News Release

Title

Intestinal bacteria, genus *Collinsella*, may mitigate the infection and exacerbation of COVID-19 by producing ursodeoxycholate

Key Points

- We generated a machine-learning model to predict the COVID-19 mortality rate by gut microbiota in 953 healthy subjects in 10 OECD countries.
- The model revealed that low genus *Collinsella* in the intestine predicts high COVID-19 mortality rates.
- *Collinsella* produces a secondary bile acid, ursodeoxycholate, in the intestine. Ursodeoxycholate was previously reported to inhibit binding of SARS-CoV-2 to its receptor, angiotensin-converting enzyme 2 (ACE2).
- In addition, ursodeoxycholate suppresses pro-inflammatory cytokines; has antioxidant and anti-apoptotic effects; and increases alveolar fluid clearance in acute respiratory distress syndrome.
- Ursodeoxycholate produced by *Collinsella* may prevent COVID-19 infection and ameliorate acute respiratory distress in COVID-19.

Summary

The mortality rates of COVID-19 are widely variable across countries, but the identity of factor X that accounts for the variable COVID-19 mortality rates remains to be determined. We aimed at the elucidation of relationship between gut microbiota and the mortality rates of COVID-19 across countries. Raw sequencing data of the V3-V5 regions of 16S ribosomal RNA (rRNA) of gut microbiota in 953 healthy subjects in 10 OECD countries were obtained from the public database. We made a generalized linear model (GLM) to predict the COVID-19 mortality rates using gut microbiota. GLM revealed that low genus *Collinsella* predicted high COVID-19 mortality rates with markedly high statistical significance. Unsupervised clustering of gut microbiota in 953 subjects yielded five enterotypes, which were defined by the enrichment and paucity of specific sets of intestinal bacteria. The mortality rates were increased from enterotypes 1 to 5, whereas the abundances of *Collinsella* were decreased from enterotypes 1 to 5 except for enterotype 2. *Collinsella* produces a secondary bile acid, ursodeoxycholate, from primary bile acids. Ursodeoxycholate was previously reported to suppress binding of SARS-CoV-2 to its receptor, angiotensin-converting enzyme 2. In addition, ursodeoxycholate suppresses pro-inflammatory cytokines; has antioxidant and anti-apoptotic effects; and increases alveolar fluid clearance in acute respiratory distress syndrome. Ursodeoxycholate produced by *Collinsella* may prevent COVID-19 infection and ameliorate respiratory distress in COVID-19.

Research Background

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The infection has rapidly spread worldwide and has a great impact on medical care and the economy. SARS-CoV-2 causes widely variable phenotypes from lack of any symptoms, mild phenotypes, rapidly progressive phenotype, to respiratory failure. The mortality rate increases exponentially with age with about one in ten patients over 80 years of age. Additional risk factors associated with high mortality rates include obesity, diabetes, tobacco smoking, and a past history of respiratory infection. These risk factors should be similar between countries. However, there are large differences in mortality rates between countries. Mortality rates are higher in the United States, Europe, and South America than in Asia. In Europe, Spain and Italy have high mortality rates, whereas Germany and Northern Europe have low mortality rates (https://ourworldindata.org/). Similarly, in Asia, Taiwan and China have lower mortality rates than Japan and Korea. The differences could be accounted for by the differences in the genome, previous exposure to less virulent corona virus, geopolitical factors, and/or gut microbiota. Temporal profiles of gut microbiota in COVID-19 patients have been reported but without consistent bacterial features.

In the course of our analysis of gut microbiota in Parkinson's disease and idiopathic rapid-eye-movement sleep behavior disorder in the world, we noticed that the mortality rates of COVID-19 may be associated with gut microbiota. We analyzed the relationship between the composition of intestinal bacteria in 953 healthy subjects in ten countries and the mortality rates of COVID-19 in these countries.

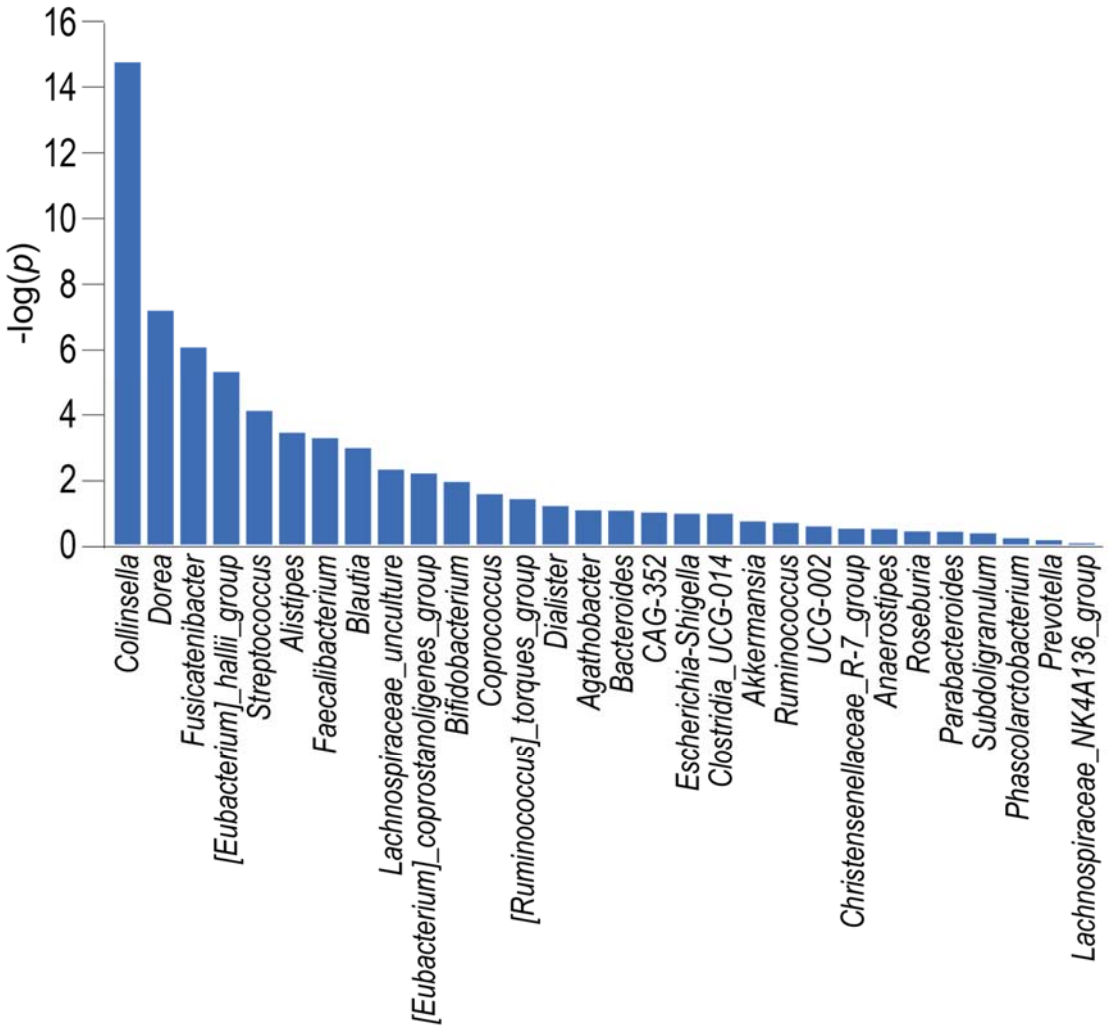

Fig. 1. Plot of p-values of 30 genera in a generalized linear model (GLM) to predict the COVID-19 mortality rates

Fig. 1. A machine-learning generalized linear model was generated by gut microbiota in 953 healthy subjects in 10 OECD countries to predict the mortality rates of COVID-19 on February 2021 when vaccination was not commonly available in these countries. Statistical significances of 30 intestinal bacteria genera are plotted by a negative logarithm of *p*-value.

Research Results

We downloaded the raw data of gut microbiota of 953 healthy subjects in 10 OECD countries from public database. We restricted our analysis to OECD countries to minimize variability due to the geopolitical factors. We predicted the mortality rates of COVID-19 with a machine-learning technique, generalized linear model (GLM) (Fig. 1). We found that low genus *Collinsella* predicted high COVID-19 mortality rates with markedly high statistical significance. Next, unsupervised clustering of gut microbiota in 953 subjects by LIGER yielded five

enterotypes (Fig. 2a). Each enterotype is comprised of a specific set of enriched intestinal bacteria. Ten countries were sorted in order of increasing COVID-19 mortality rates, and fractions of the five enterotypes were plotted in Fig. 2b. The rates of enterotype 1 were high in countries where the mortality rates were low, whereas the rates of enterotypes 4 and 5 were high in countries where the mortality rates were high. Color-coding of the COVID-19 mortality rates on the LIGER plot showed that the mortality rates were increased from the right side to the left side (Fig. 2c). Quantitative analysis showed that the average mortality rates were increased form enterotypes 1 to 5 (Fig. 2d). Color-coding of the relative abundance of genus *Collinsella* on the LIGER plot showed that *Collinsella* was decreased from the right side to the left side (Fig. 2e). Quantitative analysis showed that the average relative abundances of *Collinsella* were decreased form enterotypes 1 to 5 except for enterotype 2 (Fig. 2f).

Genus *Collinsella* produces a secondary bile acid, ursodeoxycholate, from primary bile acids in the gut. Ursodeoxycholate was previously reported to suppress binding of SARS-CoV-2 to its receptor, angiotensin-converting enzyme 2 (ACE2). In addition, ursodeoxycholate suppresses pro-inflammatory cytokines; has antioxidant and anti-apoptotic effects; and increases alveolar fluid clearance in acute respiratory distress syndrome.

Ursodeoxycholate produced by *Collinsella* may prevent COVID-19 infection and ameliorate acute respiratory distress in COVID-19.

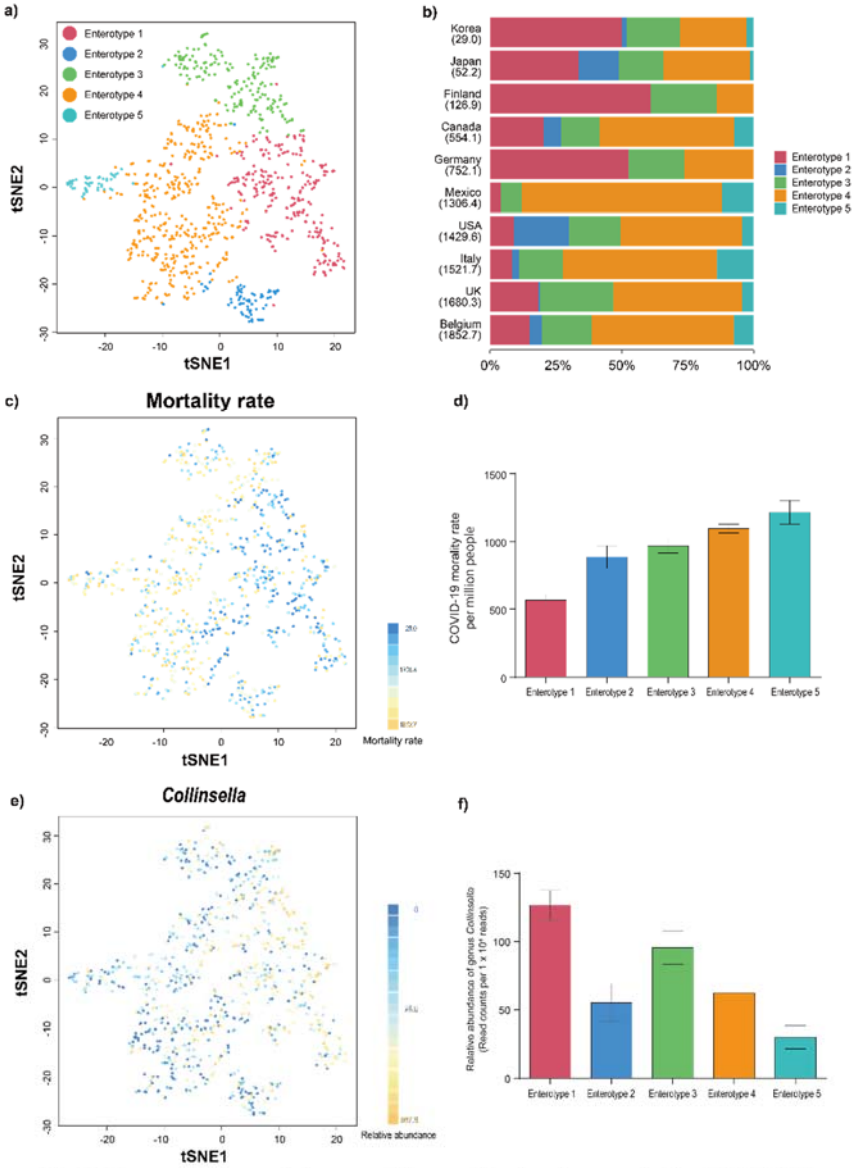

Fig 2. Five enterotypes in ten countries and their relevance to the COVID-19 mortality rates and genus *Collinsella*.

Fig. 2. (a) Five enterotypes (sets of intestinal bacteria) exist in 953 healthy subjects in 10 OECD countries. **(b)** Ten countries are shown in ascending order of the COVID-19 mortality rates. In countries where the COVID-19 mortality rates are low, enterotype 1 is dominant. In countries where the COVId-19 mortality rates are high, enterotypes 4 and 5 are dominant. **(c, d)** The COVID-19 mortality rates are increased from the right to left sides of the plot. Indeed, the mortality rates increases from enterotypes 1 to 5. **(e, f)** In contrast, genus *Collinsella* decreases from the right to left sides of the plot. Indeed, genus *Collinsella* decreases from enterotypes 1 to 5 except for enterotype 2.

Research Summary and Future Perspective

Analysis of gut microbiota in healthy subjects in ten OECD countries revealed that an intestinal bacterium, genus *Collinsella*, is high in countries where the COVID-19 mortality rates is low. A secondary bile acid, ursodeoxycholate, produced by *Collinsella*, is likely to be preventive against SARS-CoV-2 infection and to ameliorate respiratory distress in COVID-19. Although our current analysis is at the nation level, analysis of gut microbiota and fecal concentrations of ursodeoxycholate in COVID-19 patients would disclose whether *Collinsella* and ursodeoxycholate indeed prevents worsening of COVID-19 at the person level. In addition, the analysis may disclose yet unidentified intestinal bacteria that are preventive or facilitative of worsening of COVID-19. Furthermore, as ursodeoxycholate is clinically available for treating liver diseases, ursodeoxycholate may be able to prevent SARS-CoV-2 infection and to ameliorate respiratory distress in patients with COVID-19.

Title: Intestinal *Collinsella* may mitigate infection and exacerbation of COVID-19 by producing ursodeoxycholate

Authors: Masaaki Hirayama[1, ¶, *], Hiroshi Nishiwaki[2, ¶], Tomonari Hamaguchi[2], Mikako Ito[2], Jun Ueyama[1], Tetsuya Maeda[3], Kenichi Kashihara[4], Yoshio Tsuboi[5], and Kinji Ohno[2*]

Affiliation: [1]Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Division of Neurogenetics, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan, [3]Division of Neurology and Gerontology, Department of Internal Medicine, School of Medicine, Iwate Medical University, Iwate, Japan, [4]Department of Neurology, Okayama Kyokuto Hospital, Okayama, Japan, [5]Department of Neurology, Fukuoka University, Fukuoka, Japan, [¶]These authors contributed equally to this work. [*]Corresponding author

Journal：*PLOS ONE*

DOI：https://doi.org/10.1371/journal.pone.0260451

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/PLOS_ONE_20211124.pdf