Metagenomic analysis reveals the signature of gut microbiota associated with human chronotypes

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Abstract

Patterns of diurnal activity differ substantially between individuals, with early risers and late sleepers being examples of extreme chronotypes. Growing evidence suggests that the late chronotype significantly impacts the risk of developing mood disorders, obesity, diabetes, and other chronic diseases. Despite the vast potential of utilizing chronotype information for precision medicine, the factors that shape chronotypes remain poorly understood. Here, we assessed whether chronotypes are associated with different gut microbiome composition. Using metagenomic sequencing, we established a distinct signature associated with chronotype that involves two bacterial genera *Alistipes* (elevated in “larks”) and *Lachnospira* (elevated in “owls”). We have identified four metabolic pathways (e.g. gluconegonesis) that were associated with early chronotype.

Significance Statement

Studies in recent years uncover a significant relationship between the circadian system and the composition of the gut microbiota. However, the extent to which microbiome variability is associated with individual diurnal timing in humans (chronotype) is unknown. In this study we employed metagenomic sequencing and identified for the first time, a distinct microbiome signature that differs between early and late chronotypes. Given that the microbiome has been shown to be directly linked with food consumption and diet, this study may pave the way for diet-driven interventions aimed to ameliorate health problems associated with extreme chronotypes.

Introduction

Numerous biological, mental, and behavioral functions exhibit circadian oscillations that are orchestrated by a central pacemaker in the brain. While the periodicity of these rhythms under natural conditions is uniform (24 hr), the phase of these oscillations shows considerable inter-individual variability. Phase variation is often manifested as the tendency of individuals to be active during specific times of the day and is referred to as their chronotype. There is a genetic predisposition for chronotype (1), which changes over the course of development and determines individual variation in diurnal timing of various functions, such as sleep, cognitive and physical activities (2).

The gut microbiome has been shown to regulate metabolic processes and to modulate brain functions and behavior via immune, endocrine and neural pathways along the gut-brain axis (3). Diurnal oscillations in the function and composition of the gut microbiome have been reported (4, 5), and in turn, metabolites of gut microbes have been shown to mediate gene expression of the circadian clock (5). Furthermore, circadian misalignment with external light conditions induced by simulating jet lag in mice was associated with the disruption of feeding patterns, loss of diurnal microbiota rhythmicity and dysbiosis, and exacerbated metabolic imbalance including weight gain and glucose intolerance (4). Travel-induced jetlag in humans was also associated with microbial dysbiosis, and fecal transfer from jet lagged mice and humans to germ free mice created similar metabolic impairments (4). Overall, these findings allude to the bidirectional associations between gut microbiota and the circadian clock and begs the question as to whether variation in chronotype is also linked to distinct microbiota composition. Here, we sought to explore this link using metagenomic sequencing.

Results and Discussion

To investigate changes in the gut microbiome compositions of different chronotypes, we collected fecal samples from 133 individuals from across Israel (age, 32.1± 10.2 years), body mass index (BMI): 23.6 ± 3.5 data are mean ± SD). The distribution of chronotypes is shown in Fig. 1A. The mid sleep time on free-days (MSF) distribution centered on 4:33 (±SD :34 hr) and did not differ significantly between males and females (Watson-Williams, F test, p =0.2). For the analysis of microbiome composition, participants were divided into three groups based on their MSF: “Early” (n = 18), “Intermediate” (n = 29) and “Late” (n = 42) chronotypes.

DNA from the fecal samples was extracted in order to conduct shotgun metagenomics sequencing. The mean library size was 3,091,458 reads (range, 174,934 – 19,829,176). Samples were filtered to include the taxa with a minimum relative abundance of >0.01% and were identified in 20% of the samples. The identified taxa in the filtered dataset were distributed into 7 phyla, 14 classes, 17 orders, 28 families, 46 genera, and 87 species. The most ubiquitous genera were the Bacteroides, Faecalibacterium, Parabacteroides, and Eubacterium (all detected in more than 96% of the samples). The ten most abundant genera are presented in Fig. 1B.

We observed a higher α-diversity (Shannon index) in participants from the early chronotype, compared to the late chronotype ($p < 0.05$, Kruskal-Wallis rank sum test) indicating a less complex microbiota community in the latter. There were no significant differences between early and intermediate or between intermediate and late chronotypes (Fig. 1C). Chao1 indices, measuring the richness of the samples, were not significantly different between the chronotypes (Fig. 1D).

Differences in individuals’ gut bacteria relative abundances were tested using a feature-wise association approach, controlling for age, BMI, and gender. Two genera were significantly different between the early and late chronotypes, *Alistipes* (pFDR = 0.2, Fig. 2A) and *Lachnospira*

(pFDR = 0.2, Fig. 2B). Within those genera, two species were significantly different between groups. *Alistipes finegoldii* were more abundant in early compared to both intermediate and late chronotypes (pFDR = 0.2, Fig. 2C). *Lachnospira pectinoschiza* were more abundant in the late compared to early chronotypes (pFDR = 0.2, Fig. 2D).

Reads were also assigned to microbial metabolic pathways and differences in relative abundances were tested. In total, 15 pathways were significantly different between the groups (pFDR < 0.2), 11 of which showed a difference between intermediate to either early or late chronotypes. The four pathways that were different between early and late chronotypes (Fig. 3) included the super-pathway of histidine, purine, and pyrimidine biosynthesis (pathway ID PRPP-PWY), anaerobic energy metabolism (PWY-7383), super-pathway of pyrimidine deoxyribonucleotides de novo biosynthesis (PWY-7211), and gluconegonesis (PWY66-399). All four pathways were more abundant in early compared to late chronotypes (Fig. 3).

Overall, our analysis reveals modest but consistent differences between chronotypes. The finding that lower α-diversity, a marker of dysbiosis, was observed in late chronotypes, is consistent with growing evidence of increased cardio-metabolic morbidity and mortality risk in this group (6). Indeed, previous studies showed that jet-lag in both humans and mice is associated with dysbiosis (4), and since late chronotype individuals are predisposed to social jet-lag, the decreased microbial diversity that we observed is expected.

We identified two bacterial genera that differed between early and late chronotypes. Interestingly, *Alistipes,* which was enriched in early chronotypes, has previously been reported as being overrepresented in older mice (7), and humans (8). This may explain the observation that chronotype becomes earlier with age, with MSF advancing by nearly two hours between ages 20 to 70 years (9). Furthermore, *Lachnospira* was more abundant in late chronotypes. In a recent human study on microbiota and eating behavior during the day (10), a higher abundance of *Lachnospira* was found when a greater percentage of energy was consumed after 2pm. This finding aligns with our data portraying *Lachnospira* as a biomarker for the late chronotype, as energy consumption is expected to be delayed in these individuals (11).

Finally, our study underscores the role of several metabolic pathways that differ between chronotypes, particularly gluconeogenesis (Fig. 3). In fact, Gluconeogenesis is known to be under tight circadian regulation of the host, where expression of gluconeogenesis associated genes is stimulated upon anticipation of nightly fasting (12). This suggests an interaction between the host and the microbiome metabolic pathways that is likely to be chronotype-dependent.

Materials and Methods

This study was approved by the local ethics committees at the University of Haifa (#283/18). Participants provided written informed consent prior to participation.

The study population consisted of 133 individuals (51% males), evaluated for body mass index (BMI) and medication intake. Chronotype was assessed by computing mid sleep time on free-days (MSF) (2). Stool samples were collected by the participants using the DNA/RNA Shield Fecal Collection tubes (Zymo research). DNA extraction of the samples was performed using the PureLink™ Microbiome DNA Purification Kit (Invitrogen) according to the manufacturer's instructions. The genomic DNA was sheared to an average size of 300□bp with an M220 ultrasonicator (Covaris, Woburn, MA, USA). Sheared DNA samples were used for paired-end indexed library construction using Ovation Ultralow library systems V2 (NuGEN, San Carlos, CA, USA), according to the manufacturer instructions.

Next generation sequencing (Paired end, 2X150bp) was performed by the University of Illinois at Chicago, Core for Research, using the Illumina NextSeq500. Metagenomic sequencing data was processed with bioBakery workflows utilizing the bioBakery 3 tools (13). Briefly, sequence data quality control, including removal of human reads, was conducted using KneadData. Taxonomic profiles were generated using MetaPhlAn v3.0 and functional profiles were generated with HUMAnN v3.0 using MetaCyc pathway definitions (14). Relative proportions data was transformed by centered-log ratio. Microbial communities were compared between different chronotypes. The primary analysis was performed using MicrobiomeAnalyst (15) followed by comprehensive analysis using the R packages Phyloseq (16), Vegan, and MaAsLin2 v1.06. Alpha diversity indices (Chao1 and Shannon) were compared using the Wilcoxon rank sum test. Beta diversity distance matrices (Aitchison) were compared using the Vegan package's function ADONIS, a multivariate analysis of variance based on dissimilarity tests. Differences in microbial taxa and functional modules were assessed using differential abundance analyses with MaAsLin2. Results were visualized using the ggplot2 R package. The matrices were visualized using principal coordinate analysis (PCA).

Data sharing:

Raw metagenomic data are deposited in the National Center for Biotechnology Information sequence read archive: BioProject accession is PRJNA714678.

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Figures and Tables

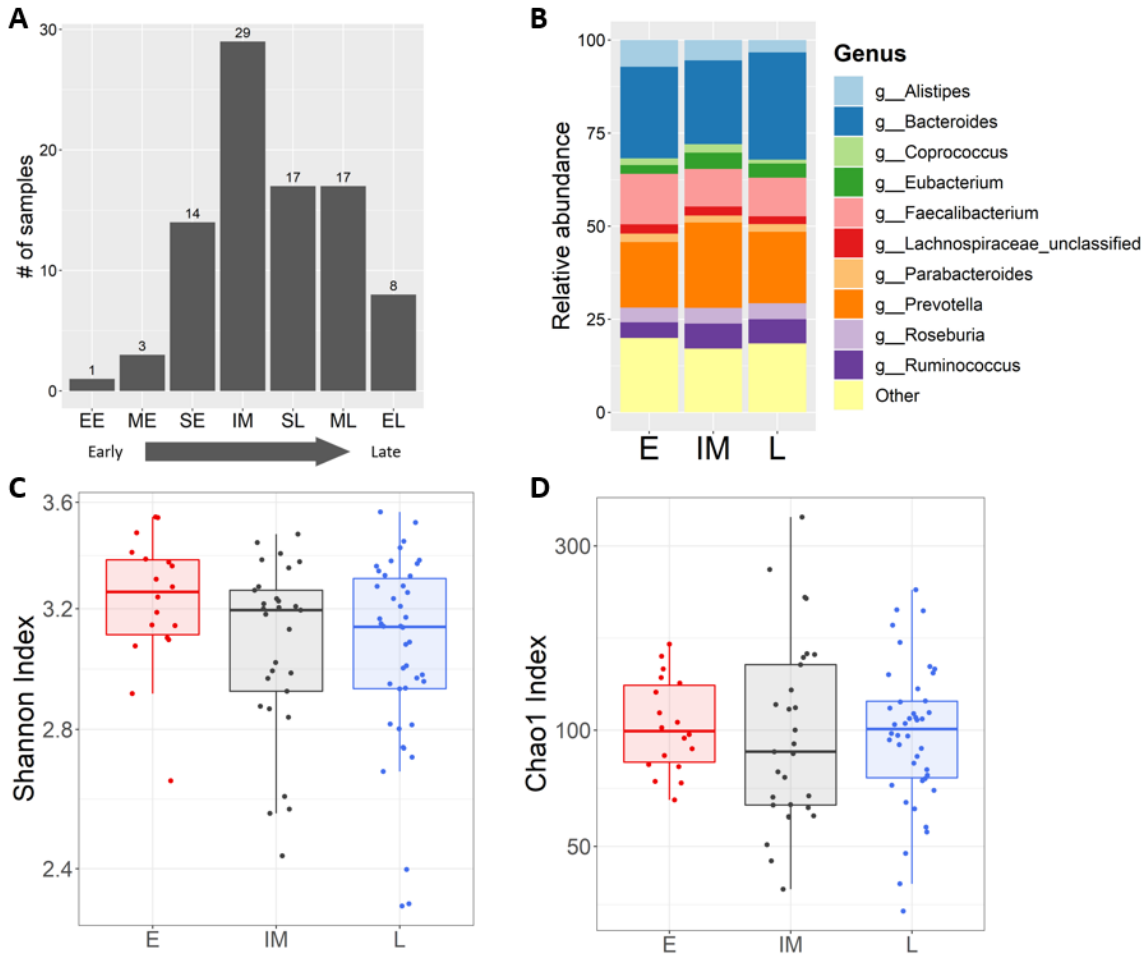

Figure 1. (A) Chronotype distribution. Chronotype were divided into 7 categories, Extremely early (EE): <01:29, Moderately early (ME) 1:30-2:30, SE 2:30-3:30, Intermediate (IM) 3:30-4:30, Slightly late (SL) 4:30-5:30, Moderately late (ML) 5:30-6:30, Extremely late (EL) > 6:30. **(B)** Microbiome composition (genus level) in the different chronotype. The relative abundance of main taxa in early (E) intermediate (IM) and late (L) chronotype is shown. **(C)+(D)** Alpha diversity was measured via Chao1 index (C) and Shannon index (D) in early (E) intermediate (IM) and late (L) chronotype. Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers). Statistical comparisons shown for separate matched-pairs tests with the Kruskal-Wallis rank sum test corrected using the Benjamini–Hochberg FDR method.

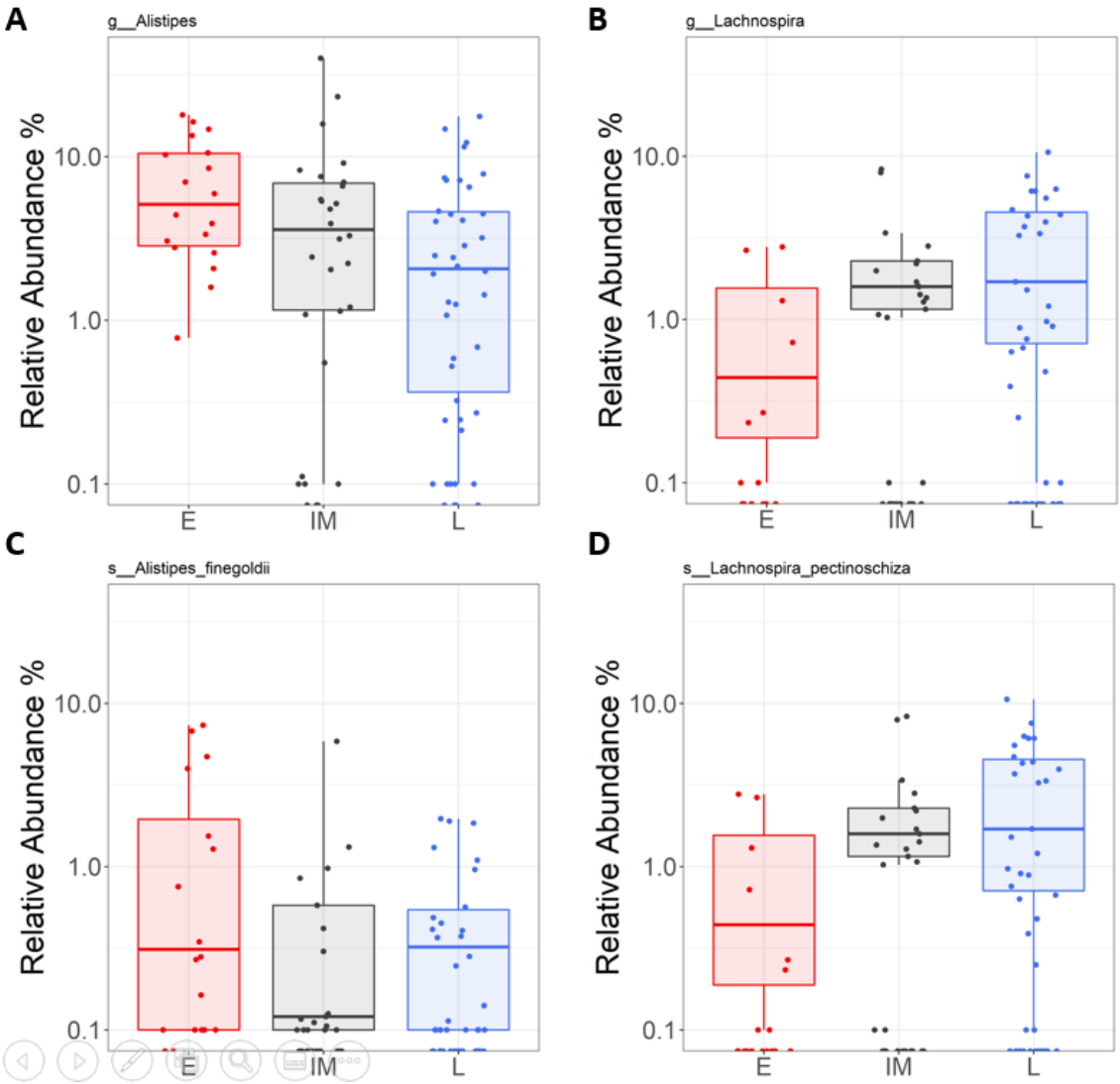

Figure 2. Relative abundances of significantly different taxa in early (E) intermediate (IM) and late (L) chronotype. Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers).

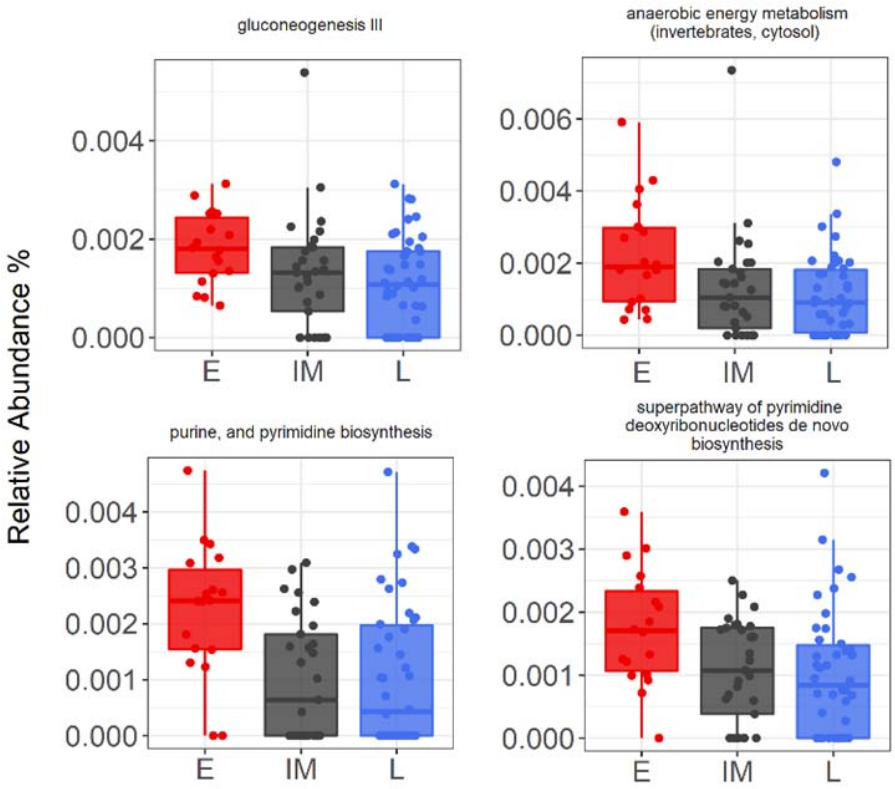

Figure 3. Relative abundances of significantly different metabolic pathways in in early (E) intermediate (IM) and late (L) chronotype. Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers).