The transition from a non-westernized to westernized gut microbiome in Indian-Immigrants and Indo-Canadians is associated with dietary acculturation

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Background: Indian immigration to westernized countries has surged in recent years, with this demographic facing a higher risk of Inflammatory Bowel Disease (IBD) after migration. Studying the gut microbiome is crucial to understanding the factors contributing to IBD, yet Indians remain underrepresented in microbiome research,

Objective: This study investigates the gut microbiome of Indians residing in India, Indian immigrants in Canada, and Canadians of Indian ancestry to understand the impact of westernization on their gut. Given the higher risk of IBD among Indo-Canadians, these results provide insight into how westernization of the gut microbiome may influence incidence of IBD.

Stool samples were collected from healthy Indians in India, Indian Immigrants in Canada, Indo-Canadians, plus Canadian-borne individuals with European descent and Euro-Immigrants as non-Indian/westernized controls (*N* = 174). 16S rRNA gene and shotgun sequencing compared microbial taxa and functional profiles across groups. Dietary and demographic data were collected to assess lifestyle patterns.

Results: Indians and Indo-Immigrants had distinct gut microbiotas from westernized groups, with high abundances of *Prevotella* spp. and a CAZyme profile reflecting their traditional high complex carbohydrate diet. The Indian microbiome also showed characteristics of high bacterial cell turnover, pathogenic potential, and stress tolerance, indicating their gut may be better primed to handle stressors on the gut. Indo-Canadians displayed a transitional microbiome towards westernization, which followed a pattern of increasing dietary acculturation.

Conclusions: The shift from a non-westernized to westernized microbiome in Indo-Canadians highlights the significant influence that westernization and dietary acculturation has on the gut microbiome. Given that 44% of the Canadian population is made up of first- and second-generation immigrants and westernized practices are being adopted worldwide, future research should investigate the health implications that such microbiome transitions have on immigrant populations and newly industrialized nations.

BACKGROUND

The growing immigrant population in North America is rapidly westernizing through the adoption of practices like reduced physical activity, changes in hygiene/medical practices, and acculturation of the westernized diet, commonly characterized by caloric dense, highly processed foods with low fiber intake. Amidst this backdrop are health disparities emerging in these populations. Furthermore, the global spread of industrialization raises concern for the increasing prevalence of modern diseases.[1] For instance, inflammatory bowel disease (IBD) is increasing in prevalence globally, and alarming evidence reveals a specifically increased risk in young Indian

immigrants and Indians born in Westernized countries.[2-4] A likely contributor to this elevated risk stems from changes in the gut microbiome as a response to living in an industrialized environment.

The industrialized microbiome has been previously characterized by the decline of VANISH (volatile and/or associated negatively with industrialized societies of humans) taxa, frequently found in populations adhering to traditional lifestyles, and the rise of BloSSUM (bloom or selected in societies of urbanization/modernization) taxa, which appear in modern, industrialized populations.[5,6] Furthermore, health consequences following the assimilation into Westernized culture have been previously documented. For example, non-industrialized Irish Travellers who adopted modern practices and Hmong and Karen migrants from Thailand who relocated to the United States (US) both experienced gut microbiome shifts that were associated with an increased risk for metabolic disorders.[7,8] Currently, there is only one study published on the Indian immigrant gut microbiome, highlighting the gap in non-western/non-European microbiome research. Indeed, over 71% of all microbiome research comes from Westernized cohorts, skewing our interpretation of a healthy human gut.[9] Here, we aimed to investigate the distinction between the Indian and Westernized gut microbiome and what changes occur in the gut when Indians migrate to Canada, alongside the adoption of the westernized diet.

METHODS

Study Design

A prospective case-control study was conducted including three Indian groups: (1) Indians residing in India ("Indians"), (2) Indians who migrated to Canada ("Indo-Immigrants") and (3) Canadians born in Canada from Indian ancestry ("Indo-Canadians"). Individuals born in Canada with European ancestry ("Euro-Canadian") were used as a control, and immigrants with European ancestry from a Westernized country ("Euro-Immigrants") as a westernized immigrant control.

Healthy participants aged 17-55 years were recruited. Exclusion criteria included pregnancy, diagnosis of any chronic inflammatory condition, antibiotics used or travel to India within three months prior to stool collection. Participants completed a demographic questionnaire to assess baseline characteristics. Immigrants also completed a lifestyle survey to assess changes in lifestyle and immigration-related stress.

Stool Collection & DNA Extraction

Stool samples were collected from India (Kolkata and Manipal) and Canada (Kelowna) and processed on site as previously described.[10] DNA was sent to Gut4Health for 16S rRNA gene sequencing on the Illumina MiSeq platform (V4-V4, 515F/806R primers) (~75,800 reads per sample) and to the Center for Health Genomics and Informatics for shotgun sequencing on the Illumina NovaSeq platform (~9.9M reads per sample). Additional details are in Supplementary Methods.

Taxonomic Analysis

Demultiplexed 16S rRNA gene reads were processed in QIIME 2 (Version 2022.2)[11] with DADA2[12] for quality control and taxonomic classification with GreenGenes2 (10.28.22).[13] Demultiplexed raw shotgun reads underwent quality assessment with FastQC/MultiQC and used KneadData (Version 0.10) for trimming and quality control. Taxonomic profiling was conducted using MetaPhlAn4,[14] then each sample was normalized to relative abundances. Diversity metrics included Shannon and Pielou's Evenness (Kruskal-Wallis), and Bray Curtis and Weighted UniFrac (PERMANOVA). Differentially abundant bacteria were determined using the Linear Discriminate Analysis (LDA) Effect Size (LEfSe) algorithm with a 3.5 threshold and alpha 0.05.[15] Using shotgun data, the normalized relative abundance MetaPhlAn4 output table was imported into MicrobiomeAnalyst 2.0 to apply a Random Forest algorithm ranked according to their Mean Decrease Accuracy score, indicating the extent to which each taxon's presence or absence

influences the overall accuracy of classifying samples into their respective cohort[16].[16] These taxa were then visualized in a heatmap. Using 16S rRNA gene data, BugBase was used to predict microbiome phenotypes,[17,18] and associations between beta diversity, dietary patterns and baseline characteristics were analyzed with distance-based redundancy analysis (dbDRA). Additional details for taxonomic analysis methods can be found in Supplementary Methods.

Functional Profiling

Shotgun reads were analyzed for functional potential using HUMAnN (Version 3.6).[19] Gene family and pathway abundances were annotated using the UniRef90[20] and MetaCyc[21] databases, respectively, then normalized to reads per kilobase to account for gene length. Both tables were further normalized to relative abundance to account for sampling depth. The normalized gene family abundance output was then regrouped to the CAZy and KEGG databases. To determine differentially abundant genes/pathways across cohorts, tables were collapsed to perform LEfSe analysis on the unstratified data using a one-against-all strategy with a threshold of 3.0 on the logarithmic LDA score and an alpha of 0.05 for Kruskal-Wallis test among classes.[15] Differentially abundant features were then plotted as a heatmap using MicrobiomeAnalyst 2.0.[16]

Nutritional Analysis

Participants filled out a 3-day food log prior to their stool collection, then researchers retrospectively inputted this data into the ESHA Food Processor® software (Version 11.11.0) to obtain the calculated nutrient intake for each subject. To mitigate bias from using a North American nutritional software, Indian and Indo-Immigrant food logs were also entered into the MDRF EpiNu® Nutritional software, tailored for Indian population. Additional details on our approach for dietary analysis can be found in Supplementary Methods.

Statistical Analysis

Statistical analyses for demographics and dietary data were performed using both R statistical software and GraphPad Prism (Version 10.0.3). We applied the Kruskal-Wallis test coupled with Dunn's post hoc analysis for nonparametric data. For data that passed normality, an ordinary one-way ANOVA with post hoc Tukey's multiple comparisons test was performed. Categorical data were analyzed using either a Chi-Square, Fisher's Exact or Fisher's Exact with Monte Carlo simulation, depending on the data structure. Results were reported as median values and interquartile range (IQR), unless otherwise specified.

RESULTS

Participant Characteristics

The study included Indians (n = 61), Indo-Immigrants (n = 32), Indo-Canadians (n= 17), Euro-Canadians (n = 41) and Euro-Immigrants (n = 23) (Figure S1). Baseline demographics were comparable across groups, though Indians were older and consumed significantly less alcohol (p_{BONF} = 3.29e-09) (Table 1). Indo-Immigrants exhibited more of a change towards westernization (p = 0.002) and higher immigration-related stress (p = 0.04), with no significant differences in age at or years since immigration (Table 2).

Indian Microbiota is Distinctive from Westernized Groups

To examine the taxa and complexity of the microbial ecosystem in Indians compared to Westerners, alpha diversity revealed the lowest diversity in Indians and highest in Euro-Canadians (Figure 1A-B). Bray Curtis dissimilarity of shotgun sequence data showed significant differences across groups, with 45.3% of variation explained on the first 2 axes (Figure 1C). Indians and Indo-Immigrants' microbiotas differed from westernized cohorts (Table 3), and each other (pseudo-F = 9.132, p_{BONF} = 0.01). Weighted UniFrac also demonstrated distinctions in the Indian and Indo-Immigrant microbiomes (pseudo-F = 25.98, p_{BONF} = 0.01), with 61.8% of the

variation explained on the first 2 axes (Figure 1D) with similar findings from 16S rRNA gene amplicon sequence data (Figure S2).

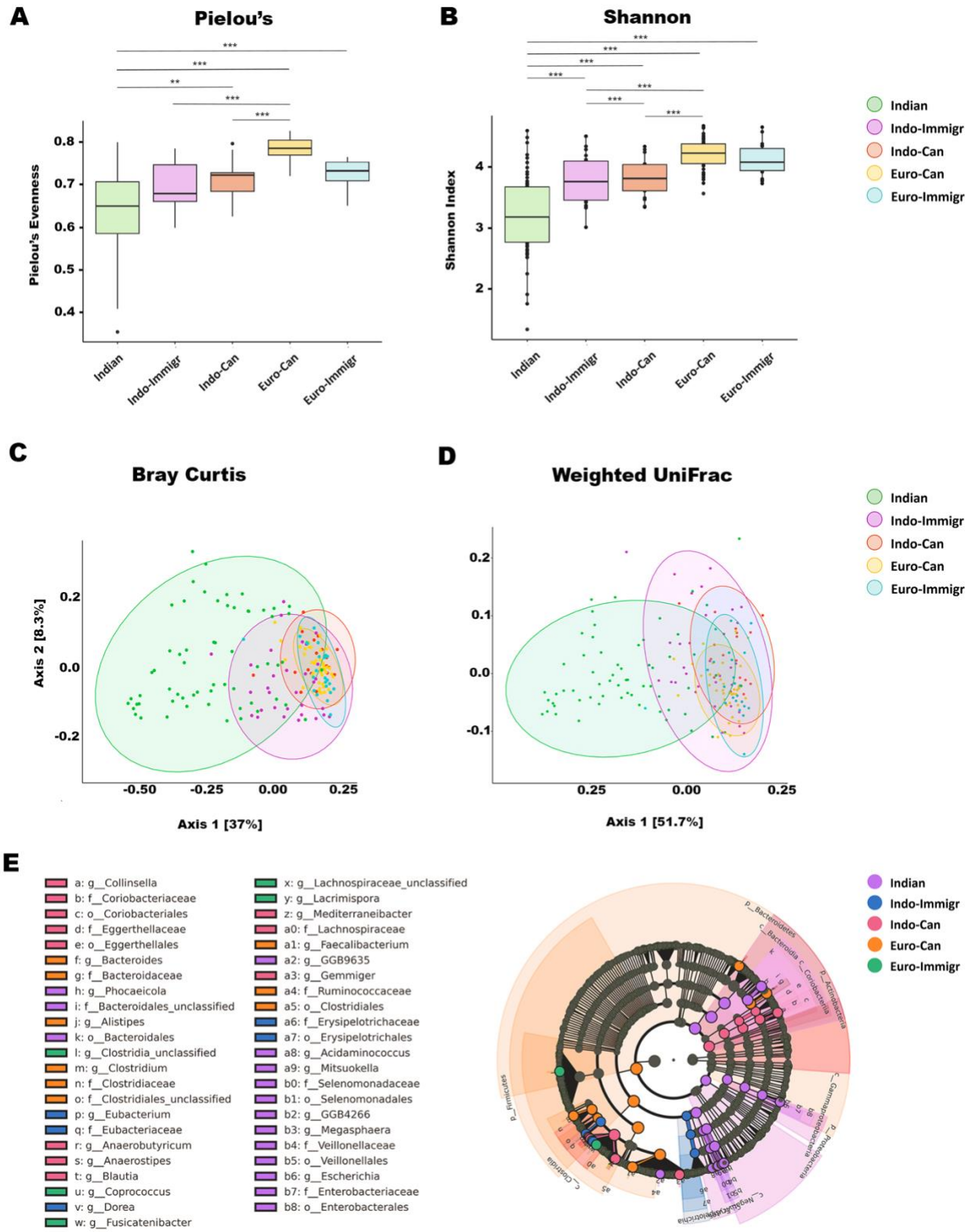

Figure 1. Indian and Indo-Immigrant Microbiotas are Distinct from Westernized Groups

Alpha Diversity - Boxes represent the interquartile range (IQR) between the first and third quartiles, the horizontal line indicates the median, and whiskers are the upper and lower values within 1.5 times the IQR. Alpha diversity was compared across groups using the Kruskal-Wallis test. **(A)** Pielou's Evenness showed significant differences (H = 85.7, p = 1.07e-17). Pairwise comparisons reveal Indians (n = 61) had significantly lower evennnes than Euro-Canadians (n = 41) (H = 56.2, q = 6.51e-13), Euro-Immigrants (n = 23) (H = 17.0, q = 7.32e-05) and Indo-Canadians (n= 17) (H = 10.8, q =1.69e-03). Euro-Canadian controls also score significantly higher evenness than Indo-Immigrants (n = 32) (H = 41.4, q = 6.09e-10) and Indo-Canadians (H = 21.7, q = 8.10e-06). **(B)** Similar results were also found with Shannon's Diversity, showing significant differences (H = 79.8, p = 1.89e-16). *** = q ≤ 0.001, ** = q ≤ 0.01, * = q ≤ 0.05

Beta Diversity - Beta diversity was explored with Bray Curtis dissimilarity and Weighted UniFrac, using Pairwise Permutational Multivariate Analysis of Variance (PERMANOVA) to test differences between groups. **(C)** Bray Curtis principal coordinate analysis (PCoA) plot shows 45.3% of variation was captured on the first two axes. Pairwise comparisons with Bonferroni correction revealed significant differences between most groups, with distinct clustering of Indians and Indo-Immigrants from westernized cohorts. **(D)** Weighted UniFrac PCoA plot shows 61.8% of the variation was captured on the first two axes, with significant differences also detected. Pairwise comparisons results can be found in Table S1.

(E) LEfSe (Linear discriminate analysis Effect Size) cladogram results, depicting differentially abundant bacteria across cohorts, with genus set as lowest taxonomic rank. Indian gut displayed a dominance of Bacteroidetes and Proteobacteria, Euro-Canadians showed high abundance of Firmicutes. Indo-Immigrants had high abundances of VANISH taxa including *Eubacterium* and Erysipelotrichaceae. Indo-Canadians harboured high abundances of both VANISH taxa like *Collinsella*, and BloSSUM taxa such as *Anaerostipes* and *Blautia*.

LEfSe identified dominant taxa in each group, with Bacteroidota (formerly Bacteroidetes) and Pseudomonadota (formerly Proteobacteria) in Indians and Firmicutes in Euro-Canadians (Figure 1E, Table S1). Indo-Immigrants displayed high abundances of *Eubacterium* and Erysipelotrichaceae, previously categorized as VANISH taxa.[22] As expected, genera common in the industrialized microbiome were enriched in the westernized European cohorts such as *Alistipes*, *Bacteroides*, and Clostridia, previously categorized as BloSSUM taxa.[22] Indo-Canadians represented a transitional gut microbiota, harbouring both VANISH and BloSSUM taxa.[23] Similar results were found in 16S rRNA gene sequence data (Figure S3, Table S1).

When assessing differences in bacterial species abundances amongst groups, LEfSe showed that Indians harboured higher abundances of *Phocaeicola plebeius*, *Megasphaera sp.*, *Dialister hominis,* and *Escherichia coli* (Figure 2). Among Indo-Immigrants, *Dorea longicatena*, *Faecalibacterium prausnitizii* and *Lachnospiraceae bacterium* were notably abundant, while Indo-Canadians had increased abundances of two *Ruminoccocus torques* SGBs, *Blautia wexlerae, Eubacterium rectale, Blautia massiliensis,* and *Anaerostipes hadrus*. Taxa enriched in Euro-Canadians included *Phocaeicola vulgatus*, *Lachnospiraceae bacterium, Clostridium sp*., and

Blautia faecis, and Euro-Immigrants showed enrichment of *Fusicatenibacter saccharivorans, Clostridia bacterium,* and *Blautia obeum.* A key distinguishing feature from Indians to Indo-

Table 1. Baseline Characteristics of Participants

Baseline characteristic	Indian *n = 61*	Indo-Immigr *n = 32*	Indo-Can *n = 17*	Euro-Can *n = 41*	Euro-Immigr *n = 23*	*P* value
Sex						
Female	37 (61)	18 (60)	6 (35)	16 (39)	10 (43)	0.10 [a]
Male	24 (39)	12 (40)	11 (65)	25 (61)	13 (57)	
Age, (years)						
Median (IQR)	31 (12)	25 (8)	23 (7)	24 (5)	29 (15)	
Min-max	17-52	18-53	18-38	19-50	19-53	
Age classification, n (%)						
17-29 years	26 (43)	27 (84)	14 (82)	32 (78)	12 (52)	0.002 *[e]
30-39 years	21 (34)	4 (13)	3 (18)	4 (10)	7 (30)	
40-49 years	10 (16)	0 (0)	0 (0)	4 (10)	2 (9)	
50-55 years	4 (7)	1 (3)	0 (0)	1 (2)	2 (9)	
Mode of birth, n (%)						
Vaginal	44 (75)	19 (63)	14 (88)	34 (83)	19 (83)	0.23 [a]
Caesarian	15 (25)	11 (37)	2 (12)	7 (17)	4 (17)	
BMI						
Mean (SD)	24 (3)	24 (4)	22 (3)	23 (3)	22 (2)	
Min-max	17-32	17-34	17-30	17-31	18-25	
BMI classification, n (%)						
Underweight (< 18.5)	3 (5.0)	1 (4.2)	2 (12.5)	3 (8.0)	1 (5.0)	0.08 [b]
Normal weight (18.5 - < 25)	40 (66.7)	14 (58.3)	12 (75)	24 (63.0)	21 (95.0)	
Overweight (25 - < 30)	13 (21.7)	8 (33.3)	2 (12.5)	10 (26.0)	0 (0)	
Obese (> 30)	4 (6.7)	1 (4.2)	0 (0)	1 (3.0)	0 (0)	
Smokers, n (%)						
Yes	6 (10)	0 (0)	0 (0)	2 (5)	2 (9)	0.2908 [c]
No	56 (90)	32 (100)	17 (100)	39 (95)	21 (91)	
Alcohol, n (%)						
Yes	15 (24)	15 (47)	14 (82)	33 (80)	18 (78)	<0.0001†[a]
No	47 (76)	17 (53)	3 (18)	8 (20)	5 (22)	
Physical activity- walking (mins)						
Median (IQR)	NA	90 (128)	90 (120)	60 (75)	60 (90)	0.56 [d]
Min-max	NA	0-330	30-300	12-480	12-420	
Physical activity- exercise (days)						
Median (IQR)	NA	3 (2)	3 (3)	4 (3)	4.5 (3)	0.18 [e]
Min-max	NA	0-7	0-7	0.5-6.5	1-6	
Socioeconomic class, n (%)						
Low	15 (25)	8 (25)	5 (33)	10 (25)	4 (17)	0.70 [e]

Middle	40 (67)	21 (66)	7 (47)	26 (65)	14 (61)	
Upper	5 (8)	3 (9)	3 (20)	4 (10)	5 (22)	
Lifestyle born/raised, n (%)						
Rural	NA	2 (6)	5 (31)	5 (12)	5 (22)	0.10 [c]
Urban	NA	30 (94)	11 (69)	36 (88)	18 (78)	

Abbreviations: SD, standard deviation; IQR, interquartile range; BMI, body mass index; [a]Chi-Square, [b]ANOVA, [c]Fisher's Exact, [d]Kruskal-Wallis, [e]Fisher's Exact (Monte Carlo); * Bonferroni correction revealed significant differences between Indian vs. Indo-Immigr (p_{BONF} = 0.006), Indian vs. Euro-Can (p_{BONF} = 0.03); † Bonferroni correct revealed significant differences (p_{BONF} = 3.29e-09).

Table 2. Characteristics of Immigrant Participants

Characteristic	Indo-Immigr *n = 32*	Euro-Immigr *n = 23*	*P* value
△ westernization, n (%)			
No westernization	0 (0)	4 (18)	0.002 [a]
Little westernization	12 (38)	13 (59)	
Moderate westernization	17 (53)	4 (18)	
High westernization	3 (9)	1 (4)	
Immigration-related stress, n (%)			
Low stress	22 (69)	21 (91)	0.04 [a]
Moderate stress	8 (25)	2 (9)	
High stress	2 (6)	0 (0)	
Age at immigration, n (%)			
0-3 years	0 (0)	1 (4)	0.19 [b]
4-17 years	9 (28)	3 (13)	
18-49 years	23 (72)	19 (83)	
Years since immigration			
Median (IQR)	4 (6)	3 (9)	0.65 [c]
Min-max	0-22	0-48	

Abbreviations: IQR, interquartile range; [a]Chi-Square for Trend, [b]Fisher's Exact, [c]Mann-Whitney

Table 3. PERMANOVA Results from Beta Diversity Analysis

	Bray Curtis		Weighted UniFrac	
Pair	**pseudo-F**	***P* value**	**pseudo-F**	***P* value**
Indo-Immigr vs. Indo-Can	4.215003	0.01	4.670629	0.01
Indo-Immigr vs. Euro-Can	8.976951	0.01	11.932016	0.01
Indo-Immigr vs. Euro-Immigr	5.313757	0.01	6.903393	0.01

Indo-Immigr vs. Indian	9.132465	0.01	25.984365	0.01
Indo-Can vs. Euro-Can	3.084292	0.01	5.409267	0.01
Indo-Can vs. Euro-Immigr	2.166793	0.01	2.360739	0.10
Indo-Can vs. Indian	8.660676	0.01	25.206253	0.01
Euro-Can vs. Euro-Immigr	1.163757	1.00	1.116192	1.00
Euro-Can vs. Indian	18.284343	0.01	49.611215	0.01
Euro-Immigr vs. Indian	11.182855	0.01	31.565399	0.01

Adjusted *P* values are displayed, which were obtained using 999 permutations and Bonferroni correction applied. Beta diversity data from shotgun sequence data.

Canadians was the decline in *Prevotella* spp., with *Prevotella copri* (clade A) being over five times more abundant in Indians (Table 4). Overall, these findings demonstrate that the gut microbiome in Indians are distinct from the West, and Indo-Canadians exhibit a transition towards the industrialized microbiome.

Indian Gut Microbiome Predicted to be More Robust

To understand potential functionality of the gut microbiota from Indians, we used BugBase predictions (Figure 3), finding Indians appeared to demonstrate higher stress tolerance driven by Pseudomonadota (Proteobacteria). The Indian cohorts had the highest potentially pathogenic bacteria (p_{FDR} = 2.07e-18) mostly dominated by Gram negative bacteria driven mainly by a combination of Pseudomonadota, Bacteroidota (Bacteroidetes), and Firmicutes. In addition, the Indian gut microbiome had the highest potential for biofilm formation. Overall, these results suggest the India gut microbiome may be more robust, as healthy individuals were predicted to harbour microbes with higher pathogenic potential and stress tolerance.

Gut Functional Potential in Indians is Distinctive from Westernized Groups

To further understand functional attributes of the Indian gut microbiome, pathway analysis using MetaCyc was used and revealed pathways enriched in lipopolysaccharide (LPS) production, peptidoglycan synthesis, microbial growth/metabolism, protein synthesis and DNA synthesis/repair (Figure 4). In Indo-Canadians, indications of increased glucose storage were detected. Euro-Canadians had increased pathways related to fatty acid biosynthesis, and Euro-Immigrants expressed pathways indicating sugar metabolism (Table S2). CAZy gene family analysis revealed distinct profiles in the Indian gut including glycoside hydrolases (GH)43, GH51, and GH10, all of which are known for their degradation of plant-based polysaccharides (Table S3). *P. copri* was the top taxa contributing to these CAZy families (Figure 5A). In addition, KEGG identities K00561 and K18220 were enriched in Indians and Indo-Immigrants, respectively, both recognized for antimicrobial resistance genes (Figure 5B). Overall, these results suggest that both environmental factors, diets and drugs, were influencing the gut microbiome of Indians.

Westernized Dietary Acculturation Observed in Indo-Canadians

To understand the role of diet associated with the gut microbiome in our Indian cohort, we collected and analyzed dietary recalls using both validated software from North America and from India to account for Indian foods. Indo-Canadians had the highest daily intake of NOVA Group 4 Ultra-Processed Food (UPF), constituting 61% of their caloric intake (95% CI = 467.9 – 780.5 kcal, per 1000 kcal), while Indians consumed significantly less than all other cohorts, reporting 12% of their daily intake (95% CI = 63.66 – 127.1 kcal per 1000 kcal) (Figure 6). Consistent with high UPF intake, total fiber was also found to be the lowest in Indo-Canadians, opposite in Indians who consumed more fiber than Indo-Canadians ($p = 0.0028$) and Euro-Canadians ($p = 0.0110$).

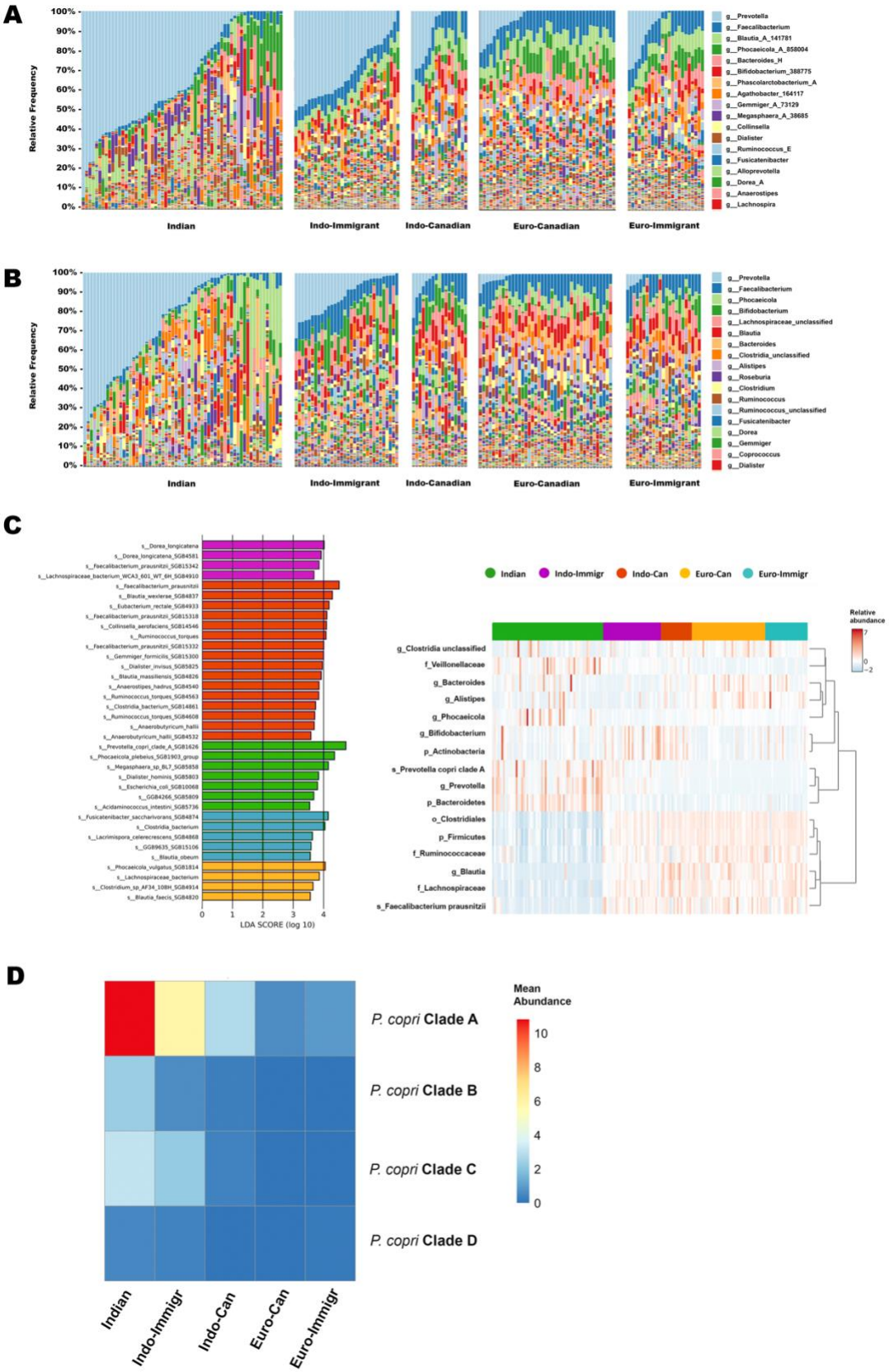
A
Relative Frequency
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Indian
Indo-Immigrant
Indo-Canadian
Euro-Canadian
Euro-Immigrant
g__Prevotella
g__Faecalibacterium
g__Blautia_A_141781
g__Phocaeicola_A_858004
g__Bacteroides_H
g__Bifidobacterium_388775
g__Phascolarctobacterium_A
g__Agathobacter_164117
g__Gemmiger_A_73129
g__Megasphaera_A_38685
g__Collinsella
g__Dialister
g__Ruminococcus_E
g__Fusicatenibacter
g__Alloprevotella
g__Dorea_A
g__Anaerostipes
g__Lachnospira
B
Relative Frequency
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Indian
Indo-Immigrant
Indo-Canadian
Euro-Canadian
Euro-Immigrant
g__Prevotella
g__Faecalibacterium
g__Phocaeicola
g__Bifidobacterium
g__Lachnospiraceae_unclassified
g__Blautia
g__Bacteroides
g__Clostridia_unclassified
g__Alistipes
g__Roseburia
g__Clostridium
g__Ruminococcus
g__Ruminococcus_unclassified
g__Fusicatenibacter
g__Dorea
g__Gemmiger
g__Coprococcus
g__Dialister
C
s__Dorea_longicatena
s__Dorea_longicatena_SGB4581
s__Faecalibacterium_prausnitzii_SGB15342
s__Lachnospiraceae_bacterium_WCA3_601_WT_6H_SGB4910
s__Faecalibacterium_prausnitzii
s__Blautia_wexlerae_SGB4837
s__Eubacterium_rectale_SGB4933
s__Faecalibacterium_prausnitzii_SGB15318
s__Collinsella_aerofaciens_SGB14546
s__Ruminococcus_torques
s__Faecalibacterium_prausnitzii_SGB15332
s__Gemmiger_formicilis_SGB15300
s__Dialister_invisus_SGB5825
s__Blautia_massiliensis_SGB4826
s__Anaerostipes_hadrus_SGB4540
s__Ruminococcus_torques_SGB4563
s__Clostridia_bacterium_SGB14861
s__Ruminococcus_torques_SGB4608
s__Anaerobutyricum_hallii
s__Anaerobutyricum_hallii_SGB4532
s__Prevotella_copri_clade_A_SGB1626
s__Phocaeicola_plebeius_SGB1903_group
s__Megasphaera_sp_BL7_SGB5858
s__Dialister_hominis_SGB5803
s__Escherichia_coli_SGB10068
s__GGB4266_SGB5809
s__Acidaminococcus_intestini_SGB5736
s__Fusicatenibacter_saccharivorans_SGB4874
s__Clostridia_bacterium
s__Lacrimispora_celerecrescens_SGB4868
s__GGB9635_SGB15106
s__Blautia_obeum
s__Phocaeicola_vulgatus_SGB1814
s__Lachnospiraceae_bacterium
s__Clostridium_sp_AF34_10BH_SGB4914
s__Blautia_faecis_SGB4820
0
1
2
3
4
LDA SCORE (log 10)
Indian
Indo-Immigr
Indo-Can
Euro-Can
Euro-Immigr
Relative abundance
7
0
−2
g_Clostridia unclassified
f_Veillonellaceae
g_Bacteroides
g_Alistipes
g_Phocaeicola
g_Bifidobacterium
p_Actinobacteria
s_Prevotella copri clade A
g_Prevotella
p_Bacteroidetes
o_Clostridiales
p_Firmicutes
f_Ruminococcaceae
g_Blautia
f_Lachnospiraceae
s_Faecalibacterium prausnitzii
D
P. copri Clade A
P. copri Clade B
P. copri Clade C
P. copri Clade D
Mean Abundance
10
8
6
4
2
0
Indian
Indo-Immigr
Indo-Can
Euro-Can
Euro-Immigr

Figure 2. Significantly Different Gut Bacterial Abundances Detected Across Cohorts

Taxonomic stacked bar plots depicting relative abundances of top 18 most abundant bacteria across all samples (N = 174; Indian (n = 61), Indo-Immigr (n = 32), Indo-Can (n = 23), Euro-Can (n = 41), Euro-Immigr (n = 23). Both **(A)** 16S rRNA and **(B)** shotgun sequence data demonstrate a high abundance of *Prevotella* spp. in Indians that declines in Indian migrant cohorts. Overall gut bacterial composition is both distinct and more heterogenous in Indians compared to all other cohorts. **(C)** LEfSe results of top differentially abundant bacteria reveals distinct patterns in bacterial abundances in those living in Canada versus India. A Kruskal-Wallis test was performed with a significance (α) at 0.05 for one-against-all comparisons. Differentially abundant bacteria were detected using a Linear Disciminate Analysis (LDA) score (equal to or greater than 3.5). In Indians, *Prevotella copri* (clade A SGB1626) was over 5 times more abundant (LDA score = 4.73, p = 9.25e-13). **(D)** Heatmap generated in MicrobiomeAnalyst 2.0, displaying taxa identified by Random Forest as key features that contribute to the predictive accuracy of classifying samples into their respective groups (Figure S4). Features were filtered for minimum 4 counts in 20% or more samples, and low variance filter of 10%. Bars on the top represent the cohorts that group the individual sample columns displayed. Taxa are labelled in rows, with taxonomic rank noted before the bacteria name. Colours on heatmap represent the relative abundances of each bacteria in a given sample. **(E)** Heatmap generated in R Studio, displaying the average relative abundances of *P. copri* clades in each cohort. Clade A relative abundances were 10.8 for Indians, 5.89 for Indo-Immigrants, and 2.75 for Indo-Canadians. A similar pattern of decreasing abundance across Indian cohorts was also observed for Clades B, C and D. All clades were lowest in abundance in Euro-Canadians.

Table 4. Differentially abundant bacteria from shotgun sequence data

Taxon	Log Score	Class	LDA	P value
Veillonellaceae GGB4266 (SGB5809)	4.023	Indian	3.680	0.003000
*Phocaeicola plebeius (*SGB1903)	4.658	Indian	4.361	2.40E-12
Prevotella copri clade A (SGB1626)	5.034	Indian	4.735	9.25E-13
Escherichia coli (SGB10068)	4.157	Indian	3.801	4.21E-12
Megasphaera sp. (BL7 SGB5858)	4.456	Indian	4.159	2.04E-10
Dialister hominis (SGB5803)	4.147	Indian	3.838	5.42E-06
Acidaminococcus intestini (SGB5736)	3.758	Indian	3.544	0.001382
Dorea longicatena	4.390	Indo-Immigr	4.019	2.99E-16
Faecalibacterium prausnitzii (SGB15342)	4.242	Indo-Immigr	3.852	3.18E-11
Lachnospiraceae bacterium (WCA3 601 WT 6H SGB4910)	4.079	Indo-Immigr	3.685	0.009908
Dorea longicatena (SGB4581)	4.301	Indo-Immigr	3.922	2.04E-07
Faecalibacterium prausnitzii	4.930	Indo-Can	4.513	1.07E-16
Ruminococcus torques	4.410	Indo-Can	4.083	4.02E-16
Anaerobutyricum hallii	4.033	Indo-Can	3.693	3.48E-21
Collinsella aerofaciens (SGB14546)	4.478	Indo-Can	4.099	1.69E-11
Dialister invisus (SGB5825)	4.270	Indo-Can	3.963	4.37E-08
Blautia wexlerae (SGB4837)	4.657	Indo-Can	4.292	5.37E-21
Gemmiger formicilis (SGB15300)	4.422	Indo-Can	4.001	3.60E-06
Faecalibacterium prausnitzii (SGB15318)	4.436	Indo-Can	4.108	3.78E-14
Blautia massiliensis (SGB4826)	4.232	Indo-Can	3.925	1.82E-12
Anaerostipes hadrus (SGB4540)	4.189	Indo-Can	3.849	3.45E-19
Ruminococcus torques (SGB4563)	4.172	Indo-Can	3.843	1.32E-10
Faecalibacterium prausnitzii (SGB15332)	4.368	Indo-Can	4.007	2.44E-09
Anaerobutyricum hallii (SGB4532)	3.918	Indo-Can	3.583	6.69E-20
Eubacterium rectale (SGB4933)	4.656	Indo-Can	4.179	5.36E-05

Clostridia bacterium (SGB14861)	4.027	Indo-Can	3.739	7.03E-18
Ruminococcus torques (SGB4608)	4.035	Indo-Can	3.710	2.92E-13
Lachnospiraceae bacterium	4.275	Euro-Can	3.861	3.12E-16
Blautia faecis (SGB4820)	3.909	Euro-Can	3.564	3.08E-19
Phocaeicola vulgatus (SGB1814)	4.488	Euro-Can	4.056	5.66E-05
Clostridium sp (AF34 10BH SGB4914)	4.083	Euro-Can	3.654	0.007673
Ruminococcaceae GGB9635 (SGB15106)	3.871	Euro-Immigr	3.588	0.014807
Clostridia bacterium	4.522	Euro-Immigr	4.047	3.81E-08
Blautia obeum	3.917	Euro-Immigr	3.566	5.24E-15
Fusicatenibacter saccharivorans (SGB4874)	4.485	Euro-Immigr	4.154	3.84E-18
Lacrimispora celerecrescens (SGB4868)	3.960	Euro-Immigr	3.635	2.47E-08

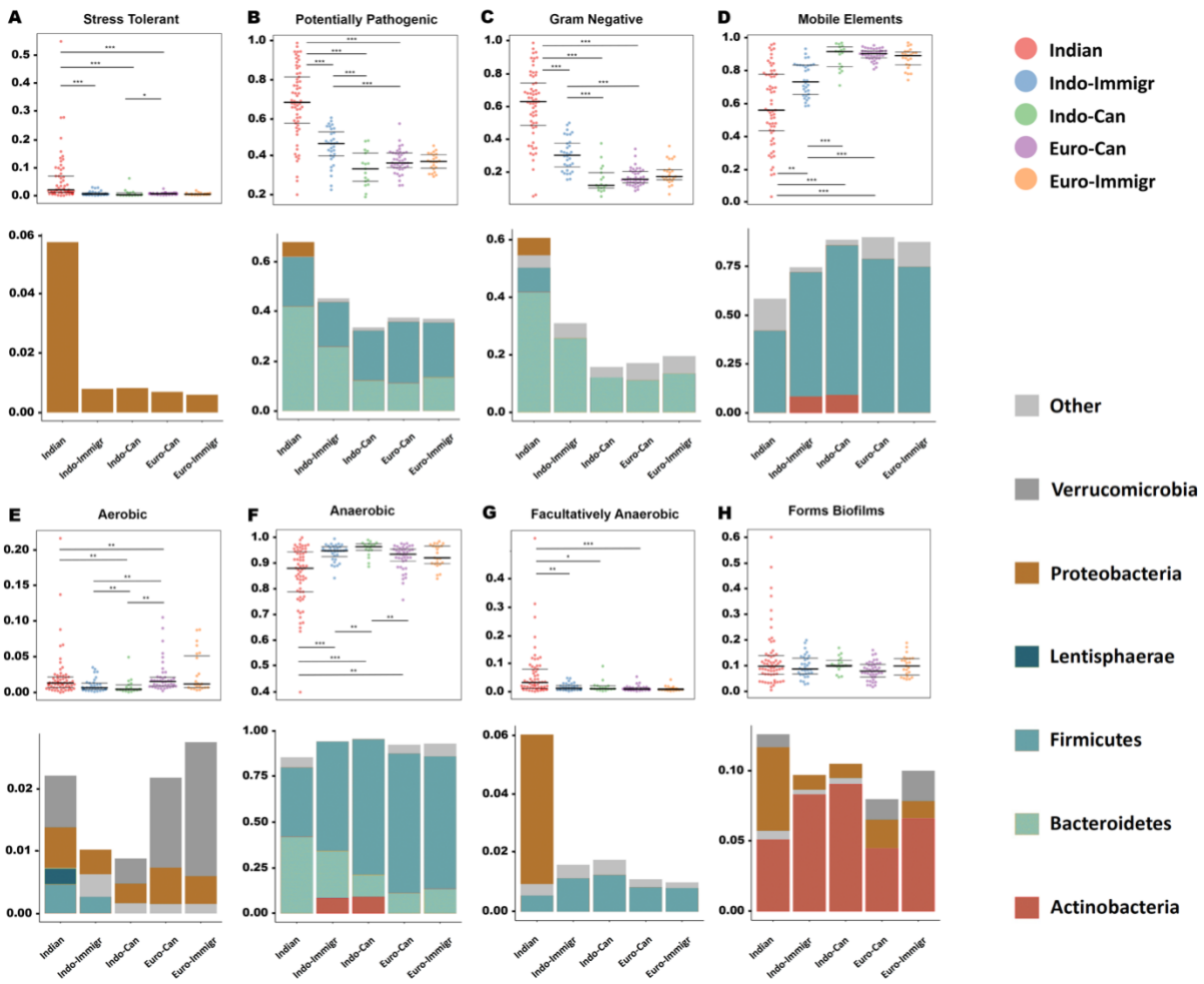

Figure 3. BugBase Predicts Higher Pathogenic Potential and Stress-Tolerant Microbiome in Indians

A Kruskal-Wallis test was performed in BugBase followed by Mann-Whitney-Wilcoxon tests for pairwise comparisons with adjusted *P* values shown. Significant phenotypic differences were predicted from the 16S rRNA gene sequence data. Relative abundance is presented on the y-axis. Indian (n = 61), Indo-Immigr (n = 32), Indo-Can (n = 23), Euro-Can (n = 41), Euro-Immigr (n = 23). **(A)** Stress tolerance was significantly higher in Indians (p_{FDR} = 2.40e-12) compared to all cohorts. **(B)** Potentially pathogenic bacteria was significantly higher in Indians (p_{FDR} = 2.07e-18) compared to all cohorts. Indo-Immigrants also showed significantly higher pathogenic potential than Indo-Canadians (p_{FDR} = 5.78e-04), Euro-Canadians (p_{FDR} = 1.41e-04) and Euro-Immigrants (p_{FDR} = 2.34e-04). **(C)** Gram negative bacteria were estimated to be significantly higher in Indians than all other cohorts (p_{FDR} = 2.01e-23). Indo-Immigrants were predicted to have a significantly higher Gram-negative bacteria abundance than Indo-Canadians (p_{FDR} = 3.32e-06), Euro-Canadians (p_{FDR} = 7.96e-10), and Euro-Immigrants (p_{FDR} = 1.81e-05). **(D)** Predictions of containing mobile elements was significantly lower in Indians than all other cohorts (p_{FDR} = 1.76e-17). Mobile elements were also predicted to be significantly lower in Indians than Indo-Canadians (p_{FDR} = 3.34e-05), Euro-Canadians (p_{FDR} = 1.53e-11), and Euro-Immigrants (p_{FDR} = 6.42e-06). **(E)** Aerobic bacteria was significantly higher in Indians than Indo-Immigrants (p_{FDR} = 0.0149) and Indo-Canadians Immigrants (p_{FDR} = 0.0090), and Indo-Immigrants were significantly lower than Euro-Canadians (p_{FDR} = 0.0023) and Euro-Immigrants (p_{FDR} = 0.0149), and Indo-Canadians were significantly lower than Euro-Canadians (p_{FDR} = 0.0019) and Euro-Immigrants (p_{FDR} = 0.0090). **(F)** Anaerobic bacteria abundance was significantly lower in Indians (p_{FDR} = 7.55e-07) compared to all cohorts. Indo-Canadians also showed significantly higher anaerobic bacteria than Euro-Canadians (p_{FDR} = 0.0060). **(G)** Facultatively Anaerobic bacteria abundance was significantly more abundant in Indians than all other cohorts (p_{FDR} = 1.57e-07). **(H)** No significant differences were detected in biofilm formation predictions amongst groups. Taxonomic contributions are also displayed for each phenotype prediction. *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$

The highest proportion of non-meat eaters were Indians at 60% (*n* = 36/61, p = <0.0001), with 30% vegetarian and 30% pescetarian. In Indo-Immigrants, 19% (*n* = 6/32), while 12% (*n* = 2/17) of Indo-Canadians, 10% (*n* = 4/41) Euro-Canadians and 39% (*n* = 9/23) of Euro-Immigrants were non-meat eaters. Cooking oil usage highlighted sunflower oil, ghee and mustard oil as the top choices of Indians, whereas Indo-Immigrants preferred olive oil, butter and ghee, with olive oil and butter being the primary fats used by the three westernized groups (Figure S5). These results highlight the westernized diet adopted by Indo-Canadians, including high levels of processed food and low levels of fiber, are potential drivers in the westernized gut microbiota.

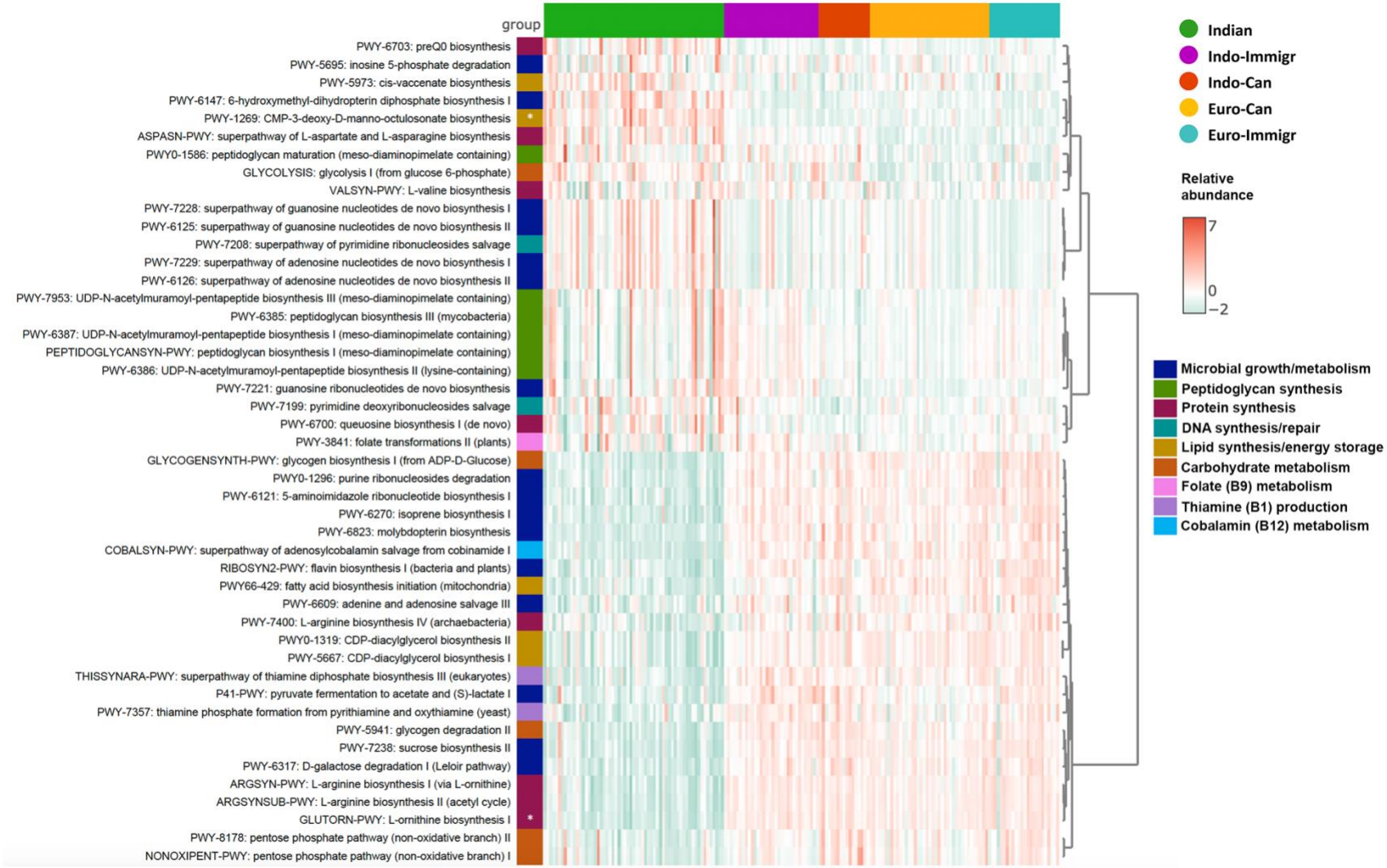

Figure 4. Microbial Metabolic Pathways Show Distinction in Functional Potential of Indian Gut Microbiome

Pathway abundances were annotated using MetaCyc, then normalized to account for gene length and sampling depth. LEfSe (Linear discriminate analysis Effect Size) was used to test for differentially abundant microbial metabolic pathways using unstratified data. A Kruskal-Wallis test was performed with a significance (a) at 0.05 for one-against-all comparisons. Discriminate features were identified using a Linear Discriminate Analysis (LDA) score, with an LDA score equal to or greater than 3.0. Using MicrobiomeAnalyst 2.0, a heatmap was generated to display the differentially abundant pathways identified from LEfSe. Each column is a sample, and the coloured bars on top represent the cohorts. Indian (n = 61), Indo-Immigr (n = 32), Indo-Can (n = 23), Euro-Can (n = 41), Euro-Immigr (n = 23). Metabolic pathways are labelled in rows and annotated with a colour that describes their general function. Colours on the heatmap represent the relative abundances of each pathway. This heatmap displays a clear distinction in microbial metabolic pathway abundances in Indians versus cohorts living in Canada. * *L-orthinine biosynthesis I* is indirectly linked with protein synthesis and also involved with urea cycle; * *CMP-3-deoxy-D-manno-octulosonate biosynthesis* is directly involved in lipopolysaccharide (LPS) production.

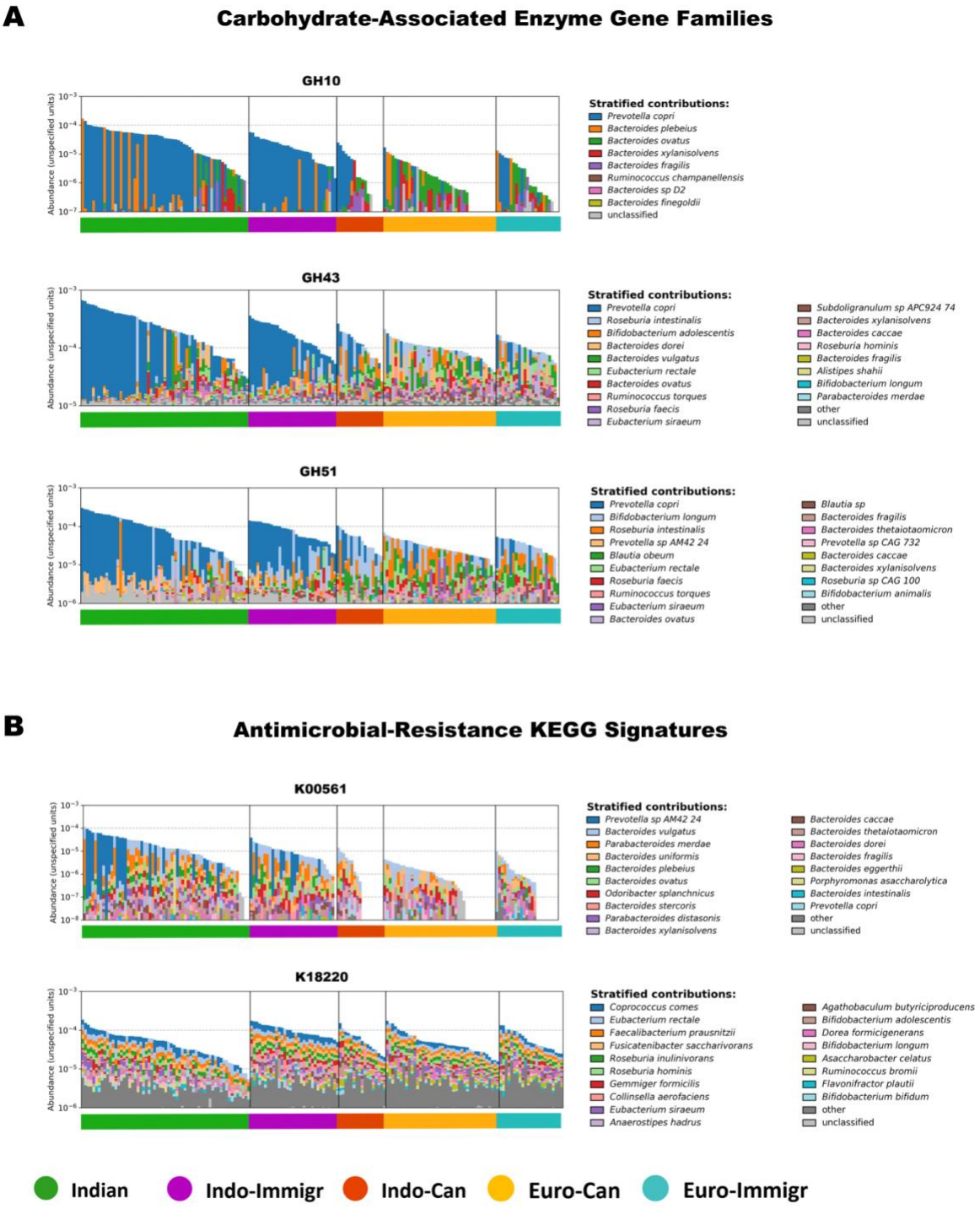

Figure 5. *Prevotella copri* is the Top Taxa Contributing to CAZy Families Abundant in Indians

Normalized gene family abundance table was regrouped to CAZy database using *human_regroup_table*. Barplots for stratified taxa contributions for each CAZy gene family was generated using *human_barplot*. Indian (n = 61), Indo-Immigr (n = 32), Indo-Can (n = 23), Euro-Can (n = 41), Euro-Immigr (n = 23). *Prevotella copri* is the top contributor to CAZyme families **(A)** GH10, GH43, GH51, all which were enriched in Indians. Top taxa contributions to antimicrobial resistance-related KEGG orthologues abundant in Indians and Indo-Immigrants. Normalized gene family abundance table was regrouped to KEGG database using *human_regroup_table*. Barplots for stratified taxa contributions for each KEGG ortholog was generated using *human_barplot*. **(B)** *Prevotella copri AM42 24* is the top contributor to K00561, enriched in Indians and *Coprococcus comes* is the top contributing taxa to K18220, enriched in Indo-Immigrants.

Table 5. Percentages of respondents who reported use of each cooking oil

Oil Type	Indian *n = 43*	Indo-Immigr *n = 18*	Indo-Can *n = 9*	Euro-Can *n = 35*	Euro-Immigr *n = 18*
Butter	5%	24%	27%	24%	26%
Ghee	12%	16%	0%	0%	0%
Coconut	9%	4%	0%	5%	10%
Olive	1%	28%	45%	44%	39%
Sunflower	30%	0%	0%	2%	0%
Canola	0%	12%	0%	13%	6%
Vegetable	5%	4%	18%	2%	10%
Mustard	11%	8%	0%	0%	0%
Avocado	0%	0%	0%	5%	6%
Groundnut	8%	0%	0%	0%	0%
Rice	8%	0%	0%	0%	0%
Other	9%	4%	9%	4%	3%

To understand what dietary factors could be driving microbial taxa, the dbRDA model, accounting for demographics and dietary patterns elucidated 16.62% of the microbial variance on the first 2 axes (PERMANOVA F = 5.9923, p = 0.001) (Figure 6C). After false discovery rate (FDR) adjustment, UPF and alcohol intake were principal determinants in differentiating the microbiota in westernized cohorts (p_{FDR} = 0.003). The pescetarian diet (p_{FDR} = 0.070) and fiber intake (p_{FDR} = 0.2268) appeared to influence clustering of Indian samples, albeit not significant. Advancing age (p_{FDR} = 0.044) influenced another clustering, whereas vegetarianism showed no significant effect (p_{FDR} = 0.371). Overall, these results suggest that UPF and alcohol intake were driving westernization of the gut microbiome in Indian migrants with this effect most prominent in Indo-Canadians. Furthermore, the higher fiber intake observed in Indians coincided with high *Prevotella* spp. and CAZy gene families tailored to the more traditional Indian diet.

Given the above data indicated that the Indo-Canadian group had adopted a westernized diet, further analysis of diet was examined including absolute macronutrient and micronutrient intakes (Table 6-7; Table S4-S5). Total energy intake was significantly higher in Euro-Canadian

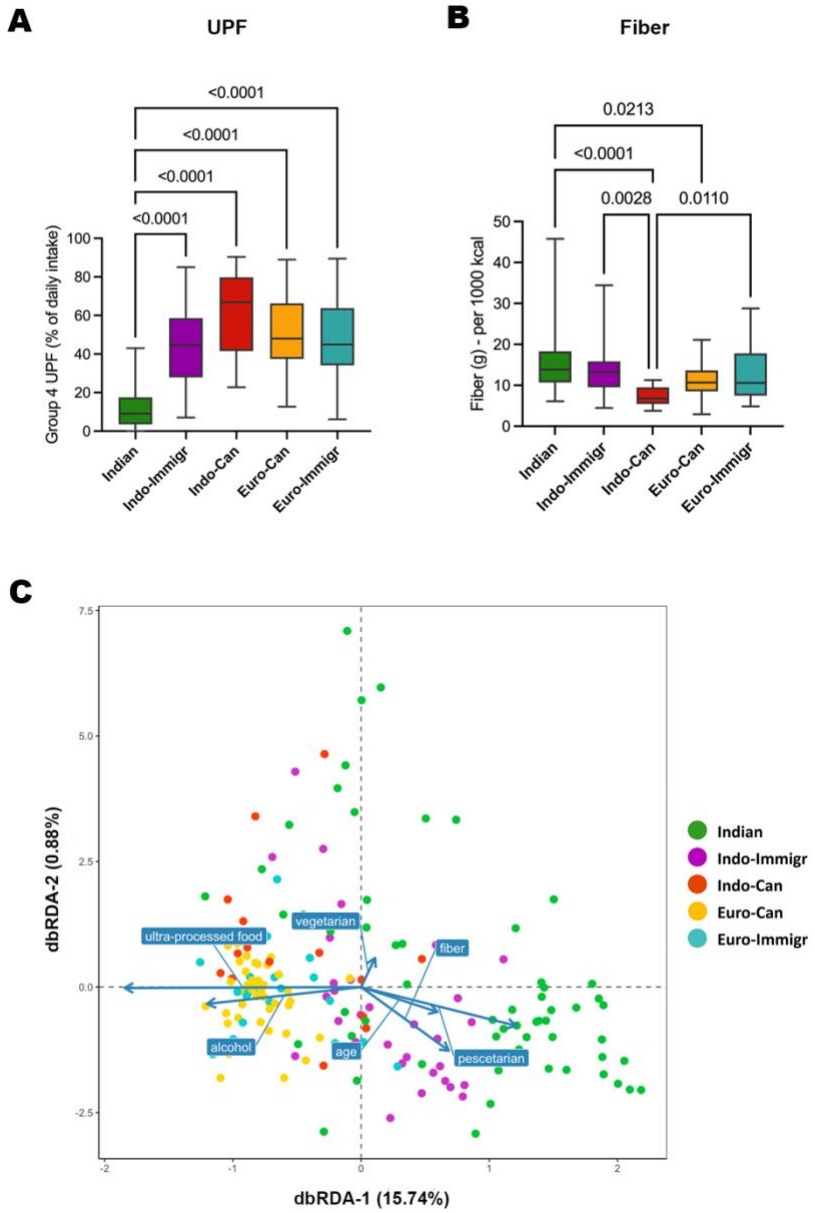

Figure 6. Significant Intake of Ultra-Processed Food and Alcohol are Drivers in the Westernized Gut Microbiota

Middle bands are the median values, top and bottom boxes display the first and third quartiles, and whiskers are the min and max values. Indian (n = 61), Indo-Immigr (n = 32), Indo-Can (n = 23), Euro-Can (n = 41), Euro-Immigr (n = 23). **(A)** Calories from Group 4 Ultra-Processed Food (UPF) was counted for each subject from the ESHA dietary reports then compared to total average daily caloric intake. A Kruskal-Wallis test was performed, followed by Dunn's multiple comparisons, with adjusted p values displayed. Percentage of daily caloric intake from Group 4 UPF was highest in Indo-Canadians and Indians consumed a significantly lower amount than all other cohorts (p = <0.0001). **(B)** Fiber intake was normalized per 1000 kcal for each subject. An ordinary one-way ANOVA was performed, followed by Tukey's multiple comparisons, with adjusted p values displayed. Indians consumed significantly more fiber than Indo-Canadians (p = <0.0001) and Euro-Canadians (p = 0.0213). Indo Canadians also consumed significantly less fiber than Indo-Immigrants (p = 0.0028) and Euro-Canadians (p = 0.0110). **(C)** Distance-based redundancy analysis (dbRDA) plot was generated using Weighted UniFrac distance matrix from 16S rRNA gene amplicon data. This dbRDA plot depicts the distribution of samples and their associations with lifestyle factors. Each dot is a sample, colour coded by cohort. Variance inflation factor values ranged between 1.14 (vegetarian) – 1.38 (pescetarian), indicating each variable uniquely contributed to the model. The explanatory variables in this model elucidated 16.62% of the microbial variation on the first two axes (F = 6.8528, p = 0.001). Permutational multivariation ANOVA (PERMANOVA) test reveals these explanatory variables collectively were significant (F = 5.9923, p = 0.001), with Group 4 UPF (pFDR = 0.003) and alcohol (pFDR = 0.003) driving distinctions in the microbiota of westernized cohorts. Distinct clustering is shown in Indians, with advancing age being a significant contribution (pFDR = 0.044).

Table 6. Male participant absolute macronutrient intake

Macronutrient	RDA/AMDR	Indian *n = 37*	Indo-Immigr *n = 18*	Indo-Can *n = 6*	Euro-Can *n = 16*	Euro-Immigr *n = 10*	*P* value
Energy, kcal (IQR)	-	2329 (1187)	2086 (933)	2274 (532)	2911 (1342)	2668 (906)	0.0343[a]
Protein							
g (IQR)	56g	82.9 (41.3)	78.8 (39.1)	138 (37.2)	119 (62.6)	103 (42.5)	<0.0001[a]
% of total energy	10-35%	13.1%	14.3%	23.1%	17.8%	16.4%	
g per 1000 kcal	-	31.3	35.1	37.5	40.4	37.6	
Fat							
g (IQR)	-	80.2 (43.1)	76.5 (40.8)	109 (39.9)	113 (63.9)	98.5 (45.2)	0.0109[a]
% of total energy	20-35%	29.2%	34.6%	39.2%	38.2%	37.1%	
g per 1000 kcal	-	34.6	38.2	44.1	42.9	40.7	
PUFA, g (IQR)	-	13.1 (13.7)	10.6 (6.33)	13.7 (6.32)	22.2 (13.3)	11.7 (9.35)	0.0254[b]
Omega-3, g (IQR)	1.6	1.44 (1.77)	0.93 (0.53)	1.24 (1.01)	2.31 (3.44)	0.85 (1.63)	0.0080[b]
Omgea-6, g (IQR)	17	11.5 (10.9)	8.77 (4.64)	10.5 (5.93)	16.7 (10.8)	9.20 (7.15)	0.0127[b]
MUFA, g (IQR)	-	17.8 (14.3)	17.7 (11.8)	17.2 (22.3)	33.0 (24.0)	24.6 (13.7)	0.0100[b]
SFA, g (IQR)	-	26.4 (20.0)	23.0 (12.2)	34.5 (15.0)	37.3 (22.3)	30.1 (19.0)	0.0055[a]
Carbohydrate							
g (IQR)	130g	336 (58.6)	267 (51.8)	207 (39.7)	268 (41.1)	284 (43.0)	<0.0001[a]
% of total energy	45-65%	58.6%	51.8%	39.8%	41.1%	43.0%	
g per 1000 kcal	-	141	131	110	113	112	
Fiber, g (IQR)	38g	34.9 (17.4)	28.3 (20.1)	13.1 (9.70)	23.9 (15.5)	22.4 (22.6)	0.0016[b]
Soluble fiber		0.37	0.59	0.03	0.66	0.56	0.1223[b]

Abbreviations: [a]ANOVA, [b] Kruskal-Wallis. Median and IQR reported.

Table 7. Female participant absolute macronutrient intake

Macronutrient	RDA/AMDR	Indian *n = 24*	Indo-Immigr *n = 14*	Indo-Can *n = 11*	Euro-Can *n = 25*	Euro-Immigr *n = 13*	*P* value
Energy, kcal (IQR)	-	1747 (630)	1976 (1136)	1812 (527)	2265 (910)	2227 (1060)	0.0332
Protein							
g (IQR)	46g	51.5 (21.8)	65.3 (47.9)	63.1 (20.5)	81.0 (20.8)	66.6 (50.7)	0.0004 [b]
% of total energy	10-35%	12.3%	13.8%	12.9%	14.1%	14.0%	
g per 1000 kcal	-	31.3	35.1	37.5	40.4	37.6	
Fat							
g (IQR)	-	62.7 (32.3)	69.8 (38.0)	73.1 (27.5)	89.2 (40.4)	103 (57.0)	0.0018 [b]
% of total energy	20-35%	33.4%	34.4%	39.7%	38.7%	36.7%	
g per 1000 kcal	-	34.6	38.2	44.1	42.9	40.7	
PUFA, g (IQR)	-	12.1 (9.49)	12.9 (11.0)	8.06 (4.33)	13.3 (8.90)	9.87 (13.0)	ns
Omega-3, g (IQR)	1.1	1.24 (0.85)	1.46 (1.02)	0.84 (0.44)	1.42 (1.25)	1.06 (1.16)	ns
Omgea-6, g (IQR)	12	10.5 (8.72)	8.80 (8.25)	7.00 (4.04)	10.0 (8.92)	8.73 (7.67)	ns
MUFA, g (IQR)	-	16.5 (10.5)	15.6 (13.4)	15.3 (9.77)	23.8 (13.7)	19.0 (23.6)	ns
SFA, g (IQR)	-	18.3 (14.9)	23.4 (10.6)	22.9 (5.66)	30.6 (14.3)	41.7 (35.1)	ns
Carbohydrate							
g (IQR)	130g	258 (86.4)	256 (116)	218 (99.5)	272 (119)	248 (141)	ns
% of total energy	45-65%	55.7%	53.6%	38.4%	47.6%	49.4%	
g per 1000 kcal	-	141	131	110	113	112	
Fiber, g (IQR)	25g	24.6 (17.8)	23.1 (18.7)	14.7 (6.30)	27.0 (15.2)	28.8 (28.4)	0.0163 [a]
Soluble fiber		0.21	0.69	0.05	0.75	0.45	0.0003 [b]

Abbreviations: [a]ANOVA, [b] Kruskal-Wallis. Median and IQR reported.
*Tukey's post hoc analysis revealed no significant differences between groups

males vs. Indo-Immigrant males (p = 0.0144, 95% CI = -1461 – -110.0 kcal). Median percentage of protein intake was highest in Euro-Canadians (16.1%, 95% CI = 13.2 – 17.5%) and lowest protein intake was in Indians (12.5%, 95% CI = 11.7 – 13.3). Indians consumed the highest percentage of carbohydrates in their diet (56.5%, 95% CI = 54.7% - 59.0%) and lowest carbohydrate diet intake was in Indo-Canadians (44.0%, 95% CI = 40.6% – 50.6%). The highest percentage of fat consumption was in Indo-Canadians (39.7%, CI 95% 33.8% – 42.2%) and the lowest was in Indians (31.1%, 95% CI = 28.5% – 34.4%). Software comparisons between North American ESHA and Indian EpiNu revealed no macronutrient composition differences however, EpiNu calculated a significantly higher intake of PUFAs and MUFAs and lower intake of SFA amongst Indians, highlighting discrepancy in dietary fat types between dietary software, which could skew dietary analysis between the countries (Fig 7A-B). When the type of oils consumed were examined, it was revealed that Indo-Canadians have the least diversity with no traditional fats added to their diet like ghee (Fig 7C).

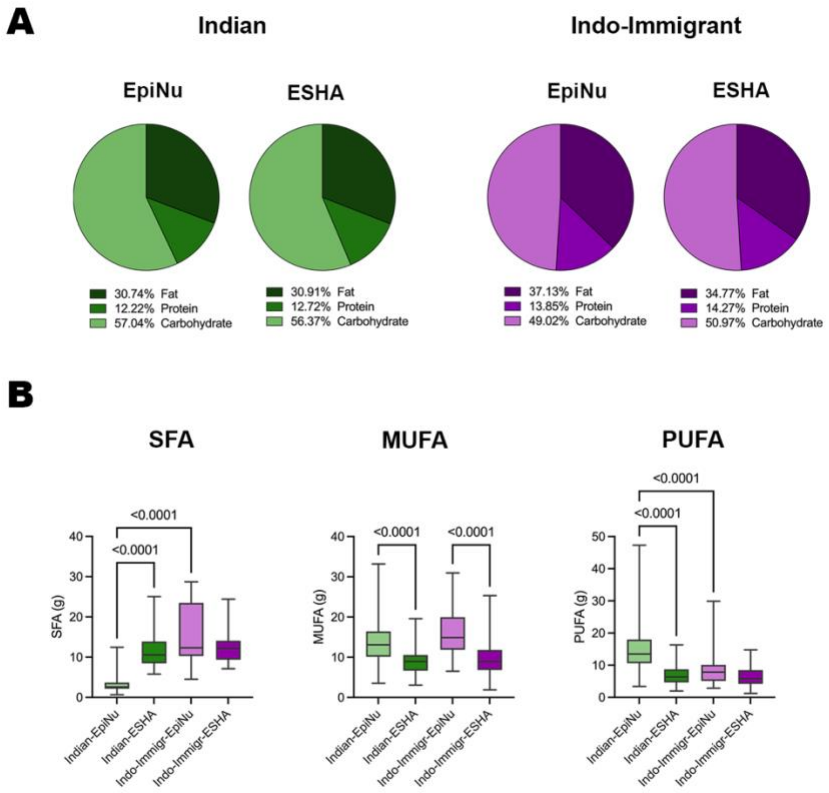

Figure 7. Dietary Fat Types are Significantly Different Between EpiNu vs. ESHA

(A) Percentages of fat, protein and carbohydrates were calculated in ESHA and EpiNu for each participant, then mean values were calculated for each cohort. An ordinary one-way Analysis of Variance (ANOVA) was used, and no significant differences in macronutrient intake were found between ESHA and EpiNu. **(B)** Significant differences in types of fat consumed by Indians (n = 61) and Indo-Immigrants (n = 32) were calculated between EpiNu vs. ESHA. Dietary fats displayed in grams per 1000 Calories (kcal). Kruskal-Wallis tests were conducted to determine differences between groups. The middle band inside of the boxes is the median, the bottom and top boxes are the first and third quartiles, and the whiskers are the min to max values. When compared to EpiNu, ESHA calculated a significantly higher saturated fatty acid (SFA) intake in Indians ($p = <0.0001$). EpiNu calculated significantly higher monounsaturated fatty acid (MUFA) intake in both Indians and Indo-Immigrants ($p = <0.0001$). Compared to ESHA, EpiNu calculated a significantly higher intake of polyunsaturated fatty acid (PUFA) in Indians.

DISCUSSION

Westernization is Linked to Both Taxonomic and Functional Transitions in the Gut Microbiomes of Indian Migrants

Earlier studies identified that the Indian gut microbiota is distinct, as it is rich in *Prevotella* spp*., Dialister* spp*.,* and *Megasphaera* spp.,[24-30] unlike westernized groups dominated by *Bacteroides* spp.[31] and *Phocaeicola vulgatus*[32]. Furthermore, the high taxonomic variation in Indians may in part reflect their diverse dietary practices in India alongside the current western influence. This heterogeneity also highlights how attempting to generalize the "Indian microbiome" may inaccurately represent this population due to the great cultural variations that exist within the country. Contrary to associations of low alpha diversity in westernization and disease, healthy Indians showed lower alpha diversity than westernized cohorts, suggesting this diversity measure may not always be a reliable marker of westernization or health status.[33]

Taxonomic data revealed high abundances of VANISH taxa in Indians and Indo-Immigrants, and BloSSUM taxa enriched in European westernized controls. Indo-Canadians exhibited a transitional microbiota containing both VANISH and BloSSUM taxa, a trend previously detected in infants living in transitional societies (intermediate between non-industrialized and industrialized lifestyles).[34] A key genus that is distinctive in this transition in immigrants is the loss of *Prevotella* spp.[8,35] In fact, this contrast in *Prevotella* spp. abundance in non-westernized versus westernized groups has sparked interest both in its relation to lifestyle and disease, previously

being linked to IBD,[36 37] rheumatoid arthritis,[38] and obesity[39]. Yet, contradictory studies also showed its correlation with reduced risks of cardiovascular disease (CVD) [40] diabetes [41], and obesity,[42] illustrating the current contradictory understanding around *Prevotella* spp.-dominance in the gut and its role in diseases.

Globally, Indians have some of the highest abundances of *Prevotella* spp. in their gut,[43] as was reflected in our data, which highlights the importance of context when investigating the role of microbes in our gut, as host-microbe interactions may divergently evolve depending on the host's genetics, geography, and lifestyle. In fact, these factors may have already driven evolutionary changes in *Prevotella* spp. strains: isolated *P. copri* strains from Indian/non-western populations showed traits that aid in digestion of plant-based carbohydrates using carbohydrate-active enzymes (CAZymes), whereas *P. copri* strains from westernized populations were enriched with virulence factors and resistance genes.[43] In our study, Indians had a significantly higher average abundance of *P. copri* Clade A, along with notable abundances of Clades B and C, which subsequently declined in Indo-Immigrants and Indo-Canadians (Figure 2D). All *P. copri* clades have previously been documented at higher abundances in non-westernized individuals than westernized,[44] and our results highlight this effect of westernization on clade abundances using groups of the same ethnicity. These findings demonstrate how specific strains can evolve with its host, cautioning against broad associations between bacterial species and diseases.

Indians, known for their high-complex-carbohydrate diet, have been previously indicated to exhibit an increased expression of carbohydrate metabolism genes for complex polysaccharides in their gut.[24,28,30] Our findings reflected this relationship, as we detected enrichment of CAZy families that aid in the digestion of xylan and xyloglucan, with *P. copri* as the top contributing taxa. A similar pattern was in fact detected in another study on immigrants who migrated from Thailand to the US, where a decrease in *Prevotella* spp. abundance corresponded

with a loss of CAZymes that break down plant fiber.[8] The subsequent loss of *P.copri* in our Indo-Immigrant and Indo-Canadian cohorts aligned with their reduction of a high carbohydrate, high fiber diet, which was a trend previously explored among Hadza and Nepali groups.[45] Plant-derived microbiota-accessible carbohydrates (MACs) are required to maintain abundances of *P. copri*, whereas other species such as *Bacteroides thetaiotaomicron* can utilize both plant and animal-derived MACs, allowing for persistence in the gut.[45] This relationship therefore suggests that the *Prevotella* spp. abundance in the Indian gut is highly linked to their specific dietary patterns. Furthermore, the generational loss of *P. copri* in Indian immigrants prompts questions about gut health implications, meriting further research on strain-specific *P. copri*-host interactions within this demographic.

A previous study found *Dialister succinatiphilus* and *Megasphaera* more abundant in Indo-Immigrants versus Indo-Canadians, who had significantly higher *Dialister invisus.* Our data align with this trend, with *D. invisus* enriched in Indo-Immigrants, *D. succinatiphilus* in Indo-Canadians, and *Megasphaera* in our Indian cohort. While little is known about these taxa, a relationship between a carbohydrate-rich diet and succinate-utilizing bacteria has been previously discussed,[46] with *D. succinatiphilus* being a well-known species in this category. Additionally, *Ruminococcus torques,* a known mucin degrader,[47] previously linked to IBD risk [48] and identified as a significant contribution in a Crohn's Disease microbiome-risk score,[49] was notably enriched in Indo-Canadians. Deeper investigations should explore the role of specific *R. torques* strains in the gut and its implications in IBD should be explored further.

India is Currently Industrializing

India's industrialization is reshaping dietary habits, as seen in our data where sunflower oil, contradictory to traditional fats like ghee, has become predominately used due to globalization[50] and dietary guidelines recommending a reduction in saturated fatty acids and

increase in PUFA-rich vegetable oils. Overtime, these vegetable oils became inexpensive and more readily available in India, therefore there has been a transition away from the use of their traditional fats.[51] Coinciding with this transition of increased PUFA consumption is the rising epidemic of several modern diseases such as IBD[52], type-2 diabetes, and metabolic syndrome (MS).[53] India is now in an accelerating incidence phase of IBD[1], doubling the number of patients from 1990 to 2019,[54] and the increased intake of linolenic (omega-6 PUFA) in Indians has been previously associated with an increased risk for UC.[55,56] These findings highlight the need to investigate the role of vegetable oil consumption in India and its potential contribution to the rising incidence of IBD.

Surprisingly, the westernized cohorts in our study reported cooking mainly with olive oil and butter, and few reported to cook with vegetable oils unlike their expected intake of oils rich in omega-6 PUFAs.[57] Though these reports are limiting it is worth highlighting that while India has adopted the use of white oils like sunflower oil and canola oil, North America may currently be shifting away from them. For decades, the westernized diet has been characterized by a much higher intake of omega-6 PUFA, leading to omega-6:omega-3 ratios of 20:1, which is far from the estimated ratio of 1:1 of our ancestors.[58] This excessive intake of omega-6 PUFAs has been associated with CVD,[59] cancer,[60] and autoimmune diseases.[61] Furthermore, with large-scale studies and meta-analyses refuting the link between saturated fatty acids and CVD[62,63] and the increasing popularity of the Mediterranean Diet,[64] a cultural shift in the North America may be occurring, once again preferring olive oil and butter,[65] which was an observation also recently reported from an Australian cohort.[66]

Another factor of industrialization that is common in India is the overuse of antibiotics. India has one of the highest threats for antimicrobial resistance,[67] as was reflective in our cohorts

with enriched KEGG identities in Indians and Indo-Immigrants that contain signatures for antibiotic resistance genes, a pattern previously documented in the Indian gut[24 68] The widespread use of antibiotics in India not only increases the prevalence of antibiotic resistance, but also accelerates microbiome turnover, which was indicative in the pathways enriched in our Indian cohort including peptidoglycan synthesis, microbial growth/metabolism, and DNA synthesis/repair. While common usage of antibiotics is a likely catalyst for cell turnover, the higher risk for infectious disease via water contamination and inadequate sanitation in India may also serve as environmental triggers. Our findings highlight India's unique transition phase, balancing Western influences with traditional lifestyle practices, all of which are shaping their gut microbiome. Future research should delve deeper into how westernization is currently influencing the gut microbiome in Indians, as this will likely pose future health challenges.

Acculturation of Westernized Diet Drives Functional Shifts in Microbiome

Coinciding with previous findings, we observed dietary acculturation from the traditional high-complex-carbohydrate Indian diet to an increased intake of NOVA Group 4 UPFs,[35,69,70] which are characterized by industrially made ingredients with non-nutritive additives like emulsifiers, flavouring and sweeteners.[71] This dietary shift might explain the enrichment of pathways tapping into glycogen storage in Indo-Canadians, suggesting microbes had inadequate nutrient availability from the host diet.[72] With a 49% rise in Group 4 UPF intake and significant fiber reduction in Indo-Canadians, concerns emerge regarding their gut health and chronic disease risks.

Increasing UPF consumption has been linked to higher risks for CVD, metabolic disorders, micronutrient deficiencies, and anxiety and depression,[71] which may partly be due to imbalances in the gut microbiome that are a triggered from UPF. While there are several potential culprits involved in the ultra-processing of foods that may have mechanistic interactions with the gut, it is

likely an interplay of several factors surrounding the consumption of UPFs. First, despite being affordable and accessible, UPFs are often high in calories and palatability, but low in essential nutrients, leading to overconsumption and displacement of nutritious meals.[73] Additionally, emulsifiers, used to mix components in UPF, have been extensively studied in murine models, showing their ability to modulate the gut microbiota, reduce mucus thickness and increase intestinal permeability, bacterial translocation and inflammation.[74] While studies translating these same mechanistic effects of emulsifiers in humans are scarce, a short-term prospective study (*N* = 588) found a positive association between dietary emulsifiers and the inflammatory biomarker glycoprotein acetyls.[75] Moreover, a randomized double-blind controlled-feeding study (*N* = 16) with the emulsifier carboxymethylcellulose (CMC) found varied responses in the treatment group, with two subjects exhibiting significant alterations in gut composition and microbial encroachment, whereas other participants seemed non-sensitive to CMC.[76,77] Although constrained by a small sample size, these findings prompt the need to further investigate in large-scale cohorts, as this may in part explain the inconsistent outcomes observed when transitioning from murine to human studies. Overall, these findings underscore that emulsifiers may be one of many components in UPF that may affect the gut microbiome, however more human studies are required to fully understand the complex role of UPF and its effects on the gut microbiome and inflammation. Future studies should also consider examining the synergistic effects of multiple food additives to mirror the composition of UPFs typically consumed by humans.

LIMITATIONS

In addition to inherit limitations of self-reported food diaries (e.g. accuracy of the subject's record), our study also faced limitations in accurately representing specific cultural diets, primarily due to inadequacies in existing databases for Indian cuisine. This led to the use of EpiNu Nutritional software, better tailored for Indian dietary data. Additionally, the shallow depth of shotgun sequencing and taxonomic classification methods used restricted our ability to identify

strain-level differences in bacteria like *P. copri*. Another limitation worth mentioning is the uneven sample sizing across groups. It was considerably more difficult to recruit Indo-Canadians for our study, which we believe may have been due to a combination of cultural, social and geographical barriers.

As this project focused on the relative abundance of *Prevotella* spp., quantitative PCR could also help to determine absolute abundances present in the gut, as this may also be a key factor influencing disease risk [78]. Additionally, while metagenomics can provide important insights into the genetic content present within the gut, these data only represent the functional potential of the gut and does not provide us information regarding genetic expression and actual function of microbiome communities. Overall, future work should aim to resolve strain-level analysis and parasite communities in the microbiomes of this population along with deeper exploration into absolute abundances of taxa and the functional activity present to better understand their increased risk to IBD. Lastly, this study merely provides associative data between westernization and gut microbiome changes; further investigation is needed to understand the mechanistic interactions of specific factors of westernization on the gut and IBD risk.

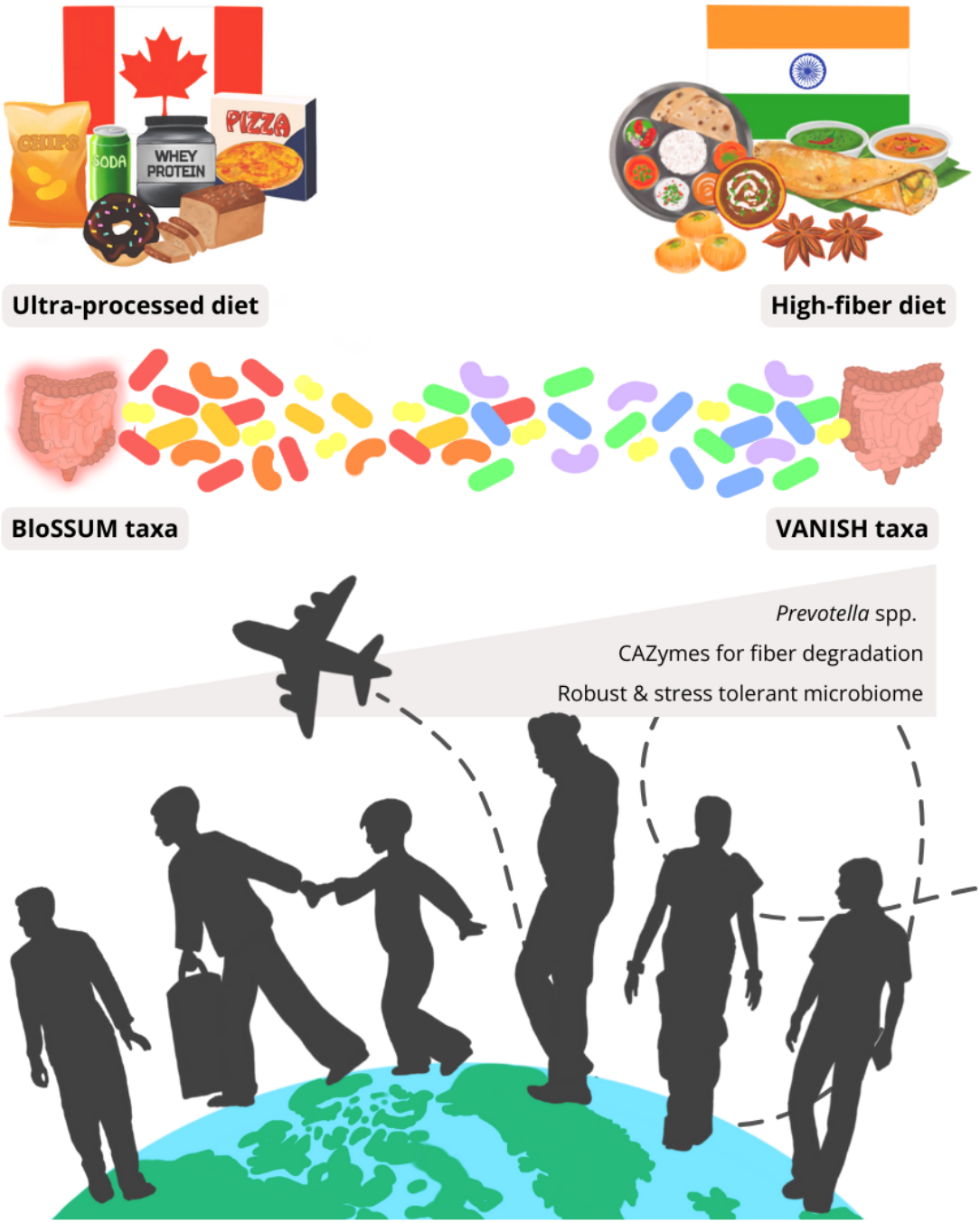

Figure 8. Adoption of Westernized Diet is Associated with a Transition Away from Indian Gut Microbiome

Graphical representation highlighting the main findings from our study. As Indo-Immigrants and Indo-Canadians subsequently increased ultra-processed food intake, their fiber consumption dramatically reduced. This change in dietary pattern was observed alongside a transition away from high *Prevotella* spp. and VANISH (Volatile and/or Associated Negatively with Industrialized Societies of Humans) taxa, which were found abundant in the Indian gut. Instead, Indo-Canadians adopted BloSSUM (Bloom or Selected in Societies of Urbanization/Modernization) taxa, which are commonly found in the westernized microbiome. High *Prevotella copri* in the Indian gut contributed to enriched carbohydrate-active enzymes (CAZymes) that are specialized to degrade complex carbohydrates common in their diet, which decline in Indian migrant cohorts. Additionally, the Indian microbiome displayed characteristics of a more robust gut, with predictive functions of higher stress tolerance and increased microbial cell turnover.

CONCLUSIONS

Consistent with previous studies, our research reveals that the Indian gut microbiome, rich in *Prevotella* spp., is adapted to their high carbohydrate, high fiber diet. We discovered that the Indian microbiome exhibits characteristics of higher bacterial cell turnover, pathogenic potential and stress tolerance, indicating its robustness against stressors posed on the gut (Figure 8). These findings highlight the importance in understanding diverse gut microbiomes, particularly in non-westernized and transitional populations, for betting predicting and managing diseases like IBD. Our study sheds light on the gut microbiome changes in a population undergoing westernization, revealing a drastic transition within the gut microbiomes of Indo-Immigrants and Indo-Canadians, extending our knowledge of the strong effect of immigration and westernization on the gut microbiome. This transition likely reflects microbial adaptation to the dietary acculturation observed, providing insight into the dynamic nature within our gut microbiome. Future investigations should examine the health effects of microbiome changes amidst global westernization, as this transition will likely redefine health outcomes for both migrating populations and those living in rapidly modernizing nations.

Acknowledgements

We thank all the individuals who took part in this study. We thank the entire Ghosh family for support. For dietary analysis, we thank Nadia Anvari. For lab support, we thank Mekenna Smith, Hephzibah Bomide, Carson McComb, Ayva Lewis, Erik Kaila, Andrea Verdugo Meza and Jessica Josephson.

Funding

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and a UBC Killam Research Award to support D.L.G. on her sabbatical in India.

Competing Interests

DLG is the co-founder and CSO of Melius MicroBiomics Inc. where LDD is the Science Communications Specialist.

Patient and Public Involvement

Not applicable.

Ethics Approval

This study was approved by the University of British Columbia Clinical Research Ethics Board (UBC CREB) (H21-01555). Approval for sample collection in India was approved under IEC No 411/2018 and by UBC CREB (H17-01324). All participants signed a consent form prior to participation. A Southeast Asian (SEA) Microbiome Biobank was created.

Data Availability Statement

Data supporting this study's findings are available within the paper and its Supplementary Information. The 16S rRNA gene sequences files and metagenomic sequence files of human fecal samples are publicly available on NCBI under accession number PRJNA1082632. Scripts used for the analysis of this study will be made available upon publication (upload pending).

Supplementary Material

Please find Supplementary Methods and Supplementary Results attached.

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