Long COVID for MDs

The microbiome aspect

Applies to ME/CFS

Agenda

Introduction To Microbiome Challenges

Where is the Associations?

One Artificial Intelligence Approach

Visual Examples

Final tips & takeaways

Microbiome Challenges

- There is a *major disconnect* between expectations and reality for most MDs
- The belief that tests are truly accurate is false.
  - They are true given some AI used to read the data.
  - A different AI (pipeline) gives different answers!
- I took one digital file (FastQ) and sent it to CosmosId, uBiome, Ombre, Biomesight, sequentia biotech.
  - Bacteria identified ranged from 253 to 632
  - The same bacteria has huge differences in amount
- This issue is compound in every peer reviewed paper. The results will be different for different AI
- General Replication is simply not there

" There are currently 97 different ways to analyze the same raw data, and they will give you 97 different answers.

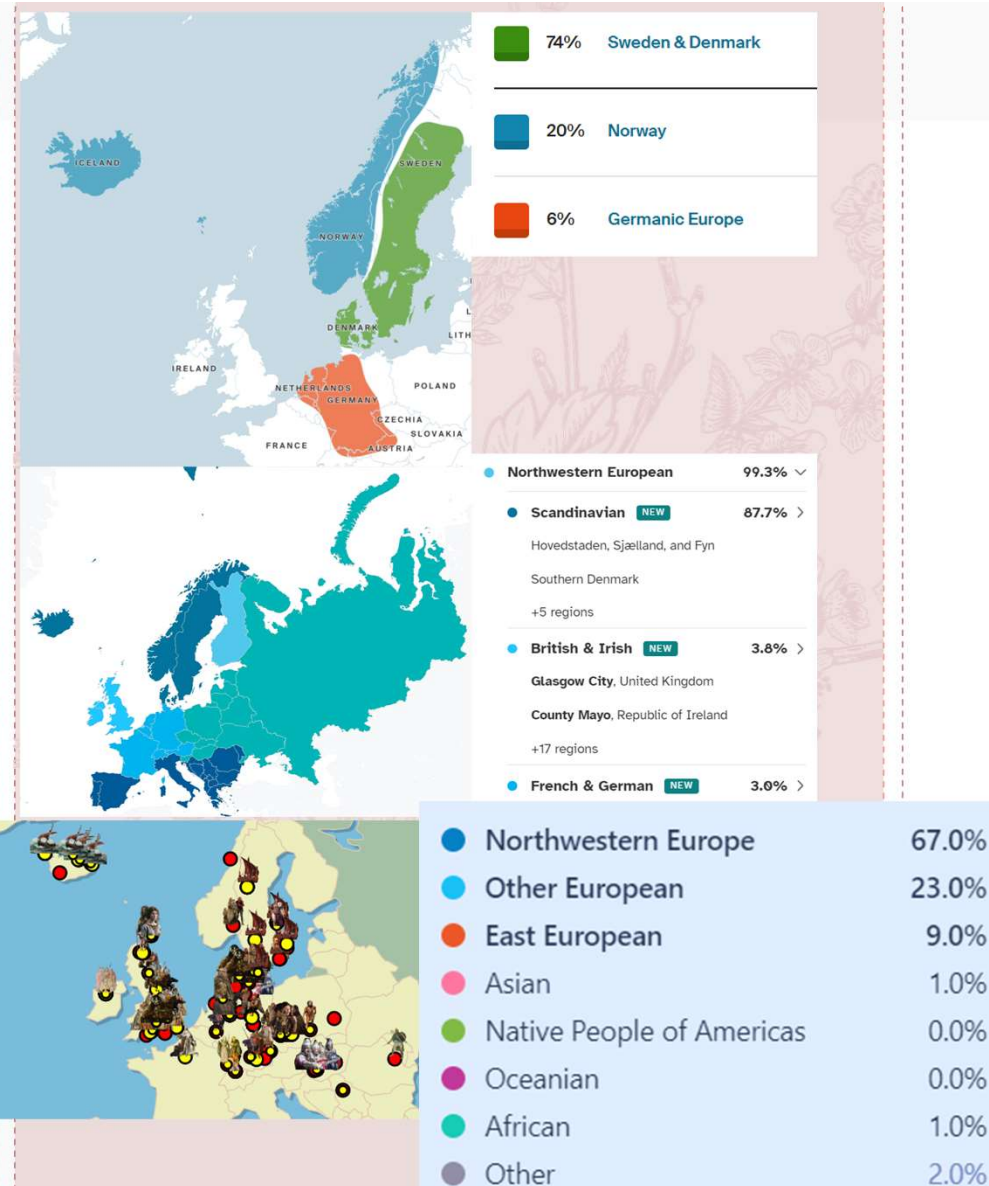— *Scott Jackson, leader, NIST Complex Microbial Systems Group*

Understanding the "AI"

Bacteria is like people. They transfer genes and RNA. Identifying is done by the amount of match against other known profiles. Like human DNA, there are *different collections* of reference profiles.

On the right are different attempts to identify my own DNA.

The data is the **same** for my DNA and for FASTQ microbiome files.

74%	Sweden & Denmark
20%	Norway
6%	Germanic Europe

Northwestern European		99.3%
Scandinavian NEW		87.7%
Hovedstaden, Sjælland, and Fyn		
Southern Denmark		
+5 regions		
British & Irish NEW		3.8%
Glasgow City, United Kingdom		
County Mayo, Republic of Ireland		
+17 regions		
French & German NEW		3.0%

Northwestern Europe		67.0%
Other European		23.0%
East European		9.0%
Asian		1.0%
Native People of Americas		0.0%
Oceanian		0.0%
African		1.0%
Other		2.0%

Patterns for Long COVID

Microbiome Prescription has over 5000+ samples donated with many having symptoms and diagnosis annotated. This data is available at https://citizenscience.microbiomeprescription.com/

There is **no consistent patterns** across labs by bacteria with statistical significance (N: 1300),

Lab	Bacteria	Rank	Direc
Thryve	Segatella maculosa	species	High
BiomeSight	Sharpea azabuensis	species	High
BiomeSight	Viridibacillus neidei	species	High
Thryve	Thermodesulfobacteriota	phylum	High
uBiome	Burkholderiales	order	Low
Thryve	Desulfovibrionales	order	High
BiomeSight	Acholeplasma	genus	High
BiomeSight	Akkermansia	genus	High
Thryve	Butyricimonas	genus	High
Thryve	Desulfovibrio	genus	High

Looking Deeper using KEGG

If we compute the **KEGG: Kyoto Encyclopedia of Genes and Genomes** compounds being produced from each species, we see consistent patterns of low or high compound production from the microbiome. (https://www.kegg.jp/). Different bacteria but matching production!

Lab	CP ID	Compound Name		Direction
Thryve	C01143	(R)-5-Diphosphomevalonate		Low
BiomeSight	C01143	(R)-5-Diphosphomevalonate		Low
BiomeSight	C01107	(R)-5-Phosphomevalonate		Low
Thryve	C01107	(R)-5-Phosphomevalonate		Low
Thryve	C00810	(R)-Acetoin		Low
uBiome	C00810	(R)-Acetoin		Low
BiomeSight	C00810	(R)-Acetoin		Low
BiomeSight	C00450	(S)-2,3,4,5-Tetrahydropyridine-2-carboxylate		Low
BiomeSight	C15565	(S)-2-Hydroxyacid		Low
BiomeSight	C00356	(S)-3-Hydroxy-3-methylglutaryl-CoA		High

Level #1 Probiotics producing missing compounds

The numbers below are how many low compounds are provided. *Some probiotics will consume compounds (making things worse)!*

Probiotic	⇕	Weight
Bifidobacterium breve		6
Bifidobacterium pseudocatenulatum		2
Bifidobacterium longum subsp. infan		2
Bifidobacterium bifidum		1

Probiotic	⇕	Weight
Bacillus pumilus		-54
Clostridium beijerinckii		-55
Bacillus subtilis		-57
Akkermansia muciniphila		-112

Level #2 Using Probabilities and Fuzzy Logic

Above we have shown two stumbling blocks to a mechanical solution:

- We do not really know what bacteria are there.

- We do not really know if some substance modifies the bacteria reported in studies.

- We see *extremely strong associations to compound production* – but 99% of these compounds are not available as supplements.

Borrowing concepts from Operations Research (mathematics applied to real world problems) we have built an expert system. Some concepts

- The more studies that report a shift, the better the odds

- The closer the match of bacteria to the study, the better the odds. If a family is reported, then genus will have less odds

- Substances impact are evaluated *across all bacteria* of concern – a holistic and not naïve approach

Problem of Identifying Bacteria of Concern

The KEGG Compound approach used *all bacteria* in the sample. It may be applied to any detailed microbiome sample that reports data at the species level where you have a reference set for.

Tracing from a KEGG Compound back to the bacteria that may produce it often becomes one compound to 3000 bacteria problem for each compound.

Using bacteria identified in published studies have unreliable bacteria identification because of different studies use different AI, Worse, the lab you use may not be using the same AI.

```
▼ Bacteria (8736)
  ► Enterobacterales (639)
  ▼ Other Gammaproteobacteria (1302)
    ► Haemophilus (23)
    ► Glaesserella (5)
    ► Histophilus (2)
    ► Pasteurella (9)
    ► Basfia (2)
    ► Mannheimia (18)
    ► Actinobacillus (14)
    ► Aggregatibacter (10)
    ► Gallibacterium (2)
    ► Bibersteinia (4)
    ► Avibacterium (2)
    ► Rodentibacter (3)
    ► Otariodibacter (1)
    ► Frederiksenia (1)
    ► Bisgaardia (1)
    ► Mergibacter (1)
    ► unclassified Pasteurellaceae (1)
    ► Xylella (9)
```

Controlling the Fuzz

You want your bacteria naming AI to match the AI used to determine patterns. MP has patterns for:

- Biomesight (largest number of samples)

- Ombre

- uBiome (no longer in business)

Bacteria as Reasonable Proxy for Compounds

- Beyond identifying the bacteria (according to the AI), we can weigh these bacteria based on their impact on the compounds of concern – giving better values

- Compounds of concern can be computed on a person-by-person basis and applied

- We can holistically address multiple symptoms at once

Official Diagnosis: Chronic Fatigue Syndrome (CFS/ME) [1299]
Neurological: Difficulty reading [596]
Neurological: Disorientation [190]
Neurological: Dysautonomia [529]
Neurological: emotional overload [786]
Neurological: Executive Decision Making (Difficulty making) [556]
Neurological: fasciculations [65]
Neurological: High degree of Empathy before onset [629]
Neurological: Impairment of concentration [1363]
Neurological: Joint hypermobility [805]
Neurological: Myoclonic jerks or seizures [207]
Neurological: Neuropathy [966]
Neurological: Seasonal Affective Disorder (SAD) [192]
Neurological: Short-term memory issues [551]
Neurological: Slowed speech [97]
Neurological: Word-finding problems [484]
Official Diagnosis: Allergic Rhinitis (Hay Fever) [724]
Official Diagnosis: Attention deficit hyperactivity disorder (ADHD) [286]
Official Diagnosis: Autism [870]
Official Diagnosis: Autoimmune Disease [776]
Official Diagnosis: Chronic Fatigue Syndrome (CFS/ME) [1299]

Demo

- We do not go with ONE way
  - Use multiple algorithms and weights
  - Combined them using the Monte Carlo Model
  - Items agrees most often are probably the most likely to have effect
- Experience to date has been very good
- Algorithm does produce strong *cross-validation*.

Thank You

The journey is just starting

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