It is becoming increasingly evident that the human gut microbiome is associated with the development of certain diseases. We have recently reviewed the link between specific bacterial taxa and colorectal cancer (CRC) (1). Numerous studies have compared the gut microbial composition of CRC patients with healthy populations, and irrespective of biogeography, the phylum Fusobacteria, the associated genus *Fusobacterium*, and more specifically, the species *Fusobacterium nucleatum* are very frequently found to be enriched in CRC patients, regardless of sample type or detection method (2-7). The role of *F. nucleatum* in CRC is largely attributed to the secretion of virulence factors FadA and Fap2 (8). Cell models have shown the virulence potential of *F. nucleatum* is mediated through FadA and Fap2 by facilitating adherence and invasion, inducing inflammatory and oncogenic pathways, and suppressing immune responses (9,10). To date, *F. nucleatum* is the only *Fusobacterium* taxon strongly associated with CRC, however, other *Fusobacterium* species such as *Fusobacterium periodonticum*, *Fusobacterium varium*, *Fusobacterium ulcerans*, *Fusobacterium necrophorum*, and *Fusobacterium gondii* have been detected in CRC patients (11). Additionally, non-*nucleatum* *Fusobacterium* have been associated with other diseases, for example, *F. varium* is associated with ulcerative colitis (12), *F. ulcerans* with tropical ulcers (13) and *F. necrophorum* with Lemierre’s syndrome (14). However, the role of non-*nucleatum* *Fusobacterium* species in CRC, if any, is yet to be elucidated.

In the study by Yeoh *et al.* (15), a shotgun metagenomics approach was used to compare the gut microbial composition of CRC and non-CRC patients (642 CRC vs. 2515 non-CRC gut metagenomes) comprising 16 populations from various biogeographies, including southern Chinese and Western cohorts, with a focus on the prevalence and relative abundances of non-*nucleatum* *Fusobacterium* sp. and the distribution of virulence genes associated with CRC. From an evolutionary and lineage perspective, this approach makes sense: the genus *Fusobacterium* contains non-*nucleatum* species implicated in several pathologies (12-14,16) and pathogenicity may be associated with specific genes or gene repertoires, which through genetic exchange mechanisms or speciation, can be acquired by several species of the same genus (17). Furthermore, it has been suggested based on in silico analyses that horizontal gene transfer may play a role in the evolution of *Fusobacterium* virulence (18). However, while the identification of virulence-related genes using computational tools is often used to infer potential pathogenicity, the establishment of a link with a virulent phenotype does not always follow (19).

There are limited studies on the distribution of *Fusobacterium* species in the gut microbiota of healthy populations. Data from Yeoh *et al.* (15) indicated that several non-*nucleatum* *Fusobacterium* species are more prevalent (39% vs. 7%) and relatively abundant (0.4% vs. 0.04%) in southern Chinese metagenomes compared to Western populations.
and rural cohorts, irrespective of CRC status. Overall, the microbial composition in stool metagenomes significantly differed between non-CRC populations. Interestingly, they observed differences in the Firmicutes-to-Bacteroidetes ratio between several populations, with Chinese and US populations having higher levels of Bacteroidetes compared with Firmicutes (63% vs. 30%), whereas European populations had higher levels of Firmicutes compared with Bacteroidetes (55% vs. 29%). Although, significant differences in the Firmicutes-to-Bacteroidetes ratio are consistently observed between diseased and healthy populations, including CRC (20), whether or not this balance contributes to disease, is yet to be fully determined. Additionally, Yeoh et al. (15) reveal differences between non-CRC cohorts, with Fusobacterium more relatively abundant in Chinese and Spanish populations (0.47% vs. 0.01%). Taking this further, Fusobacterium mortisferum, F. nucleatum, F. ulcerans and F. varium were significantly more abundant in the non-CRC metagenomes from Chinese individuals compared to their Western counterparts. While the authors acknowledge biogeography as a possible driving factor for the observed differences in taxonomic profiles between non-CRC cohorts, it is highly likely that diet and other lifestyle factors associated with the different cultures play an important role (21). The authors also compared the relative abundances of Fusobacterium species within CRC and non-CRC metagenomes. They detected F. nucleatum in all CRC metagenomes, thereby providing further evidence of an association between F. nucleatum and CRC. Interestingly, Hong Kong and French populations had higher average relative abundances of Fusobacterium taxa than those from the USA, Germany, and Austria. It was also apparent that relative abundances and the prevalence of F. nucleatum, F. gonidiaformans were higher in six CRC cohorts and F. periodonticum and F. varium were increased in five of six CRC cohorts compared with non-CRC cohorts. However, only F. nucleatum and F. varium were significantly associated with CRC. This finding suggests the existence of a genus level CRC complex, with multiple Fusobacterium species possibly implicated in the disease, rather than a F. nucleatum complex.

Notably, the last update on the taxonomic profile of Fusobacterium was in 2002, whereby Citron described 13 characterised species (22). When one considers that there are 366 Fusobacterium genome assemblies available in the Refseq NCBI database and 28 Fusobacterium species are listed by the List of Prokaryotic names with Standing in Nomenclature (LPSN) at the time of writing this commentary, an updated taxonomic profile based on a comparative genomic analysis of the Fusobacterium genus is warranted. Yeoh and colleagues (15) expanded our knowledge on the evolutionary relationship of Fusobacterium genomes by including metagenome-assembled genomes (MAGs) from Chinese populations with Western populations and 152 fusobacterial reference genomes downloaded from the Refseq database, in a phylogenetic analysis. Dereplication of 663 genomes resulted in a phylogenetic tree of 218 unique fusobacterial genomes, comprising four monophyletic lineages. Interestingly, the inclusion of MAGs from Chinese cohorts increased the phylogenetic diversity by 14.3% (characterised by branch length), indicating novel diversity within the Fusobacterium genus, and more specifically, in the gut microbiota of Chinese individuals. Furthermore, they identified potential new species based on pairwise average nucleotide identity (ANI) comparisons.

The species F. nucleatum is now considered a bacterial pathogen implicated in CRC, and, as noted above, one proposed mechanism responsible for carcinogenicity is attributed to the virulence proteins FadA (9) and Fap2 (23). Therefore, it is plausible that related taxa harbouring the same genetic determinants encoding these proteins may also contribute to the development of CRC. Yeoh et al. (15) screened fusobacterial MAGs for CRC-associated features and FadA and Fap2 homologues using ≥30% sequence identity and ≥70% alignment length to reference genes as thresholds for homology. They identified 999 FadA homologues in 311 genomes and 754 putative Fap2 homologues in 288 genomes, which included F. necrophorum, F. varium, and F. ulcerans. The identification of FadA homologues in multiple Fusobacterium lineages largely coincided with Fap2 homologues, however, FadA was identified in the absence of Fap2 and vice versa, which may also be evidence of genetic exchange events such as recombination. The authors also identify genes in multiple lineages associated with amino acid degradation and iron scavenging, as well as other genes which may be a driver of carcinogenesis. Taken together, the identification of CRC-related genes in multiple Fusobacterium lineages, especially of those encoding virulence factors implicated in the disease, gives weight to the suggestion that non-nucleatum species may contribute to CRC. The authors state that the prevalence and relative abundance of some species harbouring FadA and Fap2 homologues was increased in CRC populations compared to non-CRC cohorts, an observation that certainly requires further attention.
In summary, Yeoh et al. (15) have provided a comprehensive analysis on the relationship between CRC and *Fusobacterium* taxa, especially highlighting the potential involvement of non-*nucleatum* *Fusobacterium* in the disease. They have contributed to the knowledge of the *Fusobacterium*-CRC association and in doing so have also raised a number of interesting questions that highlight research gaps on the topic. These gaps are largely a consequence of the lack of data relating non-*nucleatum* *Fusobacterium* to disease, especially CRC. For instance, they highlight the presence of *Fusobacterium* taxa, including non-*nucleatum* species, in non-CRC southern Chinese populations that are not detected in other cohorts. A logical follow question is: are healthy populations with increased levels of *Fusobacterium* taxa at increased risk of CRC development? Furthermore, if multiple non-*nucleatum* *Fusobacterium* were enriched in CRC patients, are non-*nucleatum* strains prevalent in other CRC metagenomic datasets? Multiple *Fusobacterium* species were detected in individual CRC populations. Could the presence of multiple *Fusobacterium* species within the gut microbiota be a driver of CRC? Given that novel *Fusobacterium* taxa were identified when compared to reference *Fusobacterium* genomes, is it now time for a comprehensive update to the taxonomy of *Fusobacterium*? Genomic features and homologues of characterised virulence factors FadA and Fap2 were identified in multiple fusobacterial lineages implicating non-*nucleatum* *Fusobacterium* in CRC. Quantitative polymerase chain reaction (qPCR)-based detection of FadA and Fap2 in non-*nucleatum* *Fusobacterium* isolates from CRC patients would strengthen this association. And finally, previous studies on the mucosal (24) and tissue (25) microbiota of CRC patients have also revealed enrichment of *F. nucleatum* compared to non-diseased controls, would similar non-*nucleatum* *Fusobacterium* trends be observed? There is little doubt that further investigation into the role of non-*nucleatum* taxa harbouring FadA and Fap2 homologues in the development of CRC, and other diseases is needed.

Further to this, detailed knowledge of the association between human gut bacteria and the development and progression of CRC may be exploited in CRC prevention strategies, such as the use of antimicrobial-producing probiotics to target CRC-associated bacterial species, which may ultimately decrease the risk of CRC (1).

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**Footnote**

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**References**


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