Succession of the Gut Microbiome in the Tibetan Population of Minjiang River Basin

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Abstract

Background: Tibetans are one of the oldest ethnic groups in China and South Asia. Tibetan has a unique lifestyle and a long history, which leads to the particularity of their gut microflora in composition and function. Different from the Tibetan population on the Qinghai-Tibet Plateau, Tibetans in Minjiang River Basin have gradually increased their migration to Chengdu Plain in recent years. Based on the analysis of 1059 Tibetans in the Minjiang River Basin at an altitude of 500-4001m, we found that the dominant phylum of Tibetan population is *Bacteroides* and *Firmicutes*, and the main genera are *Prevotella* and *Bacteroides*. These findings reflect the characteristics of Tibetan population.

Results: In order to further study the factors affecting gut microbial composition of Tibetan population, 115 total parameters of 7 categories were evaluated. The results showed that altitude was the most important factor affecting the variation of microbial community in Tibetan population, and the change of altitude promoted the succession of gut microbial community. In the process of migration from high altitude to plain, the intestinal microbial composition of late immigrants was similar to that of plateau aborigines, while that of early immigrants was similar to that of plain aborigines. Migration to Tibet is related to the loss of indigenous gut microbial community species. In addition, from low altitude to high altitude, the similarity of microbial community with high altitude population increased with the reproduction of offspring after marriage. And the change of these flora will affect the metabolism, disease and cell function of Tibetan population. The other two sets (AGP and Z208) of altitude data also show the impact of altitude on the microbial community.

Conclusions: This is the first large-scale study on the influencing factors of gut microflora in Tibetan population. Our study confirmed that altitude change is the most important factor affecting the distribution of Tibetan population flora, and provided abundant and unique data to explore the interaction of impact parameter-gut microbiome-host function and disease.

Background

The gut microflora is the largest and most complex microecosystem in the human body [1, 2]. These microorganisms play important roles in digestion, vitamin synthesis, and immune system functioning. Together with the intestinal mucosa, the gut microflora constitutes an important physiological barrier against the invasion of pathogens [3, 4]. The function of the intestinal microbiota depends largely on its composition [3, 4]. Human and bacteria are in a balanced symbiotic relationship. The microbial taxa and abundances are in a dynamic balance and are influenced by environmental conditions, host diet, and genetic factors [5, 6].

A number of studies have shown that changes in the structure of the intestinal flora can lead to metabolic syndrome [7, 8]. Recent clinical studies have found that the transplantation of the intestinal flora can treat a variety of diseases, including Crohn's disease, ulcerative colitis, functional gastrointestinal diseases, type 2 diabetes, antibiotic resistance, alopecia, and neuropsychiatric diseases, such as Parkinson's

syndrome [9–13]. Intestinal microflora transplantation can also affect the efficacy of tumor immunotherapy [14–16].

Despite high similarity in the human gut microbiota among populations, different ethnic groups exhibit differences in the precise composition of the intestinal flora. In a comparative study involving 173 Caucasian infants and 182 South Asian infants, ethnicity was an independent predictor of the intestinal microbial composition of infants; for example, *Bacillus* and *Lactobacillus* are abundant in South Asian infants, while *Fusobacterium* is abundant in Caucasian infants [17]. In addition, some studies have confirmed that there are differences in the intestinal flora between Tibetan and Han populations [18, 19].

The Human Microbiome Project has resulted in increasing information about intestinal microbes. However, little is known about the intestinal microbes of the Tibetan population in China. The Tibetan population, one of 56 ethnic minorities in China, mainly resides in the Tibet autonomous region, Qinghai province, and western Sichuan province, with unique environmental conditions and culture. Ngawa prefecture, with the largest concentration of Tibetan people in Sichuan, has an average altitude of more than 3,000 m. An increase in altitude corresponds with decreases in the atmospheric pressure and oxygen partial pressure. The harsh environment of the plateau as well as the unique language and culture explain the relatively simple genetic background in the population. The high altitude of the plateau confers specific environmental characteristics, including low oxygen, low atmospheric pressure, and high radiation, as well as unique cultural, lifestyle, and dietary habits in Tibetan areas, all of which have been shown to affect the microbiome [20–22]. With a history of over 25,000 years in the area, indigenous Tibetans have adapted to the plateau and therefore serve as a good model for exploring the effects of the environment on the gut microbiota [23].

Migration from non-Western countries to the United States is associated with a reduction in gut microbiome diversity and function and an increased predisposition to metabolic disease [24]. However, the factors that influence changes in the gut microbiome in the Tibetan population are unclear. Tibetan population in Ngawa recently migrated to the Chengdu plain. This change in environmental conditions, cultural practices, and other factors has substantially altered the lifestyle of the Tibetan population. Tibetans at high altitudes mainly eat beef, milk, and highland barley, while populations at low altitudes mainly consume pork and rice as staple foods. In addition, the influence of the change of Tibetan gut microbiome on disease and physiological function also needs to be further explored.

In this study, we assessed the characteristics of the intestinal microflora of the Tibetan population. In particular, we collected fecal samples from 1059 native Tibetan individuals at altitudes of 500–4001 m. We evaluated environmental conditions, dietary habits, disease statuses, and other factors and performed blood biochemical tests. We further assessed the effects of migration on the intestinal flora using 170 Tibetan individuals who migrated from altitudes of over 1000 m to the plain, together with 36 additional migrants who migrated from plain to plateau.

Methods

Subject selection and sampling

Volunteers (natural crowd) were recruited from populations in the Ngawa Tibetan Autonomous Region and Chendu Plain of Sichuan Province. Individuals resided in the cities or towns along the main Minjiang River and tributaries, including Hongyuan, Barkam, Jinchuan, Heishui, Songpan, Wenchuan, Dujiangyan, and Chengdu (Fig. 1a), at altitudes of 500–4001 m. A total of 1251 participants were enrolled (sample information is listed in Table S1), and fecal samples from 1059 native Tibetan individuals were further analyzed. To study the effect of migration, 170 participants were recruited who migrated from the Ngawa area to the Chengdu Plain, together with 36 additional migrants, including those 1) born in the plain who moved to the plateau (Immigrant 1; n = 9), 2) born in the plateau with parents born in the plain (Immigrant 2; n = 20), and 3) born in the plateau with grandparents or ancestors born in the plain (Immigrant 3; n = 7). A questionnaire survey was filled out by all volunteers regarding basic demographic information (age, gender, birthplace, place of residence, ethnicity, etc.), health status (digestive tract diseases, type 2 diabetes, mental health, genetic diseases, etc.), diet (staple food, dietary intake, drinking status, consumption of coffee, tea, and yoghurt, etc.), and exercise (daily physical activity, exercise frequency etc.). The height, weight, and blood pressure of all participants were recorded. Fecal samples were freshly collected and transferred within 6 hours to a -80 °C freezer until use. Peripheral fasting venous blood was collected for routine blood tests (hemoglobin, erythrocyte, white blood cell, and blood platelet counts) and biochemical tests (liver and kidney function, blood glucose, and lipid levels). Standardized procedures were applied at all collection sites by the same trained personnel. Staff and procedures were regularly checked for quality throughout the data collection period. This experiment was approved by the Ethics Committee of Chengdu Medical College (No.2017009). Informed consent was obtained from all participants.

Metadata collection

Based on questionnaire surveys and blood tests, metadata were obtained, including sociodemographic characteristics, anthropometric characteristics, and information about lifestyle, diet, drug use, diseases, and biochemical parameters. In total, 115 factors were screened and further divided into the following 7 categories: Basic, Environment, Drug use, Disease, Diet, Sport, and Biochemical parameters (Table S2).

Sequencing and taxonomic profiling

DNA samples were quantified using the Qubit 2.0 Fluorometer. The V3–V4 hypervariable regions of the 16S rRNA gene from Bacteria and Archaea were amplified using the 341F/806R primer set. Sequencing was performed with a 2 × 250 paired-end (PE) configuration using the Illumina HiSeq platform. The raw PE reads were merged using FLASH (Version 1.2.7) [25], and low-quality and polyclonal sequences were filtered using QIIME (Version 1.9.1) [26]. By further comparison with the Gold database, chimeric reads were removed using Usearch (Version 8.1.1861) [27]. Resulting reads for each sample were clustered into operational taxonomic units (OTUs) at the level of 97% similarity using QIIME (Version 1.9.1). A representative sequence for each OTU was selected and annotation was performed using QIIME (Version

1.9.1) based on the GreenGenes database (Version 13.8) [28]. After random rarefaction of sequences to the minimal number of reads in all samples, microbial composition at each taxonomic level was evaluated using QIIME (Version 1.9.1).

Multivariate association

Missing values from metadata were imputed using the mice package in R (Version 3.3.3) [29], and collinear variables were detected by a Pearson correlation analysis (Pearson $|r| > 0.8$). Correlations between clinical parameters (categorical or numerical) and microbiota community ordination generated by non-metric multi-dimensional scaling (NMDS) based on Bray–Curtis distances were calculated, as previously described [30]. For collinear pairs, variables that were weakly correlated with the microbial community were filtered. Envfit was used in the vegan R package to conduct the MANOVA and to estimate linear correlations of categorical and numerical variables with the microbiota. Fifty factors were selected as significant determinants (10,000 permutations; $P < 0.05$; adjusted $P < 0.05$) of the microbial community, and the effect size (r-value) for each factor was obtained. The combined effect sizes for the 7 categories (Basic, Environment, Disease, Diet, Biochemical, Sport, and Drug) were also generated. After Bray–Curtis distance matrixes of sub-metadata and microbiota community data were generated, the correlation between the two distance matrixes was calculated ($|r|$, combined effect size) by the Mantel test in the vegan R package. In addition, clinical variables with significant contributions to core and unique genus-level community ordination were analyzed. Genera observed in more than 90% of samples were defined as the core microbiota, and genera detected in less than 10% of subjects were defined as unique. The taxonomic tree was visualized using GraPhlAn (Version 1.1.3) [31].

Datasets

Gut microbiota presented in public databases and the literature, including the AGP (American Gut Project Database), LLD (LifeLines-DEEP Database), and the flora resources published by Lan [32] (referred to as S208) and Zhang [22] (referred to as S314) were compared with our data set (referred to as Zang). Data were divided according to ethnic groups or regions to analyze the differences in the composition of the intestinal flora and specific characteristics of populations. To validate the impact of altitude on the gut microbial community, two databases with altitude information (AGP and S208) were used to obtain information for 1244 subjects and 208 Tibetans from six locations in China: Gannan, Gangcha, Tianzhu, Hongyuan, Lhasa, and Nagqu.

Statistical analyses

Alpha diversity indices (i.e., the observed OTUs, Chao1 index, Shannon index, and Simpson index) were measured using QIIME (Version 1.9.1). To quantify differences (Beta diversity) between samples, the phylogeny-based weighted and unweighted UniFrac distances between all pairs of samples were calculated using QIIME. A principal coordinate analysis (PCoA) and NMDS were used to visualize the differences between samples with the ade4 R package.

Enterotyping was performed, as described previously [33]. Briefly, all samples were analyzed by the partitioning around medoids clustering method based on the Jensen–Shannon distances for genus abundances. The optimal number of clusters was estimated using the Calinski-Harabasz (CH) index (where higher values are better). Only genera detected in at least 10% of samples were included in the analysis.

To determine significant associations between clinical variables (categorical or numerical) and genera, a multivariate association analysis was performed using MaAsLin [34]. Spearman's correlation coefficients for relationships between continuous variables and microbiota were determined. The differences in alpha diversity indices, genera, and variables between groups were tested by the Wilcoxon rank sum test or Kruskal–Wallis test, and *P*-values were calibrated by the Benjamin method. Significance was defined as an adjusted *P*-value of < 0.05 .

Results

Altitude affects the variation of microbial groups in Tibetan population

Individuals native Tibetan population to the main Minjiang River and tributaries in Sichuan province, at altitudes of 500–4001 m, were recruited (Fig. 1a). PCoA data for Tibetan samples indicated that *Bacteroidetes* and *Firmicutes* were the two most abundant phyla (Figure S1a). Five core genera were present in Tibetan individuals: *Prevotella* (22.06%), *Bacteroides* (9.08%), *Faecalibacterium* (3.54%), *Lachnospira* (1.43%), and *Ruminococcus* (1.13%), accounting for 32.75% of the total sequences, and within *Bacteroidetes*, the core species mainly belonged to the order *Bacteroidales* and class *Bacteroidia* (Figure S1b). The community richness and community diversity of the microbiome in the Tibetan population in the region were basically in line with previous literature reports (Figure S1c). Based on the CH index, the Tibetan samples were assigned to enterotype 1 (*Prevotella*) and enterotype 2 (*Bacteroides*) (Figure S1e). To assess whether the flora of the Tibetan population is unique, we compared our dataset Zang with LLD (Dutch, 1010 samples), AGP (American, 1313 samples), S314 (healthy young Chinese, 314 samples) and S208 (Tibetan, 208 samples). The 3D map of the flora distribution (Figure S2) indicated that our dataset Zang and S208 of the Tibetan population showed high similarity and were distinguished from the other three datasets, reflecting the specificity of the Tibetan population.

To further study the factors affecting the composition of the gut microbial in the Tibetan population, 115 total parameters in seven broad categories were evaluated. Drug and basic population parameters were the main factors affecting the overall flora composition in seven broad categories (Fig. 1b). With respect to the overall flora and core flora, altitude exerted the strongest effect, followed by age, antibiotic use (within 3 months), fried food, and platelet distribution width (PDW) (Fig. 1c and Figure S3a). The unique microbiota was greatly affected by the clinical indicator alanine aminotransferase (ALT) (Figure S3c). In terms of the seven categories, drug use was the most important determinant of the core flora, consistent with previous findings (Figure S3b) [35]. For the unique microbiota, the environment category had the

greatest impact (Figure S3d). Furthermore, 38 genera significantly correlated with altitude were screened using MaAsLin ($P < 0.01$). *Firmicutes* and *Proteobacteria* were the dominant phyla related to altitude (Fig. 1d).

These results indicated that altitude was indeed the most important factor affecting the gut microbiome in Tibetan populations and further supported the uniqueness of the microflora in Tibetan areas.

Altitude migration drives gut microbiome succession in the Tibetan population

The relationship between altitude and species composition in the Tibetan population was explored to further study successional patterns. The overall composition of the Tibetan microflora varied across altitudes based on an NMDS2 analysis (Figure S4a). The abundances of *Megamonas*, *Bacteroides*, *Prevotella*, *Fusobacterium*, and *Lachnospira* decreased as the altitude increased and were defined as negative with altitude (Figure S4a). In contrast, the abundances of *Coprococcus*, *Dialister*, *Succinivibrio*, *Megasphaera*, and [*Prevotella*] increased as the altitude increased, defined as positive with altitude (Figure S4a). Eight genera were further selected to analyze differences in altitude adaptability (Figure S4b). *Klebsiella* decreased as the altitude increased, while *Lachnospira* and *Megamonas* showed good adaptability to high altitudes and maintained relatively high abundances (Figure S4b). It should be pointed out that the abundance of *Lachnospira* increased significantly ($p < 0.05$) at an altitude of 1,000–2,000 m and then decreased as altitude increased. *Megamonas* showed a higher abundance at 1,000–3,000 m, with a significant ($p < 0.05$) decrease at altitudes above 3,000 m. A steady increase in the abundance of *Oscillospira* was detected with increasing altitude, indicating good adaptability to high altitudes. *Clostridium*, *Lachnobacterium*, and *Akkermansia* all showed relatively stable abundances, except at altitudes exceeding 3,000 m (Figure S4b). Taken together, these results show that alpha diversity (observed OTUs, Chao1 index, Shannon index, and Simpson index) was positively associated with altitude above 1,000 m (Figure S4c). Spearman's correlation analyses were used to evaluate relationships between genera and altitude (Figure S5a). *Clostridium*, *Oscillospira*, *WAL_1855D*, *Succinivibrio*, and *CF231* were positive with altitude, while *Bacteroides*, *Trabulsiella*, *Serratia*, *Erwinia*, and *Citrobacter* were negative with altitude. To eliminate bias due to the uneven distribution of samples at different altitudes, the relative abundances of these genera were acquired from random sampling and transformed into Z-scores (Figure S5b).

Next, we hypothesized that the intestinal flora was influenced by migration. A total of 776 Tibetan individuals were divided into three groups (Fig. 2a): plateau-born (born and living in the plateau, $n = 586$), plain-born (born and living in the plain, $n = 20$) and plateau-Trans (born in the plateau and moved to the plain, $n = 170$). An NMDS2 plot was generated based on the genus profile and the time of migration to the plain and was then used for a linear fitting analysis. As shown in Fig. 2b, the gut microbiome composition differed with respect to migration time. In particular, late migrants had similar gut microbial components to those of the indigenous population on the plateau, while early migrants had similar microbial components to those of the native population on the plain. In terms of alpha diversity (Fig. 2c), earlier

migrants exhibited higher levels of microbial diversity, consistent with that of samples from the plain. Standard deviations (SD) in prevalence in different groups revealed convergent losses in diversity from the plateau to the plain, indicating that the time of migration was correlated with the loss of alpha diversity. The ratio of *Bacteroides* to *Prevotella* is an important indicator of the status of bacteria [36]; accordingly, we analyzed the distribution of the log-normalized *Bacteroides*-to-*Prevotella* (B/P) ratio in different groups (Fig. 2d). The longer the time since migration, the higher the B/P ratio and the closer the ratio was to that of the indigenous population on the plains. Furthermore, a Spearman's correlation analysis of the overall distribution showed that the genus type was associated with the year of migration. Based on abundance profiles transformed into Z-scores (Fig. 2e), species with significantly higher abundances in the earlier migrants were also more abundant in the population in the plain.

In brief, altitude drives gut microbiome succession in the Tibetan population, and Tibetan migration is associated with the loss of diversity in the gut microbiome.

The immigration generation from plain to plateau is related to the microbiome succession

In addition, to study the effect of migration, 170 participants were recruited who migrated from the Ngawa area to the Chengdu Plain, together with 36 additional migrants, including born in the plain who moved to the plateau (Immigrant 1; n = 9), born in the plateau with parents born in the plain (Immigrant 2; n = 20), and born in the plateau with grandparents or ancestors born in the plain (Immigrant 3; n = 7) (Fig. 3a). Based on the genus profile to determine the overall microflora structure, the flora in each generation of migrants was different from that of samples from the plain and differed with respect to ethnicity (Fig. 3b). We obtained six genera in which abundance was significantly correlated with migration by the MaAsLin method. *Lachnospira* had a high abundance in samples from the plain, and the abundance gradually decreased across generations. *Klebsiella* had a high abundance in the plains group only (Fig. 3c). In terms of alpha diversity in plateau-born, plain-born, and 36 plain-to-plateau individuals (Fig. 3d), the SD in different groups revealed convergence in variation from the plain to the plateau. In general, the shorter the time since migration, the smaller the difference in sample diversity, and the longer the migration time, the greater the difference in sample diversity (Fig. 3d).

Altitude migration could affect the diversity of Tibetan gut microbiome

In order to further explore the influence of altitude migration on gut microbiome of Tibetan population. We analyzed the change of core flora in different periods from high altitude to low altitude (year of migration), and the core flora changed in different generations from low altitude to high altitude (immigration). Through correlation analysis, the correlation network showed that there were 15 bacterial communities changed in the process of altitude migration is consistent. Among them, *Lachnospira*, *Bacteroides* and *Clostridium* 2 are negatively correlated with the altitude migration, while the changes of *Lactobacillus*, [*Prevotella*], *Dialister*, *Prevotella*, *Succenivibrio*, *Catenibacterium*, *Collinsella*, [*Eubacterium*],

CF231, *Slackia*, *Oxalobacter*, *Dehalobacterium* are positively correlated with the altitude migration (Fig. 4a).

We further analyzed the influence of 115 parameters on the evolution of gut microbiome in the process of altitude migration. For people migrating from plateau to plain, altitude and fried food were significantly contributed to diversification of Chao1 index (Fig. 4b), drinking-type was significantly contributed to taxonomic composition (Fig. 4c). For people migrating from plain to plateau, altitude and fried food also had the greatest impact on the Chao1 index (Fig. 4b), and altitude was significantly contributed to taxonomic composition (Fig. 4c).

Altitude migration could affect the physiological function and disease of Tibetan population

The changes of gut microbiome are related to physiological function and disease of host. In order to study the influence of altitude migration on the physiological function and disease of Tibetan population, we conducted Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Predicted KEGG pathways revealed that metabolism, signal transduction, transcription, excretory and nervous system were positive with the year of migration from plateau to plain (Fig. 5a). While cancer, immune and digestive system, cell growth and death, replication and repair were negative with the year of migration from plateau to plain (Fig. 5a). We further analyzed the influence of gut microbial changes on Tibetan population from plain to plateau. KEGG analysis showed that metabolism, signal transduction, transcription, excretory and nervous system, poorly characterized and cellular processes and signaling were negative with the immigration generations from plain to plateau (Fig. 5b and Figure S6). While cell growth and death, replication and repair, translation, folding sorting and degradation, cancer and digestive system were positive with the immigration generations from plain to plateau (Fig. 5b and Figure S6). The above analysis showed that with the migration of altitude, the changes of microorganisms mainly affect the metabolism, signal transduction, transcription, cancer, digestive system, cell growth and death and replication and repair of Tibetan population.

Construction of the network of impact parameter- gut microbiome - function

Gram staining of bacteria is one of the important characteristics to distinguish bacterial species, which could guild for the diagnosis and treatment of diseases [37]. So, we analyzed the change of gram positive and gram-negative bacteria in the process of altitude migration. Among the people who migrate from the plateau to the plain, the shorter the migration time, the more gram-negative bacteria and the less gram-positive bacteria; the stronger the pathogenic potential of bacteria; the lower the content of mobile elements (Fig. 6a). Because the change of altitude mainly leads to the change of environmental oxygen content, we further analyzed the change of aerobic and anaerobic bacteria in the process of altitude migration. The shorter the migration time, the less anaerobic bacteria, the more facultative anaerobic bacteria and the better the oxygen tolerance (Figure S7). In addition, for people who migrate from the

plain to the plateau, the longer the migration time, the lower the content of mobile components, the more gram-negative bacteria and the less gram-positive bacteria (Figure S8).

At last, based on altitude and dietary factors that have the greatest impact on gut microbiome, 11 bacterial communities changed in the process of altitude migration and the changes of physiological functions associated with gut microbiome, we constructed the network of impact parameter-gut microbiome- function. It was found that *Prevotella* was the most closely related to the relevant KEGG pathways, and the factors most related to the genus and metabolic pathways were altitude, milktea, vegetables, friedfood, sweet (Fig. 6b).

Altitude could affect the composition of the gut microbiome in other ethnic groups

To analyze the effect of altitude on other populations, we compared our data with the two datasets AGP and S208, for which altitude information is available. We used PCoA plots based on Bray–Curtis dissimilarity at the genus level to depict the overall distribution of the intestinal flora at different altitudes. Under different data sets, altitude clearly distinguished samples (Fig. 7a). Similarly, when we analyzed AGP and S208 separately, the high-altitude samples in AGP and S208 could be differentiated in the PCoA plots (Figure S9a, b). Based on Spearman's correlation coefficients, we identified correlations between relative abundances of genera and altitude in S208 (top) and Zang (bottom) (Fig. 7b). In our data set (Zang), *Bacteroides* was more abundant in low-altitude samples; however, in the S208 dataset, the taxon was more abundant at high altitudes. In addition, other taxa that were positively correlated with altitude in our dataset were also found in S208. All samples from the S208 dataset were clustered into enterotype 1 (*Prevotella*) and enterotype 2 (*Bacteroides*) by PCoA based on Jensen–Shannon distances (Figure S9c), consistent with the results for our dataset. In another Tibetan population (dataset S208), the core genera belonging to Bacteroidetes were *Prevotella* and *Bacteroides* and in Firmicutes was *Faecalibacterium* (Figure S9d). The core genera identified in S208 were also identified in our dataset, illustrating the similarity in the intestinal flora of Tibetan populations from different regions.

Discussion

This is the first large-scale study of the gut microflora of the Tibetan population in Ngawa. In the course of human history, civilization is often formed along the river. To evaluate microbial succession in the Tibetan population, we collected 1059 samples from the Ngawa Tibetan autonomous region and Chendu plain of Sichuan province, from upstream to downstream along the Minjiang river and tributaries in Sichuan province of China. Our results showed that the gut microbiota of the Tibetan population was dominated by *Bacteroidetes* and *Firmicutes*. And the flora distribution of Tibetan population was distinguished from the other three datasets (LLD, AGP, S314). As the altitude increased, bacterial diversity increased, consistent with previous results [38]. Zhang et al. [22] studied the gut microbiome of 314 healthy young people from 7 ethnic groups in 20 villages and towns in 9 provinces of China, and obtained nine core genera, *Phascolarctobacterium*, *Roseburia*, *Bacteroides*, *Blautia*, *Faecalibacterium*,

Clostridium, *Subdoligranulum*, *Ruminococcus*, and *Coprococcus*. In comparison, the unique core genera in the Tibetan population in Ngawa were *Prevotella* and *Lachnospira*. *Prevotella* mainly participates in the metabolism of carbohydrates and plant proteins as well as short-chain fatty acid production [39]. Many *Lachnospira* strains produce butyrate, which plays a crucial role in the maintenance of human gut health [40]. These bacteria produce short-chain fatty acids, which can act as anti-inflammatory agents [40]. It may explain how Tibetans with a low dietary fiber intake maintain gut health. These bacteria may be related to adaptation to the low-oxygen environment and may be beneficial to Tibetans in high-altitude areas. The Tibetan microflora can be divided into two enterotypes, as observed in both our dataset (Zang) and S208. A previous study has reported that the human gut microbiota can be divided into three enterotypes, *Prevotella*, *Bacteroides* and *Ruminococcus* [41]. The gut microbiota of the Tibetan population mainly consisted of *Prevotella* and *Bacteroides*, while *Ruminococcus* accounted for only a small portion of sequence reads (~ 1%). We speculate that this can be explained through the unique diet of Tibetans. *Ruminococcus* is mainly related to the digestion of carbohydrates, such as sugar, starch, and potato [42]. Tibetans prefer high-protein, high-fat, and high-fiber foods, like highland barley, while carbohydrate consumption is relatively low.

Wang W et al [43] studied six regions of the plateau and found that location, altitude, BMI, age, and other factors affected the microbial composition. However, the main determinant of the flora was not established, and the study involved a small sample size. Deschasaux [44] studied the gut flora of more than 2,000 individuals of different ethnicities in the same city and showed that ethnicity contributes significantly to individual differences in the gut microbiome, independent of metabolic health. Accordingly, studies of human flora should consider ethnicity. A study about the gut microbiome of more than 7,000 individuals in 14 districts of Guangdong Province and found that regional factors have a significant impact on the flora [45].

Most Tibetans live in the harsh environment of high altitude, low pressure and hypoxia. Under such challenging conditions, the correlation between gut microbiome determinants and physiological responses and clinical problems is very important. Our study confirmed that altitude had the greatest effect on microbial composition. This is also reflected in the analysis of the immigrants from the Qinghai Tibet Plateau and the Han immigrants from the plain to the plateau [46]. The important influence of altitude on flora was further proved by other two datasets AGP and S208. High altitude is related to low pressure, hypoxia, low temperature and high radiation. These factors all affect biological processes. Hypoxia can produce reactive oxygen species through Grb2 / EGFR / PTPN11 pathway, increase the expression of HIF-1 α and iNOS, thin intestinal mucosa, irregular and less epithelial cells, and down-regulation of tight junction protein [47–48]. These changes can lead to mucosal barrier dysfunction. In addition, hypoxia can affect the intestinal mucosal immune system [49]. Low temperature can also affect the gut microbiome, which has a protective effect on obesity [50]. With regard to solar radiation, studies have proposed the intestinal skin axis and have shown that skin exposure can regulate gut microbiome [51, 52]. The effect of altitude on flora may be the result of the above factors. The effect of altitude on flora reflects the adaptability of gut microbiome. Among the groups with positive correlation between abundance and altitude, *Clostridium* was previously identified by Adak et.al [53]. *Lachnobacterium*,

Akkermansia and *Eubacterium rectum* are mucus related microorganisms that are beneficial to human health [54]. Interestingly, Vangay et. al found that the short-term response of the whole microbial community to migration varied from individual to individual, but the migration of the main native and dominant groups in the United States began within 6 to 9 months of residence in the United States [24]. Soldiers who moved from plain to high place for 15 days showed a decrease in total oxygen demand and an increase in total anaerobes and facultative anaerobes [55]. At the same time, our study confirmed that during the migration from high altitude to plain, the intestinal microbial composition of late immigrants was similar to that of plateau aborigines, while that of early immigrants was similar to that of plain aborigines. Migration to Tibet is related to the loss of indigenous intestinal microbial community species. In addition, after marriage, the similarity between the microbial community and the high-altitude area increased with the reproduction of offspring. Finally, our analysis of different datasets (Z208, USA) further proves the important influence of altitude on gut microbiome.

We confirmed that altitude is an important factor affecting the succession of gut microbiome. What are the effects of these changes on the function of Tibetan population? Our study found that in the migration process of Tibetan population, the change of gut microbiome is most related to metabolism, cell growth and death, signal transmission, cancer, immune system and digestive system. Alessia Visconti et.al studies have shown that gut genome was closely related to the host systemic metabolism. The metabolic pathway was significantly correlated with 95% of the fecal metabolites, while the microbial species were related to 82% of the fecal metabolites. The carcinogenesis of colorectal cancer was significantly correlated with gut microbiome. There are some evidences to prove the pathogenesis of *Fusobacterium nucleatum* and colorectal cancer [56, 57]. At the same time, intestinal microflora plays a very important role in the treatment of tumor. The influence of intestinal microbiota on immune checkpoint blocking reaction was studied in mouse model for the first time. In 2015, a landmark paper was published in science, which proved that the composition of intestinal microbiota can affect the immune checkpoint for cytotoxic T lymphocyte antigen-4 (CTLA-4) inhibitor response, and death receptor 1 (PD-1) [15, 16]. And, recent studies have shown that gut microbiota is associated with immune system diseases. For example, the development of autoimmune diseases, especially *P.gingivalis* [58]. Hevia et al found that the ratio of intestinal *Pachymete/Bacteroides* decreased in patients with systemic lupus erythematosus, indicating that mucosal immune dysfunction in patients with systemic lupus erythematosus affects intestinal microbiota [59]. *Bacteroides* found in the human gut fragile substances play an active regulatory role in the human immune system [60]. These studies highlight the complex interaction between gut microbiome and host function.

Gram staining of bacteria is one of the important characteristics of distinguishing bacterial species, which can guide the diagnosis and treatment of diseases [37]. Our research found that the shorter the migration time, the more gram-negative bacteria and the less gram-positive bacteria among people migrating from plateau to plain. This finding has a good clinical guiding significance with the infection of Tibetan people and the choice of antibiotic.

Conclusion

Tibetans are one of the oldest ethnic groups in China and South Asia. As an important ethnic group in China, Tibetans have formed a unique lifestyle and customs to adapt to the harsh environment. Studying the composition of the gut microbiota of the Tibetan population can provide insight into differences in microbial colonization among regions and ethnic groups as well as the contributions of the unique adaptive lifestyle, customs, and dietary habits of Tibetans to the intestinal micro-ecology. Different from the Tibetan population on the Qinghai-Tibet Plateau, Tibetans in Minjiang River Basin have gradually increased their migration to Chengdu Plain in recent years. In order to further study the factors affecting gut microbial composition of Tibetan population, 115 total parameters of 7 categories were evaluated. Our study was the first large-scale study on the influencing factors of gut microflora in Tibetan population. We indicated that altitude was indeed the most important factor affecting the gut microbiome in Tibetan populations and further supported the uniqueness of the microflora in Tibetan areas. And the change of altitude promoted the succession of gut microbial community. The other two datasets (AGP and Z208) of altitude data also show the impact of altitude on the microbial community. Furthermore, our study provided abundant and unique data to explore the interaction of impact parameter-gut microbiome-host function and disease.

Abbreviations

NMDS: non-metric multi-dimensional scaling; AGP: American Gut Project Database; LLD: LifeLines-DEEP Database; PCoA: principal coordinate analysis; CH index: Calinski-Harabasz index; PDW: platelet distribution width; ALT: alanine aminotransferase; KEGG: Kyoto Encyclopedia of Genes and Genomes. CTLA-4: cytotoxic T lymphocyte antigen-4; PD-1: death receptor 1.

Declarations

Ethics approval and consent to participate

This experiment was approved by the Ethics Committee of Chengdu Medical College (No.2017009). Informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Jun Li mainly contributes to design this study and written the original manuscript. Lin Sun, Chunfeng Mo and Xiangsheng Fu designed the study. Baijun Chen, Jing Liu, Xuemei Li, Lingmeng Song and Wen Yang responsible for the collection and processing of samples. Luo Zuo, Yan Zhou, Jingping Sun, Ling Qin and Feng He supervised and administered the project and provided funding. Li Deng mainly involved in the revision of the manuscript. Yuanqin Tang, Lin Yang, Lesiji Kang, Yonghua He and Songbo Wang involved in sample storage and processing. Junru Chen and Xian Yue Wang compiled and curated the data and performed bioinformatic analysis. Xiaofeng Qin and Xiaoan Li were responsible for project implementation and quality control. All co-authors contributed substantially to manuscript revisions. All authors read and approved the final manuscript.

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References

1. Charbonneau MR, Blanton LV, DiGiulio DB, Relman DA, Lebrilla CB, Mills DA, et al. A microbial perspective of human developmental biology. *Nature*. 2016;535:48–55.
2. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464:59–65.
3. Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol*. 2012;9:577–89.
4. Wang H, Zhang W, Zuo L, Dong J, Zhu W, Li Y, et al. Intestinal dysbacteriosis contributes to decreased intestinal mucosal barrier function and increased bacterial translocation. *Lett Appl Microbiol*. 2014;58:384–92.
5. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. *Cancer Cell*. 2018;33:570–80.
6. Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A*. 2010;107:14691–6.

7. Sedighi M, Razavi S, Navab-Moghadam F, Khamseh ME, Alaei-Shahmiri F, Mehrtash A, et al. Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals. *Microb Pathog.* 2017;111:362–9.
8. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. *World J Gastroenterol.* 2014;20:16079–94.
9. Newman KM, Rank KM, Vaughn BP, Khoruts A. Treatment of recurrent *Clostridium difficile* infection using fecal microbiota transplantation in patients with inflammatory bowel disease. *Gut Microbes.* 2017;8:303–9.
10. Vindigni SM, Surawicz CM. Fecal Microbiota Transplantation. *Gastroenterol Clin North Am.* 2017;46:171–85.
11. El-Salhy M, Mazzawi T. Fecal microbiota transplantation for managing irritable bowel syndrome. *Expert Rev Gastroenterol Hepatol.* 2018;12:439–45.
12. Guido J, Bakker M, Nieuwdorp. Fecal Microbiota Transplantation: Therapeutic Potential for a Multitude of Diseases beyond *Clostridium difficile*. *Microbiol Spectr.* 2017;5:4.
13. Bibbò S, Ianaro G, Gasbarrini A, Cammarota G. Fecal microbiota transplantation: past, present and future perspectives. *Minerva Gastroenterol Dietol.* 2017;63:420–30.
14. Chen D, Wu J, Jin D, Wang B, Cao H. Fecal microbiota transplantation in cancer management: Current status and perspectives. *Int J Cancer.* 2019;145:2021–31.
15. Ayelet Sivan L, Corrales N, Hubert, Jason B, Williams K, Aquino-Michaels, Zachary M, Earley, et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science.* 2015;350:1084–9.
16. Marie Vétizou, Jonathan M, Pitt R, Daillère P, Lepage N, Waldschmitt C, Flament, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science.* 2015;350:1079–84.
17. Jennifer C, Stearns MA, Zulyniak RJ, de Souza NC, Campbell M, Fontes M, Shaikh, et al. Ethnic and diet-related differences in the healthy infant microbiome. *Genome Med.* 2017;9:32.
18. Li L, Zhao X. Comparative analyses of fecal microbiota in Tibetan and Chinese Han living at low or high altitude by barcoded 454 pyrosequencing. *Sci Rep.* 2015;5:14682.
19. Li K, Dan Z, Gesang L, Wang H, Zhou Y, Du Y, et al. Comparative Analysis of Gut Microbiota of Native Tibetan and Han Populations Living at Different Altitudes. *PLoS One.* 2016;11:e0155863.
20. Filippo CD, Paola MD, Ramazzotti M, Albanese D, Pieraccini G, Banci E, et al. Diet, Environments, and Gut Microbiota. A Preliminary Investigation in Children Living in Rural and Urban Burkina Faso and Italy. *Front Microbiol.* 2017; 8:1979.
21. Samuel A, Smits J, Leach ED, Sonnenburg, Carlos G, Gonzalez JS, Lichtman G, Reid, et al. Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. *Science.* 2017;357:802–6.
22. Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, et al. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISME J.* 2015;9:1979–90.

23. Mark Aldenderfer. Peopling the Tibetan plateau: insights from archaeology. *High Alt Med Biol* Summer. 2011;12:141–7.
24. Vangay P, Johnson AJ, Ward TL, Al-Ghalith GA, Shields-Cutler RR, Hillmann BM, et al. US Immigration Westernizes the Human Gut Microbiome. *Cell*. 2018;175:962–72.
25. Tanja Magoč, Steven L, Salzberg. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. 2011;27:2957–63.
26. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010;7:335–6.
27. Robert C, Edgar, Brian J, Haas JC, Clemente C, Quince. Rob Knight. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. 2011;27:2194–200.
28. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol*. 2006;72:5069–72.
29. Zhang Z. Missing data exploration: highlighting graphical presentation of missing pattern. *Ann Transl Med Ann Transl Med*. 2015;3:356.
30. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, et al. Population-level analysis of gut microbiome variation. *Science*. 2016;352:560–4.
31. Francesco Asnicar G, Weingart TL, Tickle C, Huttenhower. Nicola Segata.. Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *PeerJ*. 2015;3:e1029.
32. Lan D, Ji W, Lin B, Chen Y, Huang C, Xiong X, et al. Correlations between gut microbiota community structures of Tibetans and geography. *Sci Rep*. 2017;7:16982.
33. Manimozhiyan Arumugam J, Raes E, Pelletier DL, Paslier T, Yamada, Daniel R, Mende, et al. Enterotypes of the human gut microbiome. *Nature*. 2011;473:174–80.
34. Xochitl C, Morgan TL, Tickle H, Sokol D, Gevers KL, Devaney DV, Ward, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol*. 2012;13:R79.
35. Lisa Maier M, Pruteanu M, Kuhn G, Zeller A, Telzerow EE, Anderson, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*. 2018;555:623–8.
36. Hjorth MF, Roager HM, Larsen TM, Poulsen SK, Licht TR, Bahl MI, et al. Pre-treatment microbial Prevotella-to-Bacteroides ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. *Int J Obes (Lond)*. 2018;42:284.
37. Boyanova L. Direct Gram staining and its various benefits in the diagnosis of bacterial infections. *Postgrad Med*. 2018;130:105–10.
38. Kumar S, Suyal DC, Yadav A, Shouche Y, Goel R. Microbial diversity and soil physiochemical characteristic of higher altitude. *PLoS One*. 2019;14:e0213844.
39. De Filippis F, Pellegrini N, Laghi L, Gobetti M, Ercolini D. Unusual sub-genus associations of faecal Prevotella and Bacteroides with specific dietary patterns. *Microbiome*. 2016;4:57.

40. Paggi RA, Rodríguez C, Fay JP. Growth and pectate-lyase activity of the ruminal bacterium *Lachnospira multiparus* in the presence of short-chain organic acids. *Lett Appl Microbiol.* 2005;41:434–9.
41. Roager HM, Licht TR, Poulsen SK, Larsen TM, Bahl MI. Microbial enterotypes, inferred by the prevotella-to-bacteroides ratio, remained stable during a 6-month randomized controlled diet intervention with the new nordic diet. *Appl Environ Microbiol.* 2014;80:1142–9.
42. Crost EH, Le Gall G, Laverde-Gomez JA, Mukhopadhyaya I, Flint HJ, Juge N. Mechanistic Insights Into the Cross-Feeding of *Ruminococcus gnavus* and *Ruminococcus bromii* on Host and Dietary Carbohydrates. *Front Microbiol.* 2018;9:2558.
43. Wang W, Wang F, Li L, Wang A, Sharshov K, Druzyaka A, et al. Characterization of the gut microbiome of black-necked cranes (*Grus nigricollis*) in six wintering areas in China. *Arch Microbiol.* 2020;202:983–93.
44. Deschasaux M, Huybrechts I, Julia C, Hercberg S, Egnell M, Srour B, et al. Association between nutritional profiles of foods underlying Nutri-Score front-of-pack labels and mortality: EPIC cohort study in 10 European countries. *BMJ.* 2020;370:m3173.
45. He Y, Wu W, Zheng H-M, Li P, McDonald D, Sheng H-F, et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nat Med.* 2018;24:1532–5.
46. Hauenschild F, Favre A, Schnitzler J, Michalak I, Freiberg M, Muellner-Riehl AN, et al. Spatio-temporal evolution of *Allium L.* in the Qinghai-Tibet-Plateau region: Immigration and in situ radiation. *Plant Divers.* 2017;39:167–79.
47. Li K, Gesang L, Dan Z, Lamu Gusang. Genome-Wide Transcriptional Analysis Reveals the Protection against Hypoxia-Induced Oxidative Injury in the Intestine of Tibetans via the Inhibition of GRB2/EGFR/PTPN11 Pathways. *Oxid Med Cell Longev.* 2016; 2016: 6967396.
48. Zhang F, Wu W, Deng Z, Zheng X, Zhang J, Deng S, et al. High altitude increases the expression of hypoxia-inducible factor 1alpha and inducible nitric oxide synthase with intestinal mucosal barrier failure in rats. *Int J Clin Exp Pathol.* 2015;8:5189–95.
49. Han Luo D-J, Zhou Z, Chen Q-Q, Zhou K, Wu K, Tian, et al. Establishment and evaluation of an experimental rat model for high-altitude intestinal barrier injury. *Exp Ther Med.* 2017;13:475–82.
50. Ziętak M, Kovatcheva-Datchary P, Markiewicz LH, Ståhlman M, Kozak LP, Bäckhed F. Altered Microbiota Contributes to Reduced Diet-Induced Obesity upon Cold Exposure. *Cell Metab.* 2016;23:1216–23.
51. Magdolna, Szántó. Anikó Dózsa, Dóra Antal, Kornélia Szabó, Lajos Kemény, Péter Bai. Targeting the gut-skin axis-Probiotics as new tools for skin disorder management? *Exp Dermatol.* 2019;28:1210–8.
52. Bosman E, Albert A, Lui H, Dutz J, Vallance B. Skin Exposure to Narrow Band Ultraviolet (UVB) Light Modulates the Human Intestinal Microbiome. *Front Microbiol.* 2019;10:2410.
53. Adak A, Maity C, Ghosh K, Pati BR, Mondal KC. Dynamics of predominant microbiota in the human gastrointestinal tract and change in luminal enzymes and immunoglobulin profile during high-altitude adaptation. *Folia Microbiol (Praha).* 2013;58:523–8.

54. Gillois K, Lévêque M, Théodorou V, Robert H, Mercier-Bonin M. Mucus: An Underestimated Gut Target for Environmental Pollutants and Food Additives. *Microorganisms*. 2018;6:53.
55. Howard M, Bakker-Dyos J, Gallagher L, O'Hara JP, Woods D, Mellor A. Power supplies and equipment for military field research: lessons from the British Service Dhaulagiri Research Expedition 2016. *J R Army Med Corps*. 2018;164:41–5.
56. Shang FM, Liu HL. *Fusobacterium nucleatum* and colorectal cancer: A review. *World J Gastrointest Oncol*. 2018;10:71–81.
57. Hashemi Goradel N, Heidarzadeh S, Jahangiri S, Farhood B, Mortezaee K, Khanlarkhani N, et al. *Fusobacterium nucleatum* and colorectal cancer: A mechanistic overview. *J Cell Physiol*. 2019;234:2337–44.
58. Ohtsu A, Takeuchi Y, Katagiri S, Suda W, Maekawa S, Shiba T, et al. Influence of *Porphyromonas gingivalis* in gut microbiota of streptozotocin-induced diabetic mice. *Oral Dis*. 2019;25:868–80.
59. Kim JW, Kwok SK, Choe JY, Park SH. Recent Advances in Our Understanding of the Link between the Intestinal Microbiota and Systemic Lupus Erythematosus. *Int J Mol Sci*. 2019;20:4871.
60. Thaïss CA, Levy M, Suez J, Elinav E. The interplay between the innate immune system and the microbiota. *Curr Opin Immunol*. 2014;26:41–8.

Figures

Figure 1

Altitude affects the variation of microbial groups in Tibetan population. (a) Overview of sampling regions in Sichuan province of China; red dots indicated sampling sites at different regions, blue lines represented Dadu River (left) and Min River (right). Effect size of 7 categories (b) and top 50 factors (c) found to significantly influence Tibetan gut microbiota were displayed. Factors were sorted by effect size and colored by 7 categories (Basic, Environment, Disease, Diet, Biochemical, Sport and Drug). (d) Taxonomic tree of 38 genera with significant correlations (adjusted $P < 0.1$) with altitude, as determined using MaAsLin. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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Figure 2

Altitude migration drives gut microbiome succession in the Tibetan population. (a) Plateau-born ($n = 586$), plain-born ($n = 20$), and migrants (Plateau to Plain, $n = 170$) are marked on the graphics. (b) Left: NMDS plot based on the genus profile from 170 plateau to Plain individuals; each dot represents one individual and the color associated with the year of migration. Linear fitting is based on the first NMDS dimension ($t = 4.94$, $P = 1.85e-06$). Right: Plateau (plateau-born) and Plains (plain-born) individuals were analyzed by NMDS, and the difference between groups in the first dimension is also visualized by a

boxplot ($P = 1.589 \times 10^{-11}$), the NMDS1 values for Plateau-born individuals were the averages of 50 samples by random sampling (100 times). (c) Alpha diversity (observed OTUs, Chao1 index, Shannon index, and Simpson index) between Plateau-born, plain-born, and Plateau to Plain individuals, P-values are from the Kruskal–Wallis test. The standard deviation (SD) in different groups revealed convergence in variance from the plateau to the plain, *, $P < 0.05$. (d) Distribution of the log-normalized Bacteroides-to-Prevotella (B/P) ratio between different groups, P-values are based on Kruskal–Wallis test. (e) Relative abundance of genera correlated with the year of migration (Spearman's correlation, adjusted $P < 0.05$); the abundance profiles are transformed into Z scores, where negative a Z score means a lower abundance than the mean and a positive Z score represents a higher abundance than the mean. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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Figure 3

The immigration generation from plain to plateau is related to the microbiome succession. (a) Immigrant 1 (born in the plains and moved to the plateau), Immigrant2 (born in plateau and whose parents born in plain), Immigrant3 (born in plateau and whose grandparents born in plain), plateau-born (born and living in plateau), plains-born (born and living in plain) and plateau-Trans (born in plateau and moved to plain) are marked on the graphics. (b) NMDS analysis based on the genera profile from plateau-born(n=586), plain-born(n=20) and plain to plateau(n=36) individuals, difference between nations was showed at the bottom ($P = 0.009822$) and difference between migrate generations was displayed at the top ($P = 0.04825$), P values are from Kruskal-Wallis test, the NMDS1 values for Plateau-born individuals were the

averages of 50 samples at random sampling (20 times). (c) Relative abundance of genera significantly correlated with migrate generation (Spearman's correlation, adjusted $P < 0.05$). (d) Alpha diversity between plateau-born, plain-born and 36 plain to plateau individuals, the Standard Deviation (SD) in different groups revealed convergence variant from plateau to plain, *, $P < 0.05$. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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Figure 4

Altitude migration could affect the diversity of Tibetan gut microbiome. (a) Correlation network of genera significantly correlated with the year of migration or the generation of immigration (adjusted $P < 0.05$). Factors significantly contributed to diversification of Chao1 index (b) and taxonomic composition (c) were also displayed.

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Figure 5

Altitude migration could affect the physiological function and disease of Tibetan population. (a) Abundance of predicted KEGG pathways significantly correlated with the year of migration from plateau to plain (Spearman's correlation, adjusted $P < 0.05$); colors of heatmap mean lower abundance or higher abundance in different groups. (b) Relative abundance of 20 predicted KEGG pathways which were significantly correlated with immigration generations from plain to plateau (Maaslin q value < 0.1).

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Figure 6

Construction of the network of impact parameter- gut microbiome – function. (a) Microbiome phenotypes significantly correlated with the year of migration from plateau to plain (Spearman's correlation, adjusted $P < 0.05$) and immigration generations from plain to plateau (Kendall's correlation, adjusted $P < 0.05$). (b) Correlation network between 15 common significantly genera and 20 common predicted KEGG pathways. Their correlations with factors were also displayed (Spearman's correlation, adjusted $P < 0.05$, $|r| > 0.3$).

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Figure 7

Altitude could affect the composition of the gut microbiome in other ethnic groups. (a) PCoA plot based on Bray-Curtis dissimilarity at the genus level from three datasets (Zang, n=1059; AGP, n=1244; S208, n=208), the color and size of dots represent different datasets and altitudes. (b) Relative abundance of genera significantly correlated with altitude at the dataset of S208 (top) and Zang (bottom) by Spearman's correlation (adjusted P <0.05). The abundance profiles are transformed into Z score, negative Z score represents lower abundance than mean and a positive Z score represents higher abundance than mean.

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