In this issue of the Journal, Ahn et al. (1) report on associations between fecal microbiota composition and risk of colorectal cancer (CRC). Although gut microbiota contributes to the maintenance of human health, perturbations in normal microbiota composition have been correlated with various disease states (2). In this context, the work by Ahn et al. is exciting because it may provide insights into future ways to reduce the risk of CRC. At the same time, given the complexity of these biologic systems, caution must be exercised (and a lot more research done) before proceeding too far in promoting changes in microbiota as a prevention strategy for CRC. CRC occurrence is known to be influenced by host genetics, as well as factors such as obesity, nutrition, and exercise (3–5); given that these factors also influence microbiota (2), separation of cause and effect among all of these factors may become quite difficult. Coordinated international efforts have generated a wealth of new data on the diversity of human host–associated microbiota at various anatomic sites. These studies confirmed, even in healthy individuals, a large degree of intra- and interindividual variation in microbiota composition and, albeit to a lesser degree, microbial activities. Although recent work based on analyses of both 16S rRNA and shotgun metagenomic datasets suggests the existence of three distinct enterotypes (6), other studies suggest more of a continuous spectrum of microbiota composition (7,8). The wide range in microbiota composition and associated metabolic functions among individuals provides a rational for proposing the existence of associations with disease processes, including colorectal carcinogenesis.

In the study reported by Ahn et al. (1), a 16S rRNA sequencing-based microbiota analysis was performed on rehydrated lyophilized fecal samples from 47 CRC case patients and 94 matched control subjects that had been collected in a case–control study reported on in 1989 (9). The investigators used the wealth of available information on subject characteristics to control for potential confounders in their microbiota analysis. Although the efforts to control for confounders represent an advance over previous microbiota studies, some of the conceptual issues involved will still need to be clarified. For instance, body mass index (BMI), a correlate for obesity, was used for frequency matching and also added in the multivariable regression models. However, BMI clearly appears to affect microbiota composition, and it is not only associated with CRC risk but some have argued that microbiota composition also is an important modifier of BMI (10,11). Thus, it might be more appropriate to stratify by BMI rather than adjusting for it. The same holds true for various dietary factors, such as dietary fiber. Dietary fiber can shape microbiota, but dietary fiber uptake/metabolism is also affected by the activities of the resident gut microbiota.

Although some microbial DNA degradation likely occurred in the lyophilized samples over time, the differences in overall microbial diversity and prevalence of multiple bacterial groups that were observed suggest that it is indeed feasible to obtain useful microbiota data after 25 years of storage. This is an important finding, which unfortunately could only be indirectly confirmed by suggestive similarities in microbiota composition in samples from healthy control subjects from the quarter-century-old case–control study with those in healthy individuals from a more recent study. Using this observation as a measure of sample integrity requires the assumption that microbiota composition remained fairly constant in the healthy population over time. There remains ambiguity regarding the appropriate sample collection for gut microbiota analyses. Although fecal microbiota reflects changes in both composition and activities during gut passage, biopsy samples are usually obtained only after cleansing of the gut, which removes the outer mucus layer that represents normal attachment sites. Few studies have attempted to validate what sample best reflects physiologically relevant microbiota composition. Nevertheless, the overall finding that microbiota in the CRC case patients differed from that in control subjects confirms many other similar observations from cell culture, animal model, or human observational studies that have suggested a contribution of microbiota to colorectal carcinogenesis.

Activities of the gut microbiota that have been proposed to be associated with CRC risk include bile acid metabolism, sulfate reduction, and superoxide production (12–14). Previous studies have suggested positive associations with CRC risk for specific Escherichia coli pathotypes, enterotoxigenic Bacteroides fragilis, and Streptococcus gallolyticus (bosis) and indicated protection by Bifidobacteria and butyrate-producing bacteria (15–19). Although bacterial strains (E. coli types and enterotoxigenic B. fragilis) cannot be analyzed with a 16S rRNA limited approach, the Ahn et al. report (1) does not provide additional evidence for any of the above-mentioned microbes or their activities.

Increased diversity in the Clostridium coccoides and Clostridium leptum groups has previously been reported from CRC patients (20), in contrast with the Ahn et al. report (1), which suggests lower abundance of Clostridia. Similarly, a previous finding of increased Coriobacteria and decreased Enterobacteria in CRC case patients (21) was not observed in Ahn et al. study (1). An increased abundance in Bacteroides/Prevotella (22) was only partially confirmed in this study (1). The many inconsistencies suggest that a lot of uncertainty that needs to be addressed in sufficiently powered studies in diverse human populations, preferably in those at increased CRC risk (such as black populations), remains. A microbiota feature that appears fairly consistently associated with gut inflammation and CRC is an increase in Fusobacteria, especially Fusobacterium nucleatum (23–25). The Ahn et al. study (1) confirms a statistically significantly higher Fusobacterium carriage rate in CRC case patients.
The ultimate goals of research correlating microbiota with disease states include 1) defining microbiota contributions to disease etiology (causality) and 2) developing prevention approaches directed toward screening and/or modifications in microbiota composition. How much have we progressed toward these goals and what are the next steps? It was perhaps to be expected that diseases such as CRC and inflammatory bowel disease, which change the gut environment through an influx of immune cells, compromise of epithelial barrier function, bleeding into the gut lumen, changes in transit time and nutrient uptake, and so on, strongly affect gut microbiota. Even consistent prevalence observations, such as the increased carriage of Fusobacteria in inflammatory bowel disease and CRC patients, don’t allow for an evaluation of temporality, a crucial requirement for establishing causality. Additionally, animal models are not likely to confirm strain specific associations observed in humans; few established human diarrhea pathogens cause any symptoms in murine models. Undoubtedly, mechanistic studies, such as those already reported for enterotoxigenic B. fragilis and F. nucleatum, can help to confirm carcinogenic potential. However, as Ahn et al. suggest, “[l]arge prospective studies are warranted,” not necessarily “to confirm ... findings” but to elucidate microbial agents that contribute to early steps in colorectal carcinogenesis. Our own microbiota studies in preterm infants already have shown the promise of a prospective study design in identifying novel etiologic agents [26,27] and manuscript under review]. For prospective CRC studies, a biomarker more strongly correlated with the disease than the colonic polyp, possibly one based on genetic or epigenetic changes in early preneoplastic lesions, would be crucial. Performing large cohort studies is expensive and requires long follow-up time for a sufficient number of cases to accumulate. Still, prospective microbiota studies designed to investigate temporal associations with a plethora of other disease outcomes are our best hope for developing the thorough understanding of the complexities involved that is required for harnessing the promise of improving health outcomes by modifying gut microbiota composition.

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