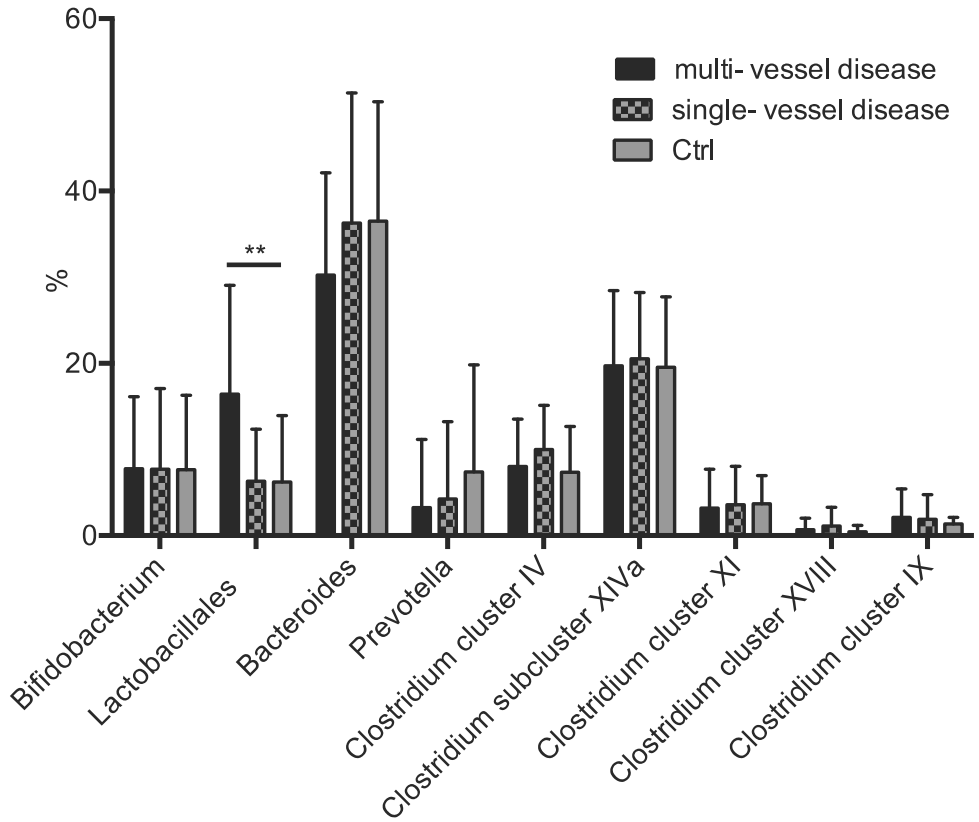
## Дополнительные Методы (к разделу "Кишечный микробиом и ИБС")

### T-RFLP

Образцы фекалий суспендировали в растворе, содержащем 100 мМ буфера трис-HCl (pH 9,0), 40 мМ этилендиаминтетрауксусной кислоты, 4 М тиоцианат гуанидина и 0,001% бромтимолового синего. Фекальные твердые частицы в суспензии разрушали с использованием прибора FastPrep FP100A (MP Biomedicals; Калифорния, США) с шариками из диоксида циркония при скорости 5 м / с в течение 2 мин. Затем ДНК экстрагировали из суспензии объемом 200 мл с использованием автоматического экстрактора нуклеиновых кислот (Precision System Science; Chiba, Япония). MagDEA® DNA 200 (Precision System Science) использовали в качестве реагента для автоматической экстракции нуклеиновых кислот. Полимеразную цепную реакцию (ПЦР) проводили с использованием тотальной фекальной ДНК и следующих праймеров: 5'-меченый FAM 516f (5'-TGCCAGCAGCCGCGGTA-3'; положения *Escherichia coli* 516-532) и 1510r (5'-GGTTACCTTGTTACGACTT-3'; Положения *E. coli* 1510−1492). Полученные ампликоны 16S рДНК обрабатывали 10 ед. фермента *Bsl* I (New England BioLabs) в течение 3 часов, фракционировали с использованием автоматического анализатора последовательностей (ABI PRISM 3130xl Genetic Analyzer; Applied Biosystems) и анализировали с использованием программного обеспечения для анализа ДНК Gene Mapper. Поскольку кажущийся размер идентичных концевых рестрикционных фрагментов может варьироваться от 1 до 3 пар оснований (п.н.), основные фрагменты, различающиеся по размеру на 1-3 п.н., были классифицированы в операционные таксономические единицы (OTU) [1, 2)].

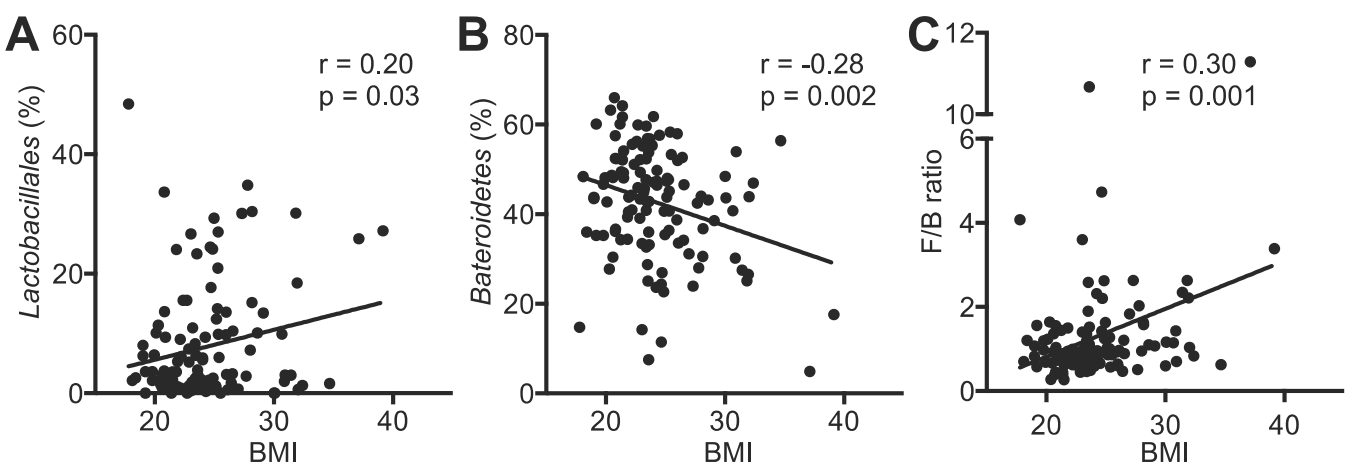
### Литература

1. Nagashima K, Hisada T, Sato M, Mochizuki J. Application of New Primer-Enzyme Combinations to Terminal Restriction Fragment Length Polymorphism Profiling of Bacterial Populations in Human Feces. Applied and Environmental Microbiology 2003; 69: 1251-1262
2. Nagashima K. Mochizuki J, Hisada T, Suzuki S, Shimomura S. Phylogenetic Analysis of 16S Ribosomal RNA Gene Sequences from Human Fecal Microbiota and lmproved Utility of Terminal Restriction Fragment Length Polymorphism Profiling. Bioscience Microflora 2006; 25: 99-107



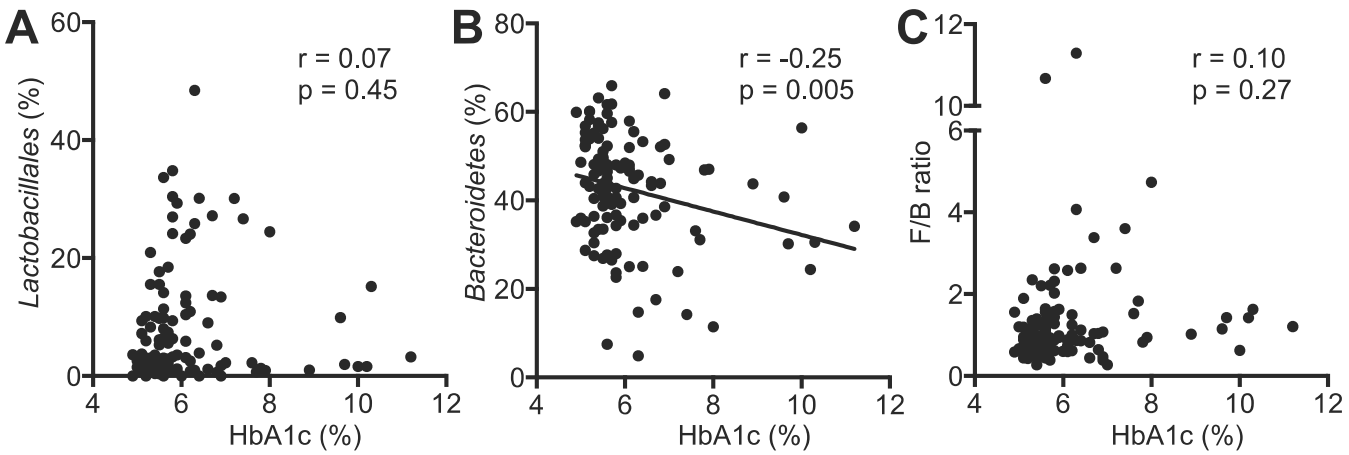

**Supplemental Fig.1.**
Relationship between the severity of coronary artery disease and each group of gut microbiota.

The percentage of each group of gut microbiota was compared between Ctrl, CAD with single- vessel disease and CAD with multi- vessel disease. Single- or multi- vessel disease referred to the number of major coronary vessels demonstrating >75% stenosis on diagnostic coronary angiography. Kruskal-Wallis test followed by Dunn's post-hoc analysis was used to calculate *p*-values ($^{**}p<0.01$).




**Supplemental Fig. 2.** Obesity and distribution of gut microbiota.

Correlations between BMI and (A) the order *Lactobacillales*, (B) the phylum *Bacteroidetes* (*Prevotella* + *Bacteroides*) or (C) the *Firmicutes*/*Bacteroidetes* (F/B) ratio were shown. Pearson's correlation analysis was used for statistical correlation between two parameters.




**Supplemental Fig. 3.** Type 2 diabetes and distribution of gut microbiota.

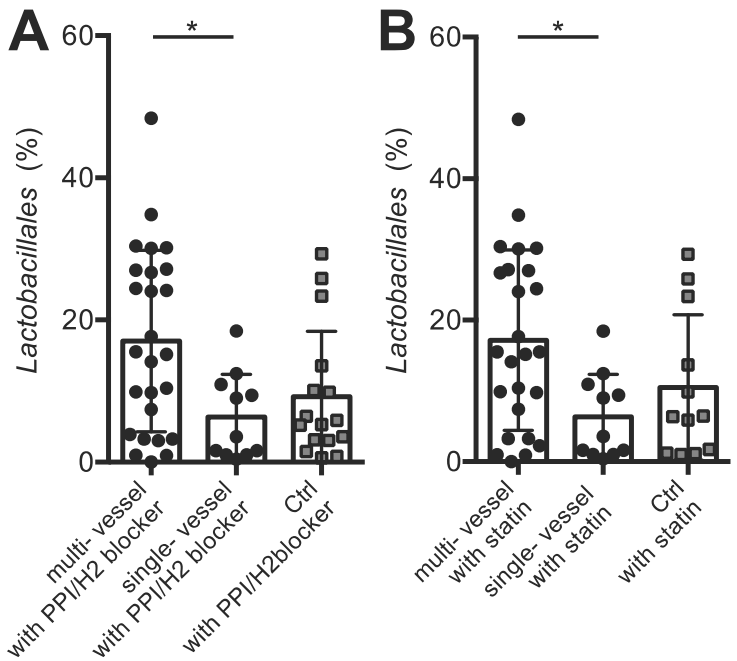
Correlations between HbA1c (%) and (A) the order *Lactobacillales*, (B) the phylum *Bacteroidetes* (*Prevotella* + *Bacteroides*) or (C) the *Firmicutes*/*Bacteroidetes* (F/B) ratio were shown. Pearson's correlation analysis was used for statistical correlation between two parameters.

**Supplemental Table.**
Logistic regression analysis for the presence of multi- vessel disease in the coronary artery disease (CAD) and control (Ctrl) groups ($n$=69).

| (A) Variables | Univariate regression | | Multivariate regression | |
|---|---|---|---|---|
| | OR (95%CI) | $p$ value | OR (95%CI) | $p$ value |
| Age | 0.95 (0.90-1.01) | 0.11 | 0.93 (0.86-1.00) | 0.066 |
| Sex | 1.69 (0.46-6.14) | 0.43 | 0.73 (0.12-4.53) | 0.74 |
| BMI (kg/m$^2$) | 1.03 (0.91-1.15) | 0.68 | Not selected | |
| DM | 1.67 (0.62-4.47) | 0.31 | Not selected | |
| HT | 1.03 (0.26-4.04) | 0.97 | Not selected | |
| DL | 4.77 (0.97-23.5) | 0.055 | Not selected | |
| History of smoking | 3.49 (1.17-10.4) | 0.025$^*$ | 9.85 (1.72-56.5) | 0.010$^*$ |
| ln CRP (mg/dL) | 1.54 (0.57-4.16) | 0.40 | Not selected | |
| *Lactobacillales* (%) | 1.11 (1.05-1.18) | $<$0.001$^{***}$ | 1.16 (1.06-1.26) | $<$0.001$^{***}$ |

| (B) Variables | Univariate regression | | Multivariate regression | |
|---|---|---|---|---|
| | OR (95%CI) | $p$ value | OR (95%CI) | $p$ value |
| PPI/H2 blocker | 6.74 (1.39-32.6) | 0.018$^*$ | 6.80 (1.05-43.7) | 0.043$^*$ |
| Statin | 6.52 (1.70-25.1) | 0.006$^{**}$ | 1.14 (0.17-7.75) | 0.89 |
| $\beta$ blocker | 2.48 (0.92-6.70) | 0.072 | Not selected | |
| ACE-I/ARB | 2.59 (0.90-7.43) | 0.076 | Not selected | |
| anticoagulant | 0.09 (0.02-0.43) | 0.002$^{**}$ | 0.02 (0.003-0.25) | 0.002$^{**}$ |
| *Lactobacillales* (%) | 1.11 (1.05-1.18) | $<$0.001$^{***}$ | 1.11 (1.03-1.20) | 0.007$^{**}$ |

Because the sample size was small, we divided this analysis into two models; (A) variables = known risk factors and *Lactobacillales* (%) (B) variables = medication and *Lactobacillales* (%). Factors with univariate $p<0.05$ were entered into the multivariate analysis ($^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$). (A) Age and sex were entered into the multivariate model as an exception. Abbreviations are same as Table 1 and 2.




**Supplemental Fig. 4.**
The comparison of *Lactobacillales* after adjusting medication.

(A) The percentage of the order *Lactobacillales* tended to be increased in CAD patients with PPI/H2 blocker, compared with Ctrls with PPI/H2 blocker (CAD with PPI/H2 blocker vs. Ctrls with PPI/H2 blocker; 13.8 ± 12.1% vs 9.2 ± 9.2%; $p = 0.24$, Figure not shown), especially in those with multi- vessel disease (multi-, single- vessel disease vs. Ctrl; 17.0 ± 12.8%, 6.3 ± 6.1% vs. 9.2 ± 9.2%; $p = 0.025$). (B) The percentage of the order *Lactobacillales* was also tended to be increased in CAD patients with statin, compared with Ctrls with statin (CAD with statin vs. Ctrls with statin; 13.9 ± 12.1% vs. 10.5 ± 10.3%; $p = 0.41$, Figure not shown), especially in those with multi- vessel disease with statin (multi-, single- vessel disease vs. Ctrl; 17.2 ± 12.7%, 6.3 ± 6.1% vs. 10.5 ± 10.3%, $p = 0.034$). Kruskal-Wallis test followed by Dunn's post-hoc analysis or Mann-Whitney *U* test were used to calculate *p*-values ($*p < 0.05$). Single- or multi- vessel disease referred to the number of major coronary vessels demonstrating >75% stenosis on diagnostic coronary angiography.