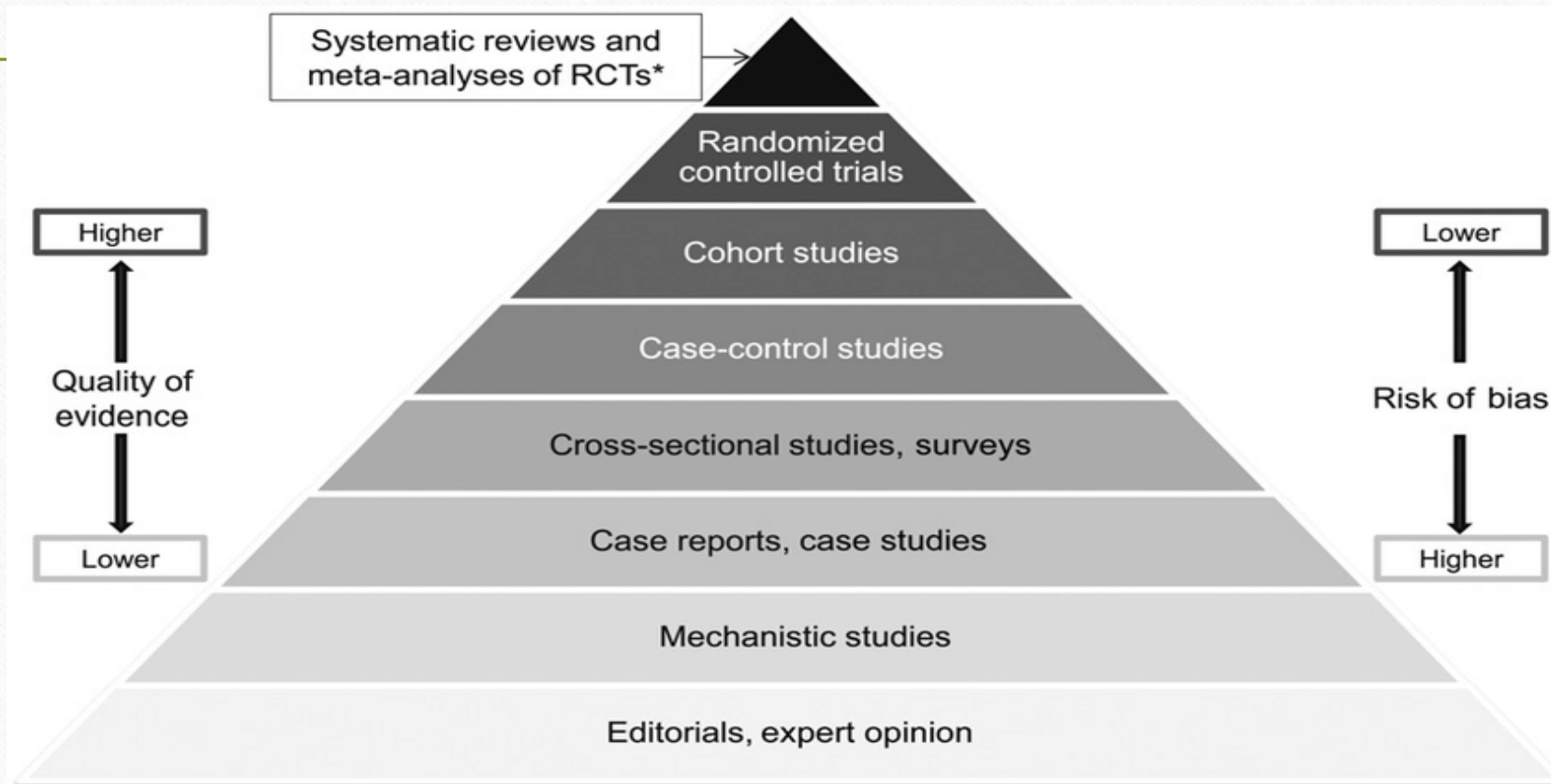
OUTLINE

- Study Design
- Measurement
- Statistical Issues of Microbiome Data Analysis

Study Design

- The goal of scientific research → Cause-Effect Relationships
- How? → Experiments
- How to plan an experiment? → Study Design

Hierarchy of Strength of Evidence



Current State of Study Designs used in Microbiome Studies

- Cross-Sectional Studies ➔ Profiling and Association
- Case Control Studies ➔ Differential Abundance
- Cohort Studies ➔ Longitudinal Microbiome Studies
- RCTs ➔ Intervention to alter gut microbiome to change certain health outcomes
- Meta Analysis ➔ Aggregate findings from multiple studies

Cross-Sectional Microbiome Study



RESEARCH ARTICLE
Host-Microbe Biology



A Cross-Sectional Study of Compositional and Functional Profiles of Gut Microbiota in Sardinian Centenarians

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ABSTRACT Sardinia, Italy, has a high prevalence of residents who live more than 100 years. The reasons for longevity in this isolated region are currently unknown. Gut microbiota may hold a clue. To explore the role gut microbiota may play in healthy aging and longevity, we used metagenomic sequencing to determine the compositional and functional differences in gut microbiota associated with populations of different ages in Sardinia. Our data revealed that the gut microbiota of both young and elderly Sardinians shared similar taxonomic and functional profiles. A different pattern was found in centenarians. Within the centenarian group, the gut microbiota was correlated with the functional independence measurement of the host. Centenarians had a higher diversity of core microbiota species and microbial genes than those in the young and elderly. We found that the gut microbiota in Sardinian centenarians displayed a rearranged taxonomic pattern compared with those of the young and elderly, featured by depletion of *Faecalibacterium prausnitzii* and *Eubacterium rectale* and enriched for *Methanobrevibacter smithii* and *Bifidobacterium adolescentis*. Moreover, functional analysis revealed that the microbiota in centenarians had high capacity for central metabolism, especially glycolysis and fermentation to short-chain fatty acids (SCFAs), although the gut microbiota in centenarians was low in genes encoding enzymes involved in degradation of carbohydrates, including fibers and galactose.

IMPORTANCE The gut microbiota has been proposed as a promising determinant for human health. Centenarians as a model for extreme aging may help us understand the correlation of gut microbiota with healthy aging and longevity. Here we confirmed that centenarians had microbiota elements usually associated with benefits to health. Our finding of a high capacity of glycolysis and related SCFA production represented a healthy microbiome and environment that is regarded as beneficial for host gut epithelium. The low abundance of genes encoding components of pathways involved in carbohydrate degradation was also found in the gut microbiota of Sardinian centenarians and is often associated with poor gut health. Overall, our study here represents an expansion of previous research investigating the age-related changes in gut microbiota. Furthermore, our study provides a new perspective for potential targets for gut microbiota intervention directed at limiting gut inflammation and pathology and enhancing a healthy gut barrier.

KEYWORDS centenarian, gut microbiota, longevity, metagenomic sequencing

Longevity is a complex biological phenotype determined by genetic, epigenetic, and environmental factors such as diet, lifestyle, and even geographic location (1–4). These factors have also been shown to affect the gut microbiota in humans (5–8). It has been demonstrated that gut microbiota is tightly linked to human health and disease (9). There is evidence showing that the gut microbiome contributes to the regulation

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A Case Control Microbiome Study

Case-Control Study of the Effects of Gut Microbiota Composition on Neurotransmitter Metabolic Pathways in Children With Attention Deficit Hyperactivity Disorder

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Background: Attention-deficit/hyperactivity disorder (ADHD) is a neuropsychiatric condition that may be related to an

A Cohort Microbiome Study

Microorganisms
Multidisciplinary Digital Publishing Institute (MDPI)

Socioeconomic Status and the Gut Microbiome: A TwinsUK Cohort Study

Ruth C. E. Bowyer, Matthew A. Jackson, [...], and
Claire J. Steves

[Additional article information](#)

Associated Data

▸ [Supplementary Materials](#)

Abstract

Socioeconomic inequalities in health and mortality are well established, but the biological mechanisms underlying these associations are less understood. In parallel, the gut microbiome is emerging

A Microbiome RCT



Advanced

Structured Exercise Alters the Gut Microbiota in Humans With Overweight and obesity-A Randomized Controlled Trial

Timo Kern et al. Int J Obes (Lond). 2020 Jan.

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Abstract

Objectives: Studies suggest that exercise affects the composition and function of the human gut microbiota, yet this has not been investigated in a randomized controlled trial. The primary aim of this study was to assess if exercise alters the diversity, composition and functional potential of the gut microbiota in free-living humans. A secondary aim was to test whether alpha diversity was associated with phenotypical outcomes.

Meta Analysis of Microbiome Studies

nature
COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-017-01973-8

OPEN

Meta-analysis of gut microbiome studies identifies disease-specific and shared responses

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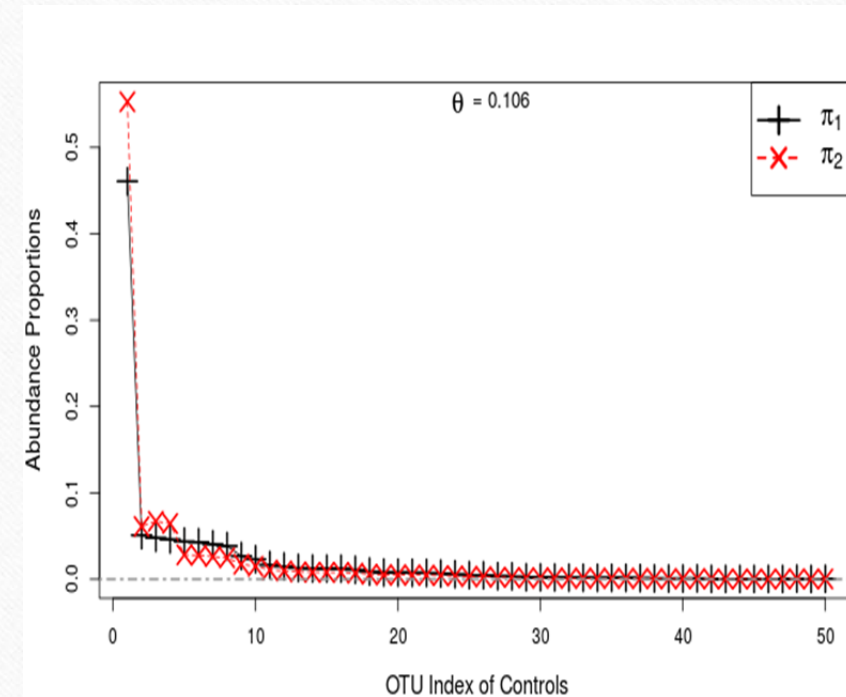
Hundreds of clinical studies have demonstrated associations between the human microbiome and disease, yet fundamental questions remain on how we can generalize this knowledge. Results from individual studies can be inconsistent, and comparing published data is further complicated by a lack of standard processing and analysis methods. Here we introduce the MicrobiomeHD database, which includes 28 published case-control gut microbiome studies spanning ten diseases. We perform a cross-disease meta-analysis of these studies using standardized methods. We find consistent patterns characterizing disease-associated microbiome changes. Some diseases are associated with over 50 genera, while most show only 10–15 genus-level changes. Some diseases are marked by the presence of potentially pathogenic microbes, whereas others are characterized by a depletion of health-associated bacteria. Furthermore, we show that about half of genera associated with individual studies are bacteria that respond to more than one disease. Thus, many associations found in case-control studies are likely not disease-specific but rather part of a non-specific, shared response to health and disease.

Sample Size and Power Calculation of Microbiome Studies

- How many subjects do I need for my study?
- Keep in mind that sample size and power are only meaningful when you have the following:
 - 1. Study Design
 - 2. Hypothesis
 - 3. Hypothesis Test
 - 4. Effect Size or Sample Size

Power & Sample Sizes Tool for Case-Control Microbiome Studies

- Study Design: Case Control
- Effect Odds Ratio
- A Range of Sample Sizes for Simulating Effect Size
- <https://fedematt.shinyapps.io/shinyMB/>



Power and sample-size estimation for microbiome studies using pairwise distances and PERMANOVA

- Study Design: ANOVA
- Hypothesis Test: Permutation
- Simulate Distance Matrix (Unifrac and Jaccard Distance)
- URL:
<https://github.com/brendankelly/micropower>
- A detailed description of the methods underlying the micropower package has been submitted for publication. Please see: Kelly BJ, Gross R, Bittinger K, Sherrill-Mix S, Lewis JD, Collman RG, Li H, Bushman FD. Power and sample-size estimation for microbiome studies using pairwise distances and PERMANOVA.

Hypothesis Testing and Power Calculations for Taxonomic-Based Human Microbiome Data

- Parametric Model to Specify Hypothesis (Dirichlet Multinomial Distribution)
- R Implementation HMP
- <https://cran.r-project.org/web/packages/HMP/HMP.pdf>
- La Rosa PS, Brooks JP, Deych E, Boone EL, Edwards DJ, et al. (2012) Hypothesis Testing and Power Calculations for Taxonomic-Based Human Microbiome Data. PLoS ONE 7(12): e52078.
- doi:10.1371/journal.pone.0052078

powmic: an R package for power assessment in microbiome case–control studies

- A simulation-based strategy and R package powmic to estimate the empirical statistical power while considering the complexity of data characteristics.
- R package
- <https://github.com/lichen-lab/powmic>
- Li Chen, powmic: an R package for power assessment in microbiome case–control studies, Bioinformatics, , btaa197, <https://doi.org/10.1093/bioinformatics/btaa197>

Measurement

- Measurement is a critical issue in any scientific study.
- Two most important properties of a measurement system are: Reliability and Validity
- Measurements for Microbiome Studies: 16S rRNA or Shotgun Sequencing
- Question: Are they reliable and valid?
-

Biases and Variabilities in Measuring Microbiome

- Sample Selection (e.g. Human body site)
- Sample Method (e.g. swab vs stool samples)
- Sample Storage (e.g. fresh vs frozen)
- Sequencing reads (End1, End2, or pairs)
- Quality Scores (Illumina Q1-40)
- Removal of Chimeric Sequences
- Clustering Algorithm
- Reference Gene Library (GreenGenes vs RDP vs SILVA)
- Technological and Biological Variations (semiauto DNA extraction, biomass)

Statistical Issues in Microbiome Data Analysis

- Measurement Errors (Error Models)
- Zero-Inflation (Zero-Inflated Model)
- High-Dimensionality (Dimension Reduction)
- Big Data (Size of the data sets, Multi-omics, Epidemiologic studies ...)
- Complex Modeling (Predicting functions, predicting phenotypes, modeling host interactions, longitudinal microbiome data analysis, mediational analysis, etc)

Popular Workflow

