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ADDENDUM

Colonic biogeography in health and ulcerative colitis

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ABSTRACT

The relevance of biogeography to the distal gut microbiota has been investigated in both health and inflammatory bowel disease (IBD), however multiple factors, including sample type and methodology, microbiota characterization and interpersonal variability make the construction of a core model of colonic biogeography challenging. In addition, how phylogenetic classification relates to immunogenicity and whether consistent alterations in the microbiota are associated with ulcerative colitis (UC) remain open questions. This addendum seeks to review the human colonic microbiota in health and UC as currently understood, in the broader context of the human microbiome.

Spatial ecology, or biogeography, is the study of how organisms are dispersed throughout their native environment and how these patterns change with time.[1] While the field developed initially in relation to macroecology, many of the concepts have been fruitfully applied to microbial communities. With respect to the microbiota, biogeography seeks to establish the community structure of the microbiota across different body habitats and niches within those habitats, elucidating the environmental and biological selection pressures responsible for shaping these communities, quantifying their stability over time and across diverse ethnogeographic populations and ultimately, discovering how disturbances in the overall community structure are associated with disease states. These goals are exemplified by the results of the human microbiome project (HMP), which provides a fundamental reference point for any study of organ-specific biogeography.[2,3]

The microbiota across body habitats

The HMP, which cataloged the healthy microbiomes of 242 individuals across different body sites at differ-

ent time points, identified clear clustering based on biogeographical location, with specific skin, oral and vaginal sites as well as stool samples representing the gut microbiota. For stool samples, the degree of α diversity, or within-subject diversity, was high, while the degree of β-diversity, or between-subject diversity, was low with respect to other habitats in the human body.

When investigating the effects of location on the microbiota, it is important to consider how degrees of variation between the spatial sites relate to inter-personal variability. For instance in the HMP, the major variation in community structure was accounted for by differences between body habitats, making it easy to observe separation between the communities of different body sites by aggregating individuals together, findings evident also in another study of the microbiome across body habitats.[4] For example, a person's oral microbiota would usually be more similar to another person's oral microbiota than their own skin or stool microbiota. In these examples, differences occur due to explicit driving mechanisms, such as oxygen and environmental factors influencing skin communities and fermentation influencing anaerobic

communities in the distal gut. This combination of environmental and biological pressure accounts for the dominant community structure and function demonstrated between the habitats selected in this pivotal study.

However in studies involving changes in the microbiota within single organs or habitats, the variation between individuals can be significantly greater than the variation between different regions within the habitat (Fig. 1). This poses a significant challenge to both characterization and interpretation of different samples from throughout the same body habitat or organ.

While it is well established that single body habitats can have "sub-habitats," such as in the oral cavity, with clear niches in very closely apposed areas, such as the hard palate, the sub-and supra-gingival plaque and saliva[5] and similarly clear differences along the gastrointestinal tract as a whole,[6] the colon appears to be more homogenous. In assessing the degree of interpersonal variability, Zhou and colleagues applied a model, the Dirichlet-Multinomial Distribution, which was able to characterize variation between individuals by using an over-dispersion parameter to quantify variation of the whole community between individuals for each body habitat. Using this method, stool and oral samples had the lowest interpersonal variability of the body habitats studied in terms of community structure.[3,7] Despite this, variation between individuals is still significantly greater than the variation between different colonic regions.[8-10] Notably, this variation was preserved in UC (Fig. 2A). Keeping in mind another major finding of the HMP, that large interpersonal variation at a phylogenetic level was countered by marked similarity at a genetic or microbiome level, the relevance of small differences in biogeography within an organ must be considered in a larger perspective.

Spatial structure of the colonic microbiota

Biogeography within the human colon can refer to radial (or cross-sectional) and longitudinal structure (Fig. 2B). Additionally, the effect of inflammation on community structure and

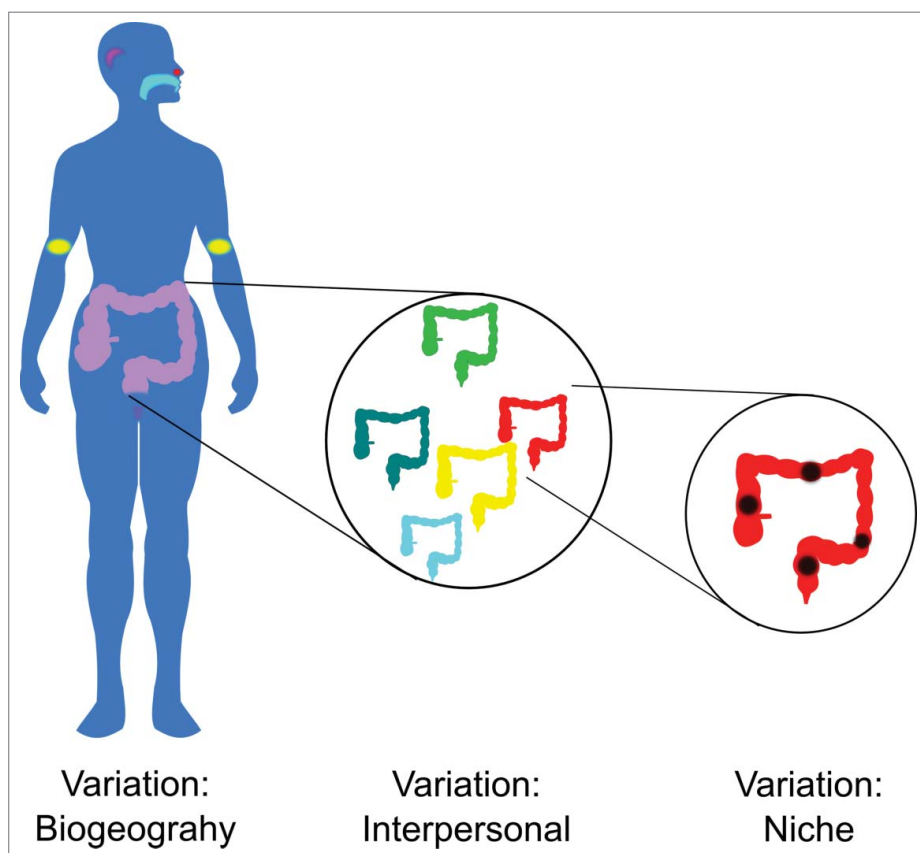| Variation: Biogeograhy | Variation: Interpersonal | Variation: Niche |

Figure 1. Clear distinctions exist between the microbial communities in different body habitats. However, within the same body habitat, variation between individuals can be much larger than variations between different niches or locations within that habitat.
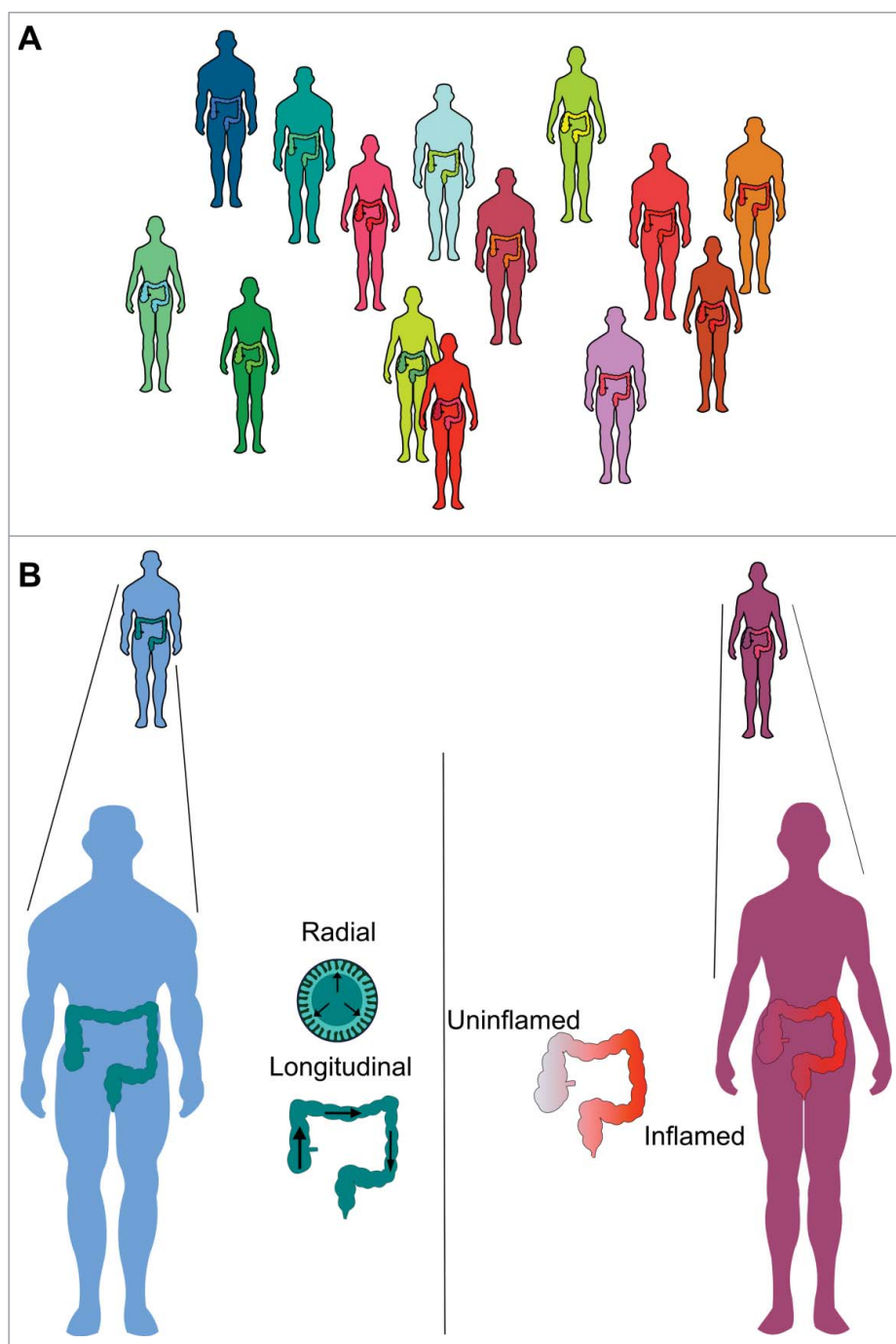
Figure 2. Interpersonal variation is a characteristic feature in health and UC (A), with significant overlap between health (green-blue figures) and UC (yellow-red figures). Within healthy individuals, bigeography may relate to radial or longitdunal distributions, while local inflammation is an additional feature that could influence the biogeography in UC (B).

biogeography will be considered here. Various potential mechanisms have been put forward to explain purported cross-sectional gradients, including gradients in radial oxygen tension, alterations in pH at the luminal interface or adaptation of butyrate producers to metabolizing liberated glycans from syntrophic mucin degrading organisms, while proposed mechanisms for longitudinal variation include changes in pH, substrate availability and host expression of toll-like receptors.[11-13]

Radial structure within the colon

Radial structure involves comparing paired luminal and mucosal communities in a region-specific manner. The most abundant luminal bacteria relative

to the mucous gel in control volunteers in our study were *Bacteroidaceae, Porphyromonadaceae, Alcaligenaceae, Lactobacillaceae* and *Rikenellaceae*, while the most differentially abundant mucous gel bacteria relative to the lumen were *Coriobacteriaceae, Planctomycetaceae, Lachnospiraceae, Family XIII Incertae Sedis* and *Ruminococcaceae*. A previous study in mice, which also used laser capture microdissection and 454 pyrosequencing to interrogate the cross-sectional colonic axis, identified gradients similar to those in our study, with increased abundance of *Lachnospiraceae* and *Ruminococcaceae* in the inter-fold region of the mucosa, dominated by secreted mucus gel and increased proportion of *Bacteroidaceae, Porphyromonodaceae, Prevotellaceae, Rikenellaceae* and *Lactobacillaceae* in the digesta, corresponding to the luminal compartment.[14,15] The similarities, both in terms of methodology and findings, suggest overlap in terms of mice and humans and support the hypothesis of the authors in terms of bacterial groups that have evolved to inhabit the mucus gel niche.[15,16]

The finding of an increase in mucosal associated *Lachnospiraceae* and *Ruminococcaceae* has been suggested in another murine models as well,[17] while a recent murine study demonstrated increases in Bacteroidetes in the lumen with corresponding increases in clostridia and *Lactobacillus* in the mucus of the distal colon of mice.[18] An *in vitro* model of lumen and mucosal environments (L-SHIME and M-SHIME, respectively) also found differential enrichment of Firmicutes in the mucosal relative to the luminal model when inoculated with the same donor fecal samples.[12] These results support some studies in humans,[19,20] particularly the pyrosequencing studies of Willing *et al* of healthy twins and twins with inflammatory bowel disease and the pyrosequencing study of Hong *et al* in health.

The findings of differential enrichment of the mucosal environment are however not universal as some studies have demonstrated fecal enrichment of Firmicutes when compared to the mucosal niche microbiota identified in colonic biopsies.[21-23] Particularly, bowel preparation may have an effect on biogeography and work using mucosal biopsies from unprepared colons demonstrated increased Firmicutes and Proteobacteria in stool samples.[23] It is worth noting that bowel preparation has been shown to reduce stool quantities of Clostridium cluster XIVa, while increasing both Proteobacteria and Clostridium cluster IV.[24] While this has

not been compared across the luminal and mucosal niches, it is possible that colonic lavage has a greater effect on the luminal and in turn faecal communities. The results also stand in contrast to those of a recently published study examining the biogeography of the Macaque intestine, demonstrating a reversal of this pattern, with a high abundance of Firmicutes in the lumen and an increased abundance of facultative anaerobes and gram negative bacteria associated with the mucosa.[25] Notable differences between this study and human studies include the predominance of mucosal ε Proteobacteria families (largely *Helicobacter* but also *Treponema*), the lack of a gradient in *Bacteroidaceae* and the prominence of sampling location over interpersonal variation as the main variable.

Longitudinal gradients

Some studies have demonstrated longitudinal structure, including a tendency for increased γ proteobacteria[9] and *Lactobacillus* levels in distal colon[26] and increased proportions of *Streptococcus* and *Enterococcus* in the proximal colon[9] and it has been suggested that bacterial dispersion in the colon may occur in a non-linear fashion.[27] In the murine colon, regional changes in the mucosa-associated microbiota have been linked to host expression of toll-like receptors.[13] Other studies however have not demonstrated conserved longitudinal gradients in health or IBD[20,21,28-31] and our study did not demonstrate any consistent alterations in the community structure of the colonic microbiota across the long axis of the colon or any regional associations with single organisms.

Community alterations associated with UC

Whether inflammation causes alterations in the microbiota, or the microbiota instigates inflammation in IBD, the 2 processes become inextricably linked.[32] As there is no evidence of a "core microbiota" in health,[2] there is consequently no core disturbance characteristic of inflammation in IBD. This is particularly true in UC. Certain bacterial families have, however, been more strongly implicated and a consensus regarding key members that are enriched or depleted is beginning to emerge.[33-35]

Bacteria that have been associated with an increase in UC include *Fusobacterium* spp,[35,36] *Peptostreptococcus*,[8,37] *Desulfovibrio* spp[38] and the *Enterobacteriaceae*[8,35]

Ruminococcus gnavus and *R. torques*, reclassified as *Lachnospiraceae*, have been shown to be increased in one study[39] while another study has demonstrated reductions in multiple members of *Lachnospiraceae*.[40] A notable reduction in the butyrate-producing bacteria *Faecalibacterium prausnitzii* and *Roseburia hominis* have been both associated with UC and correlated with the severity of inflammation,[34] while overlapping members of *Ruminococcaceae* and *Lachnospiraceae*, including *Clostridium leptum, Ruminococcus bromii, Roseburia* and *Eubacterium rectale* were reduced in proportional abundance in another study.[35] In one of the most important recent papers published, only 9 of 350 clades were altered in UC, with only *Odoribacter* being reduced in patients with pancolitis. Other observered alterations in UC included increases in *Clostridiaceae* and reductions in *Roseburia, Veillonellaceae* and *Leuconostocaceae*, although these findings were far less dramatic than those demonstrated in ileal Crohn's disease.[33] Finally, a number of studies have noted a reduction in *Akkermensia* spp, a key mucin degrading species in health.[8,35,39] Other studies have demonstrated a reduction in Firmicutes:Bacteroidetes ratio.[10] It is important to note however that based on a recent population-level microbiome paper, powering studies to detect alterations due to clinical conditions may require significantly larger cohort sizes than have previously been employed.[41]

Differences associated with local inflammation

A natural extension of sampling the mucosa in IBD is to compare inflamed and uninflamed regions to determine if specific associations are evident (Fig. 2B). A number of studies using different techniques, including culture-based methods,[42] TTGE,[43] clone libraries[44,45] and pyrosequencing[8,19] have not demonstrated significant differences between inflamed and uninflamed tissue within individuals with IBD.[46,47] In contrast, other studies using T-RFLP and ARISA[48] and clone-library analysis[10] demonstrated differences, although this could not be attributed to specific bacterial groups, while a study using FISH demonstrated increased clostridia and *E. coli* in inflamed vs. uninflamed tissue in UC.[49] Morgan *et al* looked at anatomical regions rather than inflamed vs. uninflamed regions and noted a reduction in abundance in *Roseburia, Allistipes* and *Ruminococcaceae* in the terminal ileum and right colon with an increase in

Enterobacteriaceae and *Fusobacteria* in these regions.[33] While this question remains to be fully answered, no clear correlations between specific bacterial groups and inflamed tissue have thus far been demonstrated.

Taking a radically different approach, colitogenic bacterial species in an inflammasome murine model of colitis were identified by the avidity of IgA binding in a recent study.[50] Subsequently, the authors isolated bacteria from human subjects with IBD and created inocula for germ free mice with high and low IgA binding bacterial isolates, demonstrating a dramatic increase in the severity of dextran sodium sulfate colitis in mice inoculated with the colitogenic bacteria. Another remarkable finding of this study was that 2 bacterial isolates of the same species (*B. fragilis*) had dramatically different pro-inflammatory effects, based on their degree of IgA binding, suggesting that even at the species level, taxonomic classification cannot comprehensively identify the key bacterial mediators in inflammation. While IgA binding identified the bacteria already associated with murine colitis, the message applied to human IBD is clear: alternative approaches will be required to identify colitogenic members of the microbiota based on their functional activity, rather than their taxonomy.

Biopsy vs. stool

Most studies have demonstrated differences between the mucosal and stool microbiota.[21,28] Yasuda *et al* found that stool was highly representative of the rhesus macaque luminal and mucosal communities in the large intestine, being within 2 standard deviation for 95% of detected OTUs, although this dropped to 50% and 66% for small intestinal luminal and mucosal samples, respectively.[25] However in the patients with new-onset Crohn's disease, the associated dysbiosis was only weakly detected in stool samples when compared to tissue samples.[22] Morgan *et al* demonstrated that the most important covariates in determining community structure was whether samples were faecal or mucosal, as well as patient age, drug therapy and disease phenotype.[33]

Summary

What can be said about the findings to date and where do our results fit in with these? Inter-personal variation remains the dominant variable in studies of the colonic

microbiota. Our results suggest that this variation is retained even in advanced UC. While no single group or species has consistently been associated with UC, reductions in members of butyrate-producing clostridial species, as well as reductions in *Akkermensiaceae* with an increase in *Enterobacteriaceae* seem to be frequently described. However in one of the most comprehensive studies to date, none of these groups were altered in patients with the most severe form colonic IBD, pancolitis.[33] It is certainly possible that the enormous diversity in the fecal microbiota hides to some extent the signal of disease-associated microbes, something that has been established in pediatric Crohn's disease, making mucosal sampling an important step in focusing sequencing efforts on the mucosal niche[22] and potentially reducing the requirement for large cohort sizes implied by a recent population study based on fecal sampling.[41]

Specific to our study, we found no evidence of longitudinal changes or changes associated with local inflammation, in keeping with the larger body of evidence in health and IBD. Our results do point to a radial structure, suggesting a degree of segregation between the luminal and mucosal niches. Although we have emphasized differences between health and UC, perhaps more remarkable are the similarities, in terms of the retained inter-personal variation, the lack of regional variation associated with local inflammation and the preservation of radial structure despite profound disruption to the mucosal barrier. Ultimately, constructing a full picture of the colonic microbiota will require the integration of spatial, temporal and functional components, their interaction with the mucosa and the host immune system. While consistent community and biogeographic associations with inflammation have been lacking, targeted mucosal sampling further refined by metagenomic analysis and immunogenicity profiling will advance our understanding of the inflammatory landscape of IBD.

Abbreviations

ARISA　Automated Ribosomal Intergenic Spacer Analysis
FISH　Fluorescent In-Situ Hybridization
HMP　Human Microbiome Project
IBD　Inflammatory Bowel Disease
OTU　Operational Taxonomic Unit
T-RFLP　Terminal Restriction Fragment Length Polymorphism analysis
TTGE　Temporal Temperature Gradient gel Electrophoresis
UC　Ulcerative Colitis

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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