

# 健康と疾患のヒトマイクロバイーム解析

“Our second genome - Human microbiome”

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(Laboratory for Microbiome Sciences, RIKEN Center for Integrative Medical Sciences)

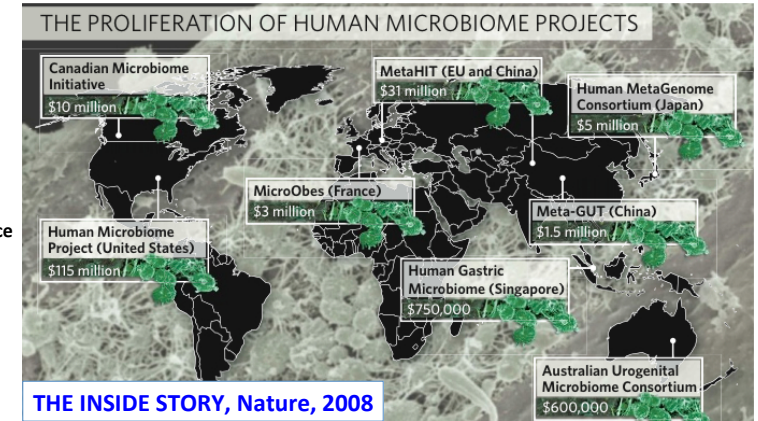
2018年7月3日

大阪医科大学 研究ブランディング事業「健康寿命をのばす たかつきモデル」学内シンポジウム

## International Human Microbiome Consortium

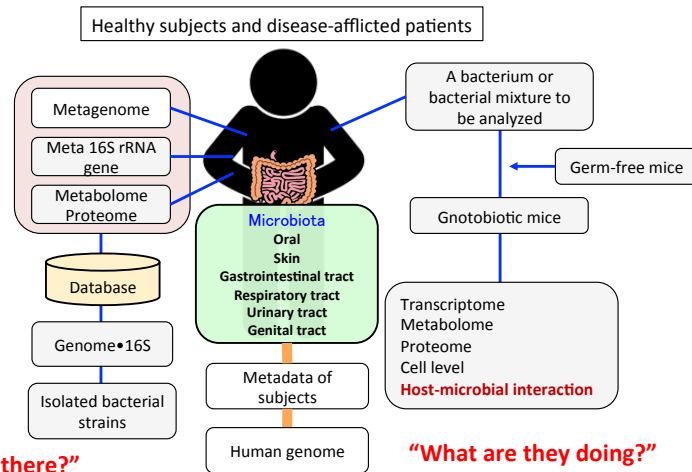
IHMC  
2008~

IHMC Conference  
2016@The US  
2018@Ireland  
2020@Spain  
2022@Japan



HMGJ (Human MetaGenome consortium, Japan) launched in 2005  
NIH HMP (Human Microbiome Project) launched in 2008  
EU MetaHIT Project with BGI (China) launched in 2008

## Outline of human microbiome research



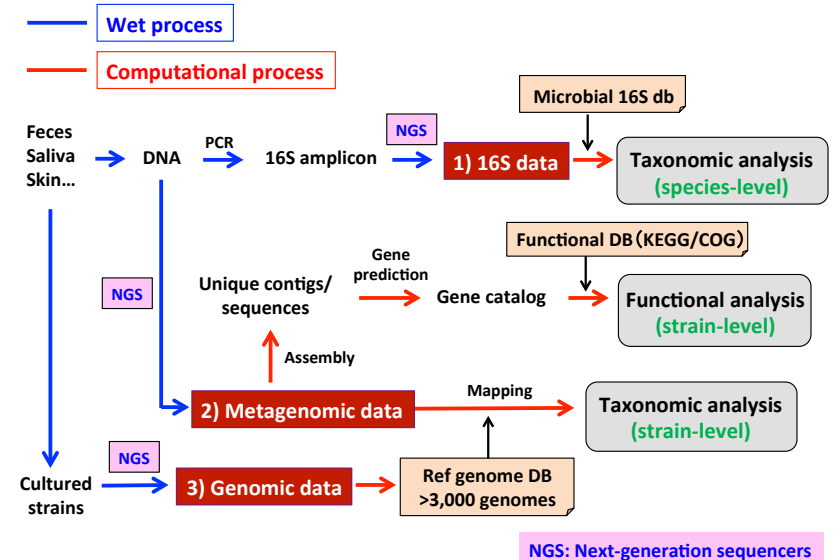
“Who’s there?”

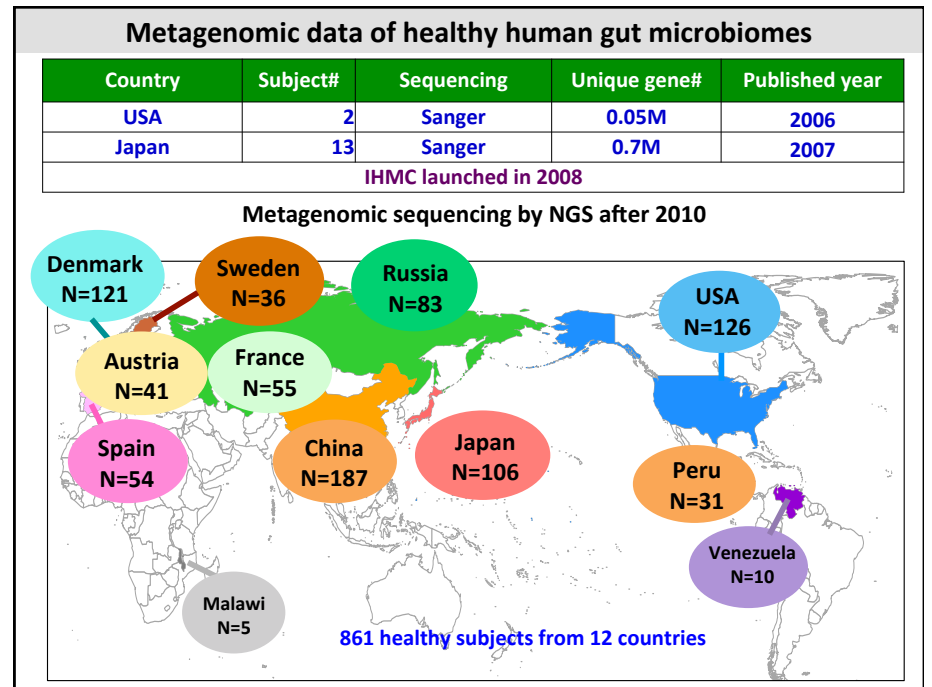
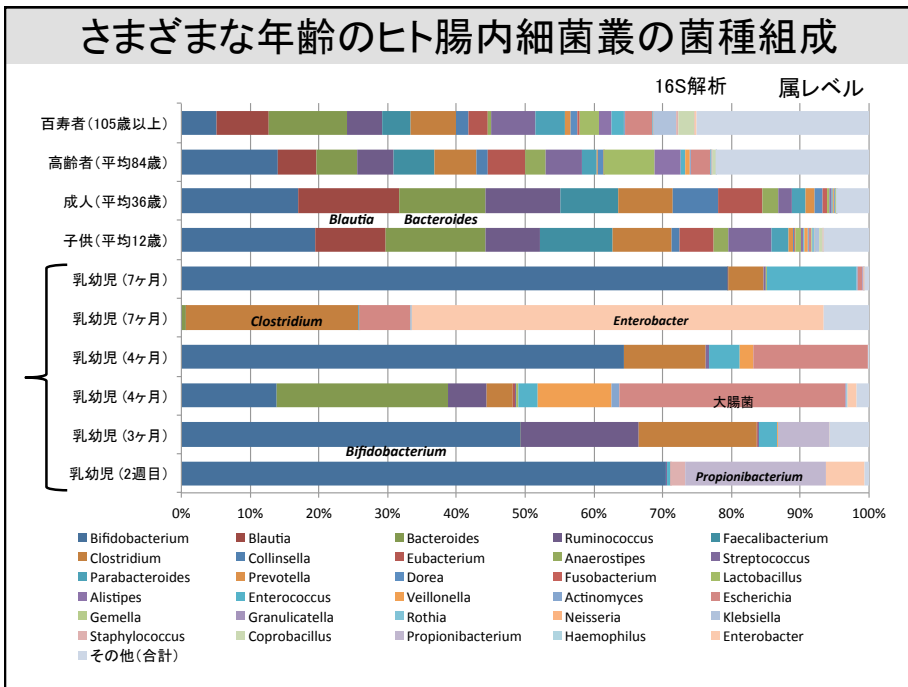
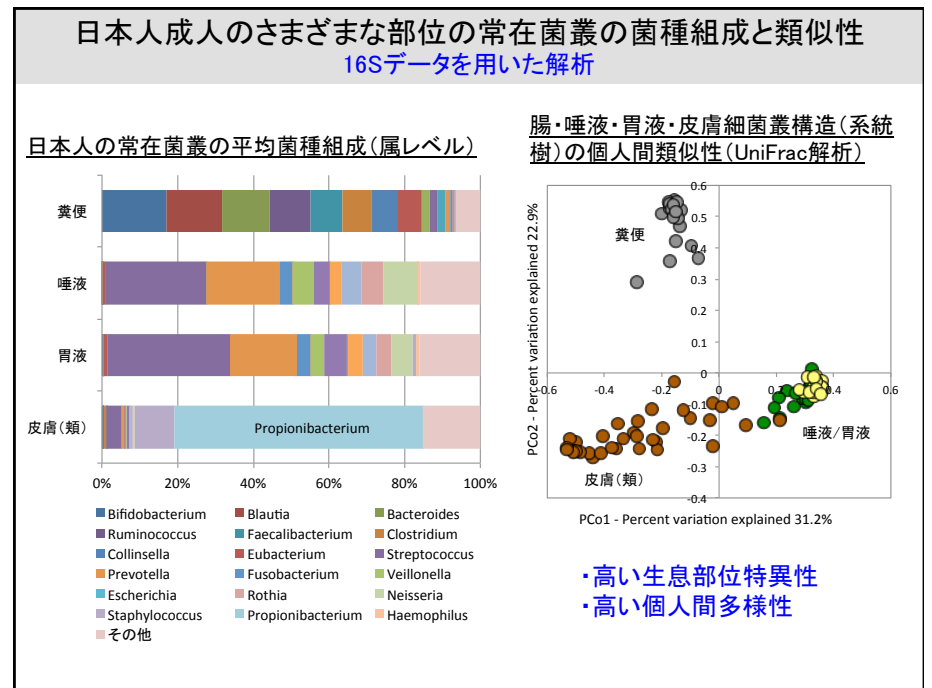
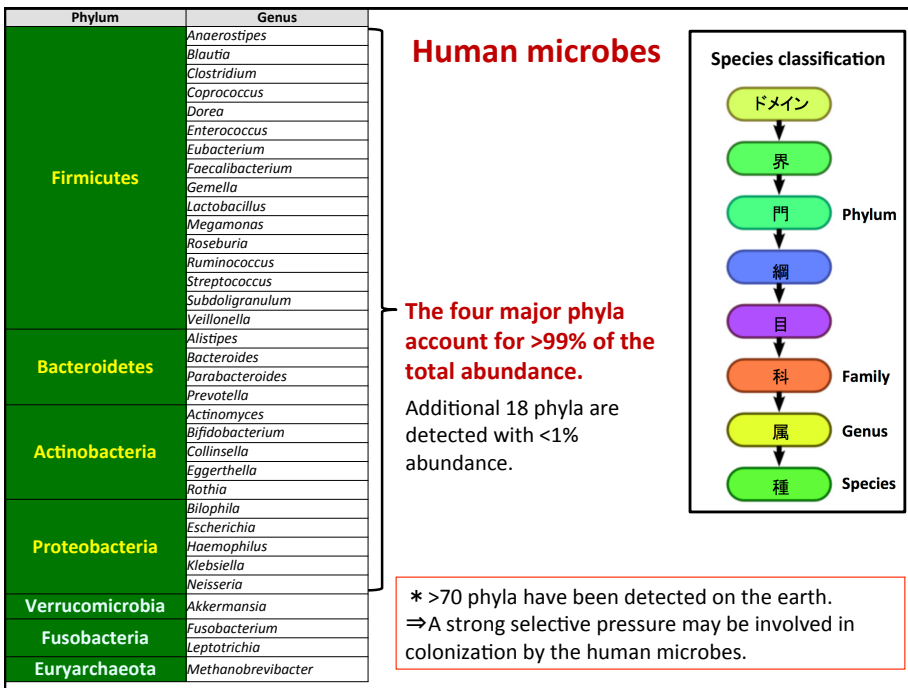
Data-driven study  
(Technology development,  
Database construction)

“What are they doing?”  
“What are they making?”

Functional study  
Multi-omics approach

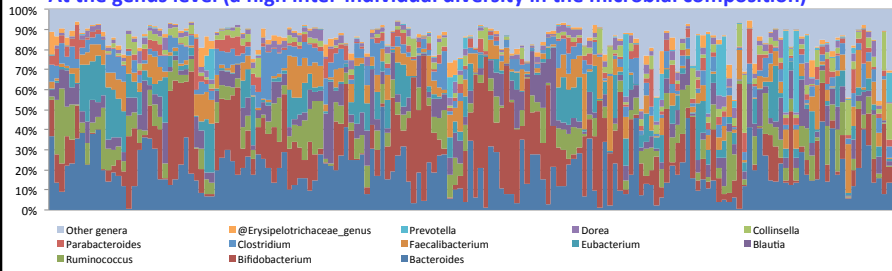
## Analytical pipeline for human microbiomes



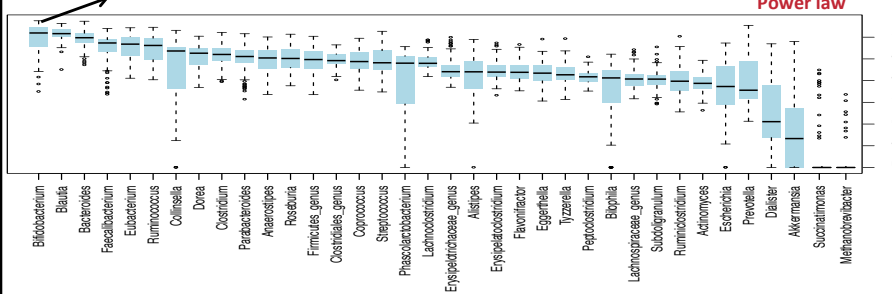


## Microbial composition of 106 Japanese gut microbiomes

At the genus level (a high inter-individual diversity in the microbial composition)



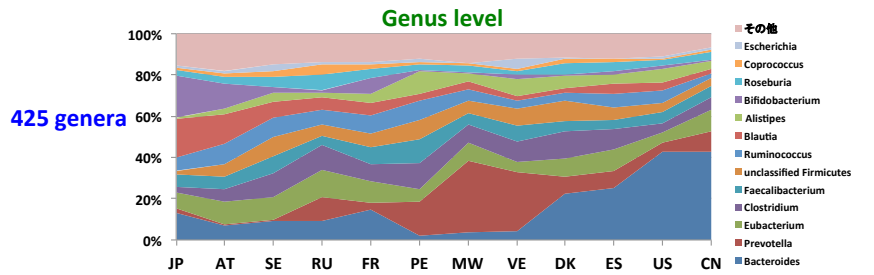
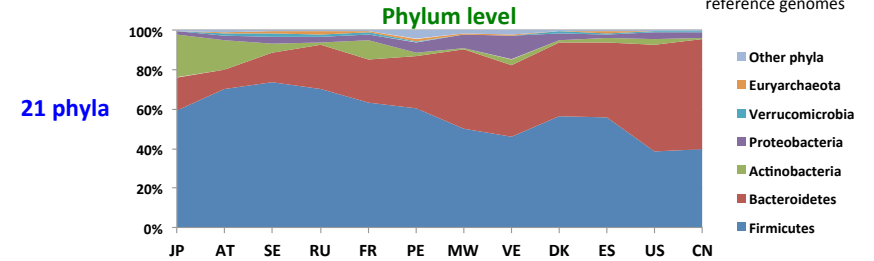
**Bifidobacterium: most abundant**



## Variations in human gut microbiomes across the 12 countries

Ave. 3.4 Gb/individual (350 Gb/106 Japanese)/MiSeq, IonPGM/Proton, 454

Mapped 1 M reads to reference genomes



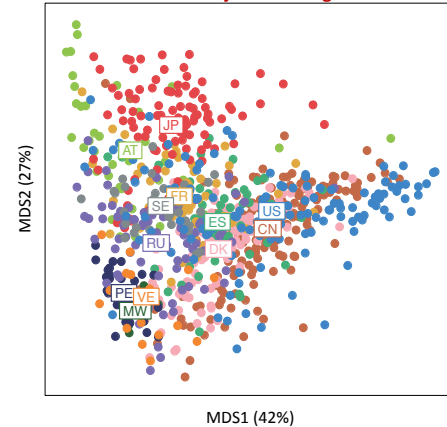
Nishijima S et al. 2016

## Comparison of gut microbial compositions between individuals

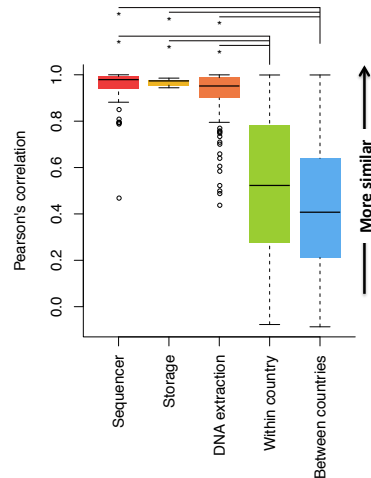
The overall gut microbiome structure of individuals in the same country is significantly more similar than those between different countries, indicating existence of **the population (country)-level diversity** in the human gut microbiome.

The observed diversity is not significantly affected by methodologies (sequencers, fecal storage conditions, and DNA extraction methods).

MDS of all 861 subjects at the genus level



MDS: Multi Dimensional Scaling

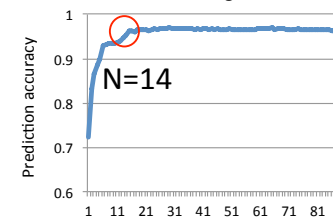


Nishijima S et al. 2016

## 機械学習プログラムRandomForestを用いた国間の違いに大きく寄与する菌種の特異性とそれらによる国の識別

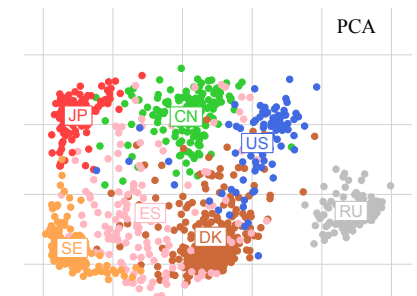
A set of  $\geq 14$  genera or  $\geq 18$  species has the ability to significantly differentiate the countries

All countries 90 genera tested

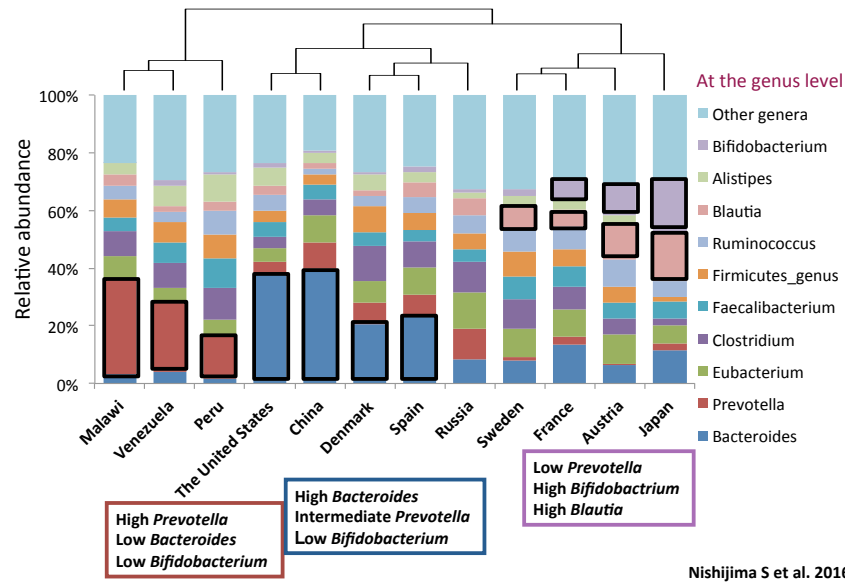


Average accuracy rate of the prediction: 83% (Genus)

| Sample | Predicted country |     |      |     |     |     |     |
|--------|-------------------|-----|------|-----|-----|-----|-----|
|        | CN                | DK  | JP   | RU  | ES  | SE  | US  |
| CN     | 87%               | 1%  | 2%   | 2%  | 4%  | 0%  | 4%  |
| DK     | 3%                | 78% | 0%   | 3%  | 2%  | 3%  | 11% |
| JP     | 0%                | 0%  | 100% | 0%  | 0%  | 0%  | 0%  |
| RU     | 4%                | 1%  | 0%   | 93% | 1%  | 0%  | 0%  |
| ES     | 14%               | 21% | 0%   | 0%  | 49% | 7%  | 9%  |
| SE     | 1%                | 0%  | 2%   | 0%  | 0%  | 98% | 0%  |
| US     | 2%                | 17% | 0%   | 0%  | 2%  | 0%  | 79% |



## Hierarchical clustering of the 12 countries based on the average microbial abundance



## Meal information of the 12 countries from FAOSTAT

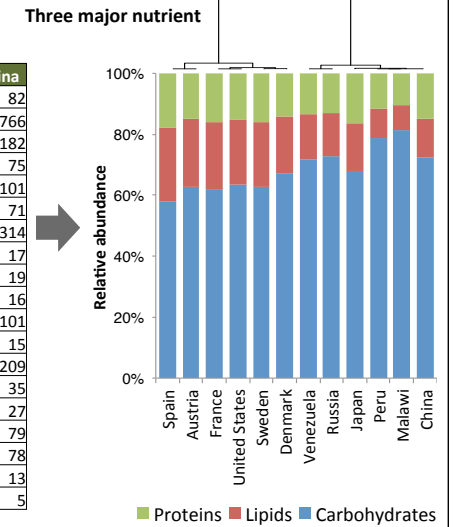
**Meal information in FAOSTAT**  
(g/capita/day)

Food and Agriculture Organization of the United Nations

119 food items of 245 countries from 1961

| 119 food items           | Japan | Russia | Sweden | USA | China |
|--------------------------|-------|--------|--------|-----|-------|
| Milk - Excluding Butter  | 203   | 481    | 979    | 700 | 82    |
| Vegetables, Other        | 228   | 240    | 170    | 186 | 766   |
| Wheat                    | 131   | 358    | 218    | 222 | 182   |
| Milk, Whole              | 127   | 350    | 193    | 310 | 75    |
| Potatoes                 | 58    | 312    | 159    | 147 | 101   |
| Beer                     | 73    | 211    | 113    | 229 | 71    |
| Rice (Paddy Equivalent)  | 222   | 21     | 23     | 34  | 314   |
| Sugar, Raw Equivalent    | 75    | 97     | 109    | 175 | 17    |
| Maize                    | 31    | 2      | 3      | 35  | 19    |
| Sugar (Raw Equivalent)   | 47    | 87     | 101    | 91  | 16    |
| Pig meat                 | 54    | 54     | 99     | 82  | 101   |
| Sugar, Refined Equiv     | 43    | 80     | 93     | 84  | 15    |
| Rice (Milled Equivalent) | 148   | 14     | 16     | 23  | 209   |
| Poultry Meat             | 46    | 62     | 42     | 134 | 35    |
| Oranges, Mandarines      | 28    | 22     | 139    | 83  | 27    |
| Fruits, Other            | 28    | 60     | 62     | 62  | 79    |
| Tomatoes                 | 23    | 64     | 61     | 121 | 78    |
| Bovine Meat              | 24    | 48     | 68     | 109 | 13    |
| Cassava                  | 0     |        | 0      | 0   | 5     |

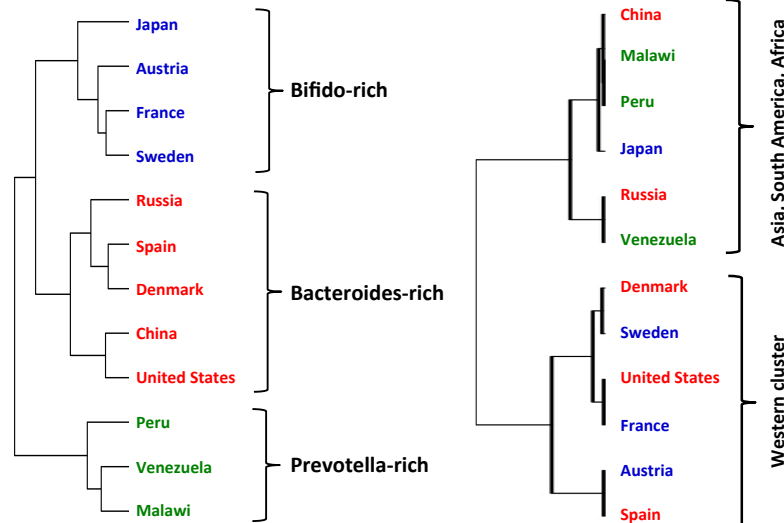
**Average food intake from 2003 to 2012**



## Food seems not to be the primary factor affecting the human gut microbiome

Relation based on microbiome data

Relation based on food intake data\*



Nishijima S et al. 2016

## Effect of traditional vegetable dishes (VD; 精進料理) on the human gut microbiome

Comparison of gut microbiomes between healthy monks of Sojiji Temple (総持寺) and ordinary adults

\* Sojiji healthy monks (SJ20) with VD for three months

\* Ordinary healthy adults (HC60) with free diet

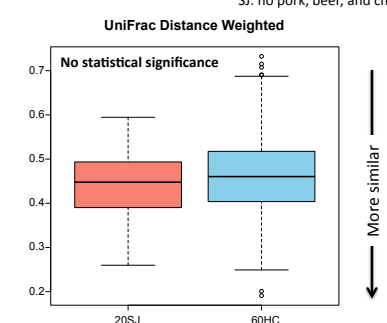
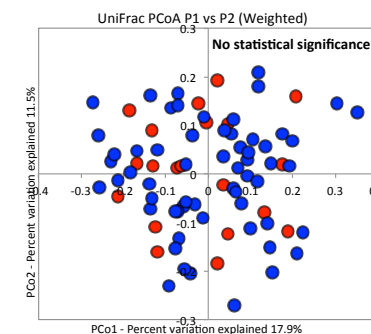
\* Many had large weight loss.

\* Some felt reduction of allergic manifestations.

\* But, no large difference in the gut microbiome structure between the two groups

| Daily intake               | SJ20   | HC60   |
|----------------------------|--------|--------|
| Total energy (kcal)        | 1,311  | 2,222  |
| Proteins (g)               | 34.4   | 78.3   |
| Lipids (g)                 | 26.6   | 72.0   |
| Carbohydrates (g)          | 232.5  | 298.9  |
| Dietary fiber (g)          | 20.3   | 13.1   |
| Vitamins A, B1, B2, C (mg) | 42.912 | 73.743 |

SJ: no pork, beef, and chicken



\* High stability and robustness of the healthy gut microbiome against change in diet?

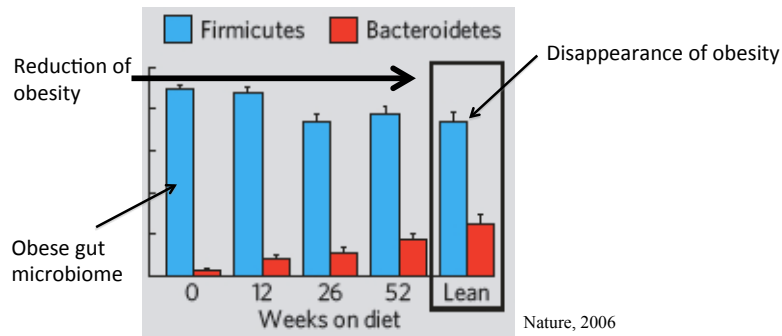
\* Same foods did not always make the gut microbiomes more similar than different foods?

\* Traditional Japanese vegetable dishes (精進料理) are sufficient for health maintenance of Japanese?

## Dietary restrictions changed the gut microbiome of obesity patients

In American people, the ratio of Firmicutes/Bacteroidetes is significantly higher in obesity patients than that of leans

### Change to lean-type from obese-type gut microbiome with low-calories diets (low carbohydrates) for 52 weeks

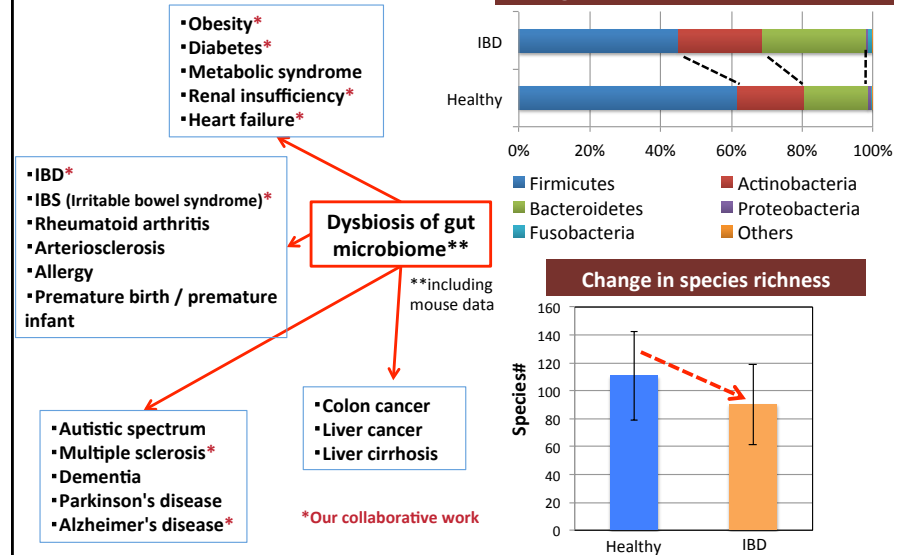


\*Diet largely affected the gut microbiome in obesity patients.

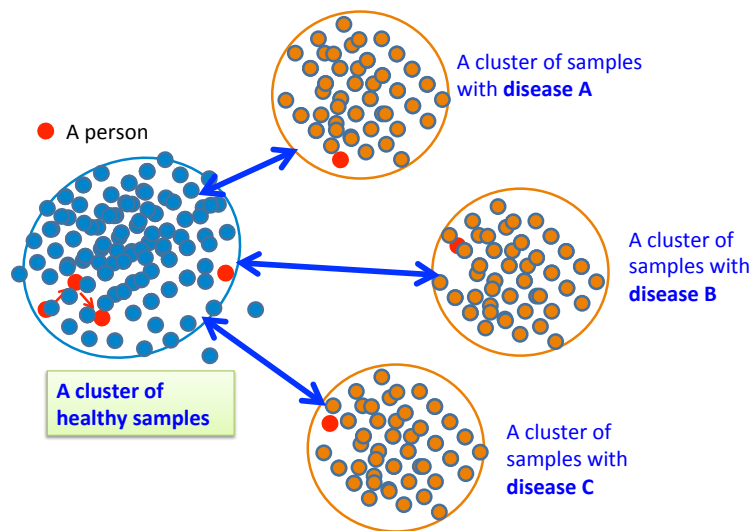
\*Dysbiotic (obese) gut microbiome is more sensitive (unstable) to change in diet than healthy gut microbiome?

## Association between dysbiosis of gut microbiome and various diseases

Metabolism system, immune system, and nervous system (brain function)

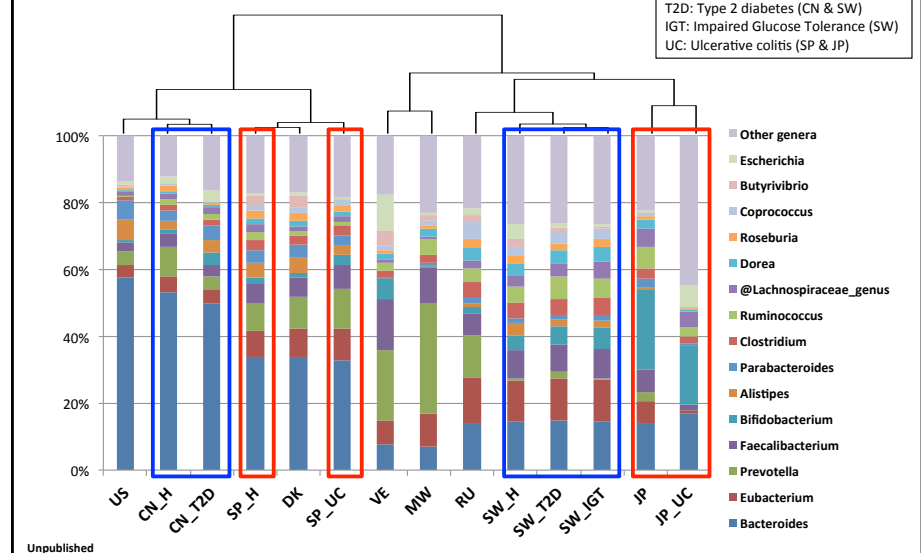


## Schematic view explaining "dysbiosis" of gut microbiome



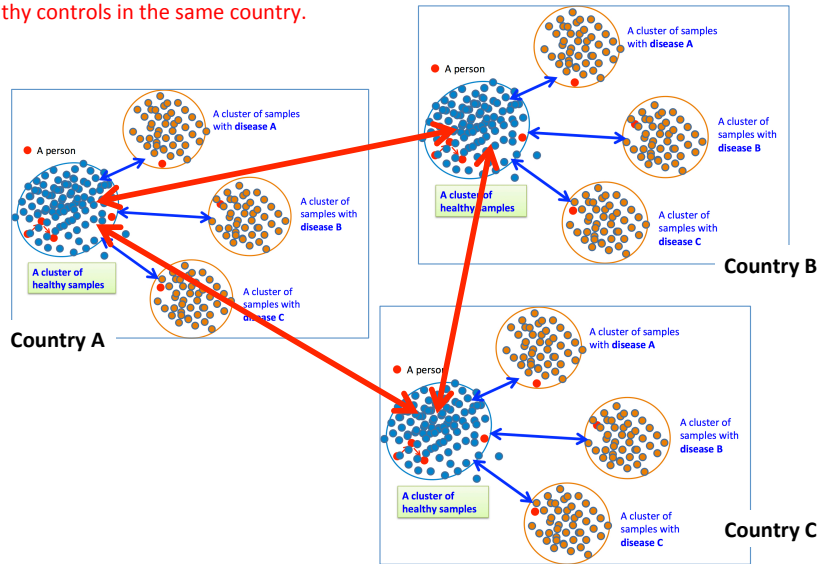
## Clustering of gut microbiomes of healthy subjects in 12 countries with those of Swedish and Chinese T2D, and Japanese and Spanish IBD patients

Inter-country variations in the gut microbiome are higher than variations between disease and healthy gut microbiomes in the same country



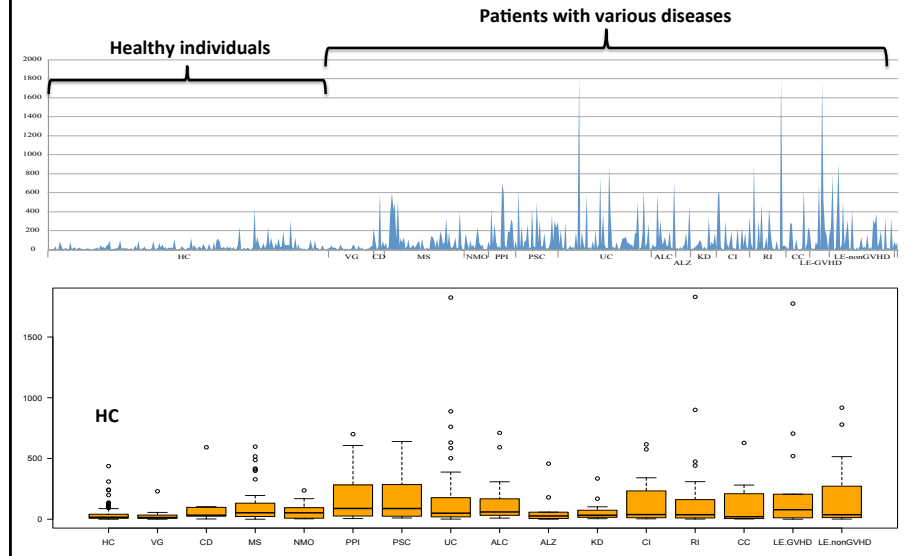
**Schematic view explaining “population-level variations between different countries are higher than those between healthy individuals and patients with diseases in the same country”**

Evaluation of gut microbiomes of patients with diseases requires data of healthy controls in the same country.

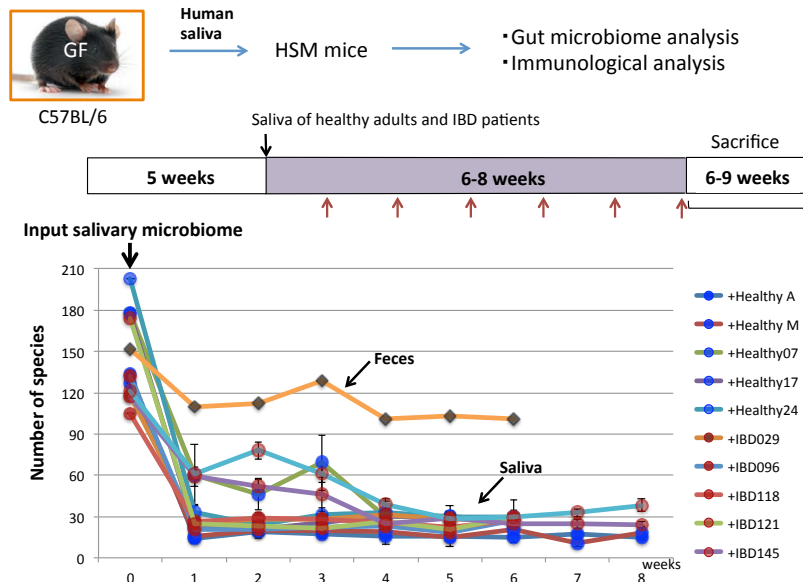


**Ectopic colonization of oral bacteria in the intestine drives TH1-cell induction and inflammation. Atarashi K. et al, Science, 2017**  
 Collaboration with K. Honda, Keio Univ.

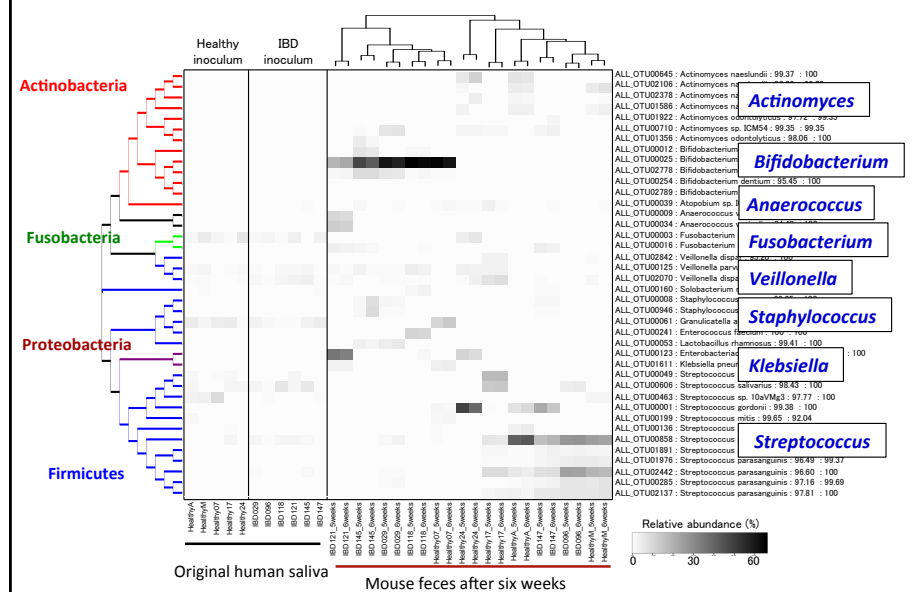
More salivary species in fecal samples of patients with various diseases than those of healthy control.



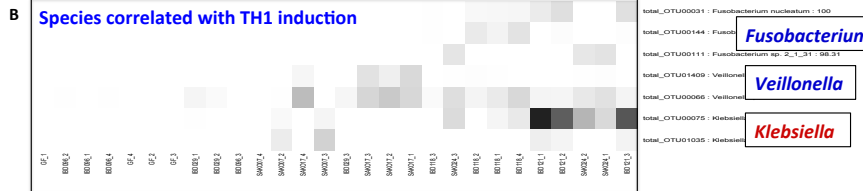
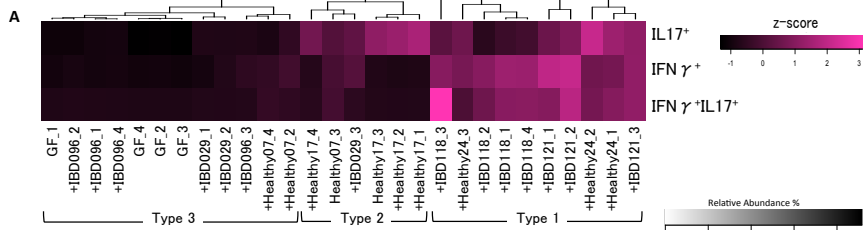
**Human salivary microbiome-derived gnotobiotic mice (HSM)**



**Human salivary species that stably persist in the mouse gut**



## Intestinal inflammation in mice colonized by salivary species

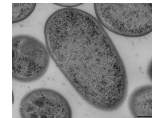


Some of isolated *Klebsiella* strains induced TH1 cells (Other *Klebsiella* strains did not.)

→ Cultured strains → Germ-free mice → *Klebsiella* ⇒ SPF mice ⇒ No TH1 induction  
*Klebsiella* ⇒ SPF mice ⇒ TH1 induction w/o inflammation  
*Klebsiella* ⇒ SPF mice ⇒ TH1 induction with inflammation

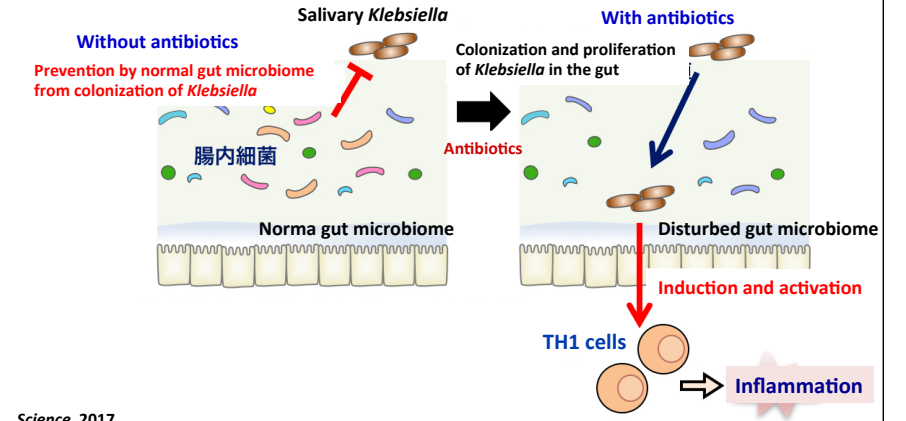
+antibiotics  
*Klebsiella* ⇒ Multi-drug resistance

## Possible mechanism for TH1 induction by a *Klebsiella* strain in SPF mice with or without antibiotics administration



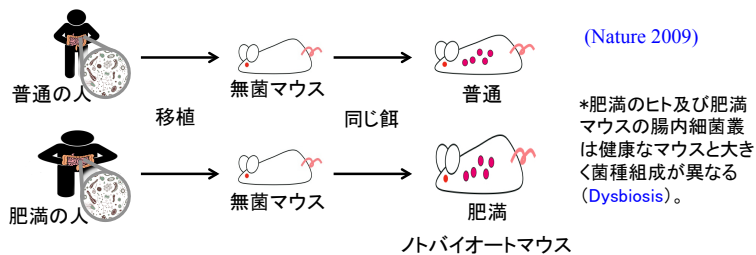
<In oral cavity> Multidrug-resistant *Klebsiella* relatively increases by antibiotics administration, resulting in increase in *Klebsiella* reaching the gut.

<In the gut> Antibiotics administration disturbs normal gut microbiome to reduce the ability of colonization resistance, resulting in increase in *Klebsiella* in the gut.



Science, 2017

## 疾患の(異常)腸内細菌叢は病気発症の原因

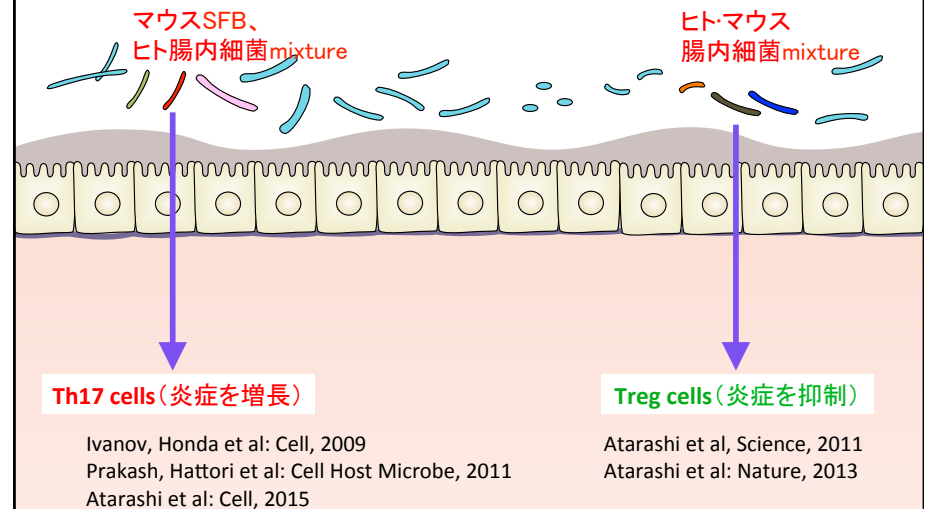


## 便微生物移植(FMT): 健康者の(正常)腸内細菌叢は薬になる

- van Nood E et al: Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med*. 368: 407-415 (2013).
- Mole B: FDA gets to grips with faeces. *Nature* 498: 147-148 (2013).



## 腸内細菌は腸免疫細胞のTh17とTreg分化誘導を制御する



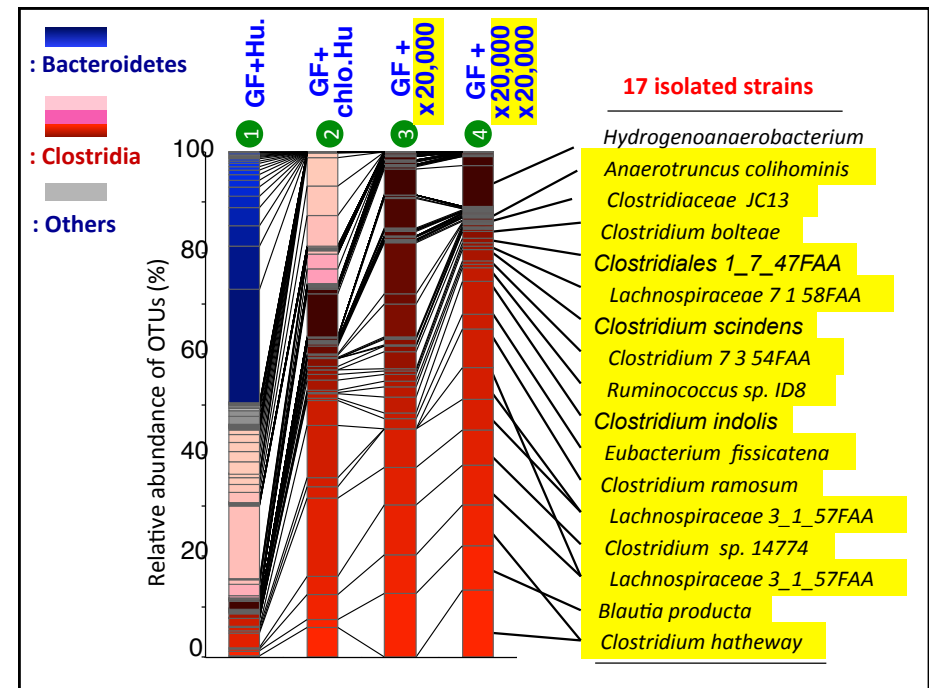
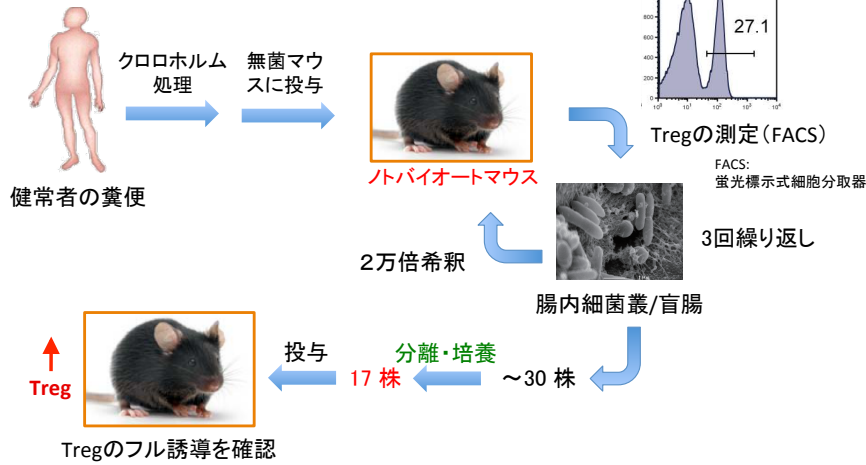
本田賢也博士(慶応義塾大)との共同研究

## 制御性T細胞 (Treg) を誘導するヒト腸内細菌の同定と分離

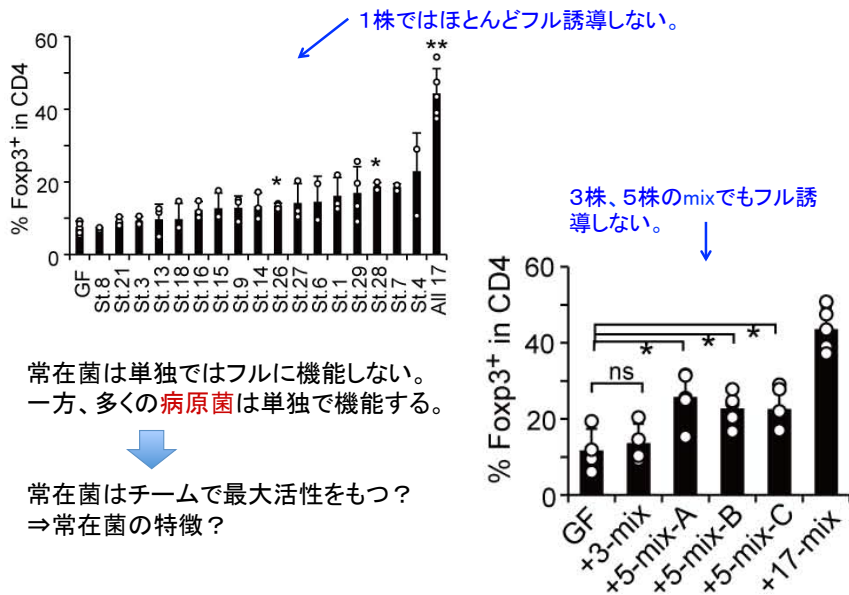
Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota.  
Atarashi K, Honda K, et al: *Nature* 500, 232-236 (2013)

Treg: 炎症を抑える機能  
(自己免疫疾患で減少)

慶應大・本田賢也ラボとの共同研究

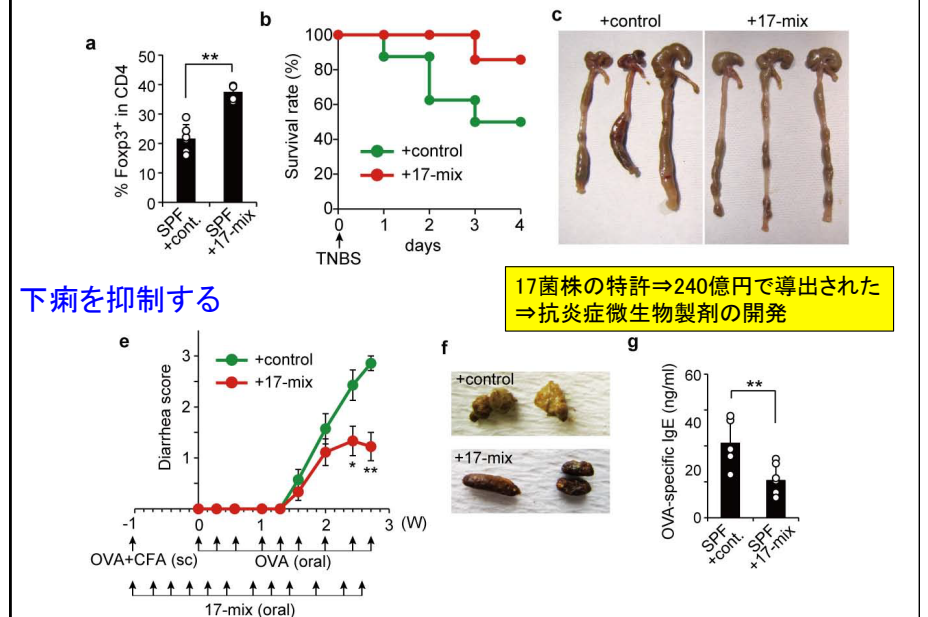


## ヒト腸内細菌17株のTreg分化能



## 腸炎を抑制する

17菌株のカクテルをマウスに投与



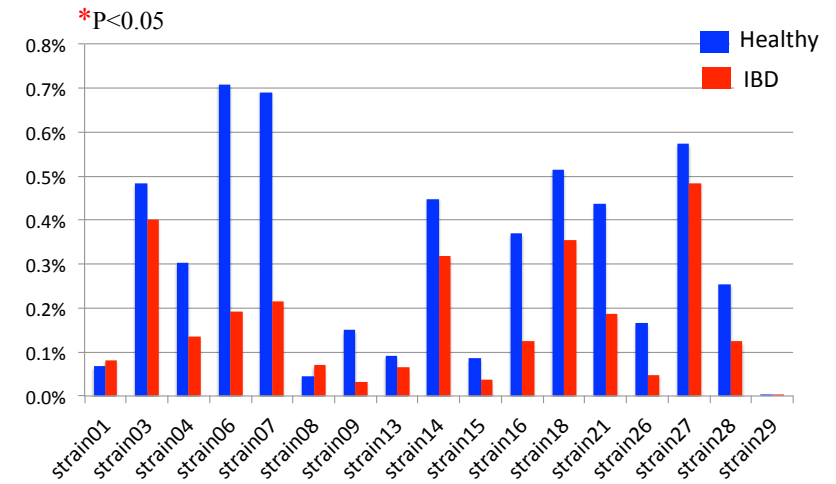


## Genome sequencing of 17 Treg-inducing strains

| Strain    | Total contig length in Mb | Number of predicted genes | Most similar strain                     | Genome size of similar strain in Mb | 16S identity in % |
|-----------|---------------------------|---------------------------|---|-------------------------------------|-------------------|
| Strain_01 | 3.28                      | 3,171                     | Clostridium ramosum DSM 1402            | 3.23                                | 96.6              |
| Strain_03 | 5.16                      | 4,897                     | Flavonifractor plautii ATCC 29863       | 3.81                                | 99.8              |
| Strain_04 | 7.40                      | 6,717                     | Clostridium hathewayi DSM 13479         | 6.63                                | 95.3              |
| Strain_06 | 6.21                      | 5,662                     | Lachnospiraceae bacterium 6_1_63FAA     | 2.72                                | 96.5              |
| Strain_07 | 6.06                      | 5,968                     | Clostridium bolteae ATCC BAA-613        | 6.56                                | 99.5              |
| Strain_08 | 3.39                      | 3,312                     | Erysipelotrichaceae bacterium 2_2_44A   | 4.97                                | 92.9              |
| Strain_09 | 3.51                      | 3,619                     | Anaerostipes caccae DSM 14662           | 3.61                                | 97.7              |
| Strain_13 | 3.56                      | 3,660                     | Anaerotruncus colihominis DSM 17241     | 3.72                                | 100.0             |
| Strain_14 | 3.16                      | 3,251                     | Coprococcus comes ATCC 27758            | 3.24                                | 93.3              |
| Strain_15 | 6.27                      | 6,076                     | Clostridium asparagiforme DSM 15981     | 6.22                                | 99.7              |
| Strain_16 | 5.44                      | 5,275                     | Clostridium symbiosum WAL-14163         | 5.35                                | 99.9              |
| Strain_18 | 3.60                      | 3,692                     | Clostridium ramosum DSM 1402            | 3.23                                | 100.0             |
| Strain_21 | 5.40                      | 5,139                     | Clostridium sp. D5                      | 5.35                                | 98.9              |
| Strain_26 | 3.93                      | 3,935                     | Clostridium scindens ATCC 35704         | 3.62                                | 99.6              |
| Strain_27 | 7.05                      | 6,724                     | Lachnospiraceae bacterium 3_1_57FAA_CT1 | 7.69                                | 97.5              |
| Strain_28 | 6.96                      | 6,736                     | Clostridiales bacterium 1_7_47FAA       | 6.51                                | 99.7              |
| Strain_29 | 7.55                      | 6,912                     | Lachnospiraceae bacterium 3_1_57FAA_CT1 | 7.69                                | 99.6              |

ほとんどがクロストリジウム系のクラスター IV, XIV, VIIIに属する菌

## 日本人炎症性腸疾患 (IBD) 患者の腸内細菌叢ではTreg誘導菌種が有意に減少 (15/17株が減少、うち7株が有意に減少)



- VE202-01 (Clostridium saccharogumia DSM 17460)
- VE202-06 (Clostridiales bacterium VE202-06)
- VE202-09 (Clostridiales bacterium VE202-09)
- VE202-13 (Anaerotruncus colihominis DSM 17241)
- VE202-14 (Clostridiales bacterium VE202-14)
- VE202-15 (Clostridium asparagiforme DSM 15981)
- VE202-21 (Clostridiales bacterium VE202-21)
- VE202-26 (Clostridium scindens ATCC 35704)
- VE202-27 (Clostridiales bacterium VE202-27)
- VE202-28 (Clostridiales bacterium VE202-28)

### Treg-inducing 17 strains

Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota.

Atarashi K, et al: *Nature* 500, 232-236 (2013)

\*ほとんどがクロストリジウム系のクラスター IV, XIV, VIIIに属する菌

- 2H6, VE202-18 (Erysipelatoclostridium ramosum DSM 1402)
- 2H11, VE202-03 (Flavonifractor plautii ATCC 29863)
- 1F8, VE202-04 (Clostridium hathewayi DSM 12489931)
- 1F7, VE202-07 (Clostridium bolteae DSM 90B8)
- 1C12, VE202-08 (Dielma fastidiosa)
- 1A9, VE202-16 (Clostridium symbiosum WAL-14163)
- 2G4 (Clostridium innocuum DSM 2959)
- 2G11 (Bacteroides dorei DSM 10212C06)
- 2F7 (Clostridiales bacterium 2F7)
- 2E3 (Clostridiales bacterium 2E3)
- 2E1 (Anaerostipes caccae DSM 14662)
- 2D9 (Ruminococcus gnavus DSM 5001C)
- 1E3 (Coprococcus sp. D6)
- 1E11 (Clostridiales bacterium 1E11)
- 1D4 (Clostridiales bacterium 1D4)
- 1D2 (Clostridiales bacterium 1D2)
- 1D10 (Clostridiales bacterium 1D10)
- 1D1 (Clostridiales bacterium 1D10)
- 1C2 (Bifidobacterium breve DSM 20213 = JCM 1192)
- 1B11 (Bifidobacterium pseudolongum DSM 1192)

← Six strains are overlapping

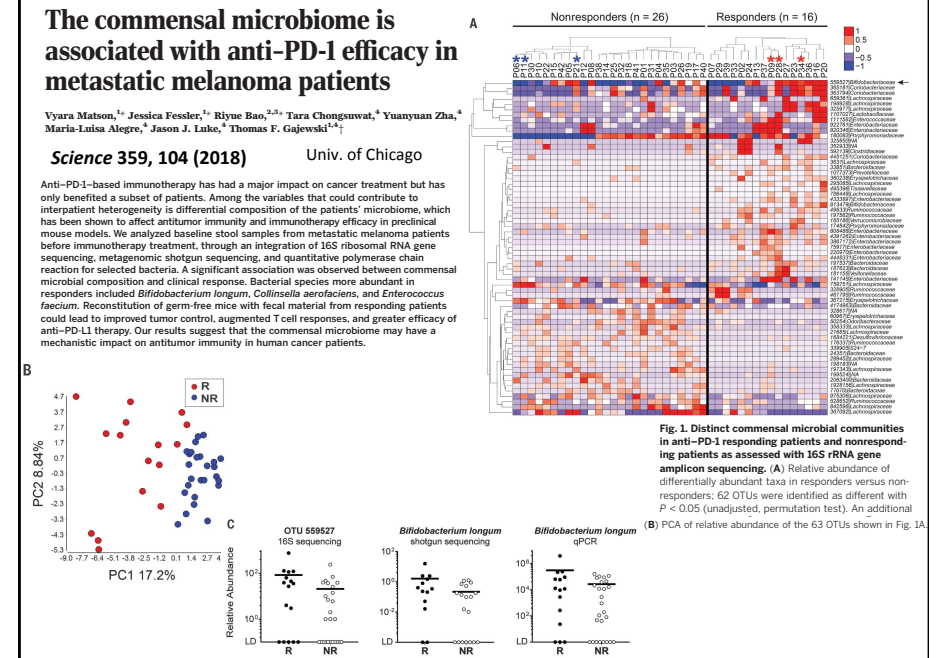
### Th17-inducing 20 strains

Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. Atarashi K, et al: *Cell* 163, 367-380 (2015).

## The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients

Science 359, 104 (2018) Univ. of Chicago

Anti-PD-1-based immunotherapy has had a major impact on cancer treatment but has only benefited a subset of patients. Among the variables that could contribute to interpatient heterogeneity is differential composition of the patients' microbiome, which has been shown to affect antitumor immunity and immunotherapy efficacy in preclinical mouse models. We analyzed baseline stool samples from metastatic melanoma patients before immunotherapy treatment, through an integration of 16S ribosomal RNA gene sequencing, metagenomic shotgun sequencing, and quantitative polymerase chain reaction for selected bacteria. A significant association was observed between commensal microbial composition and clinical response. Bacterial species more abundant in responders included *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*. Reconstitution of germ-free mice with fecal material from responding patients could lead to improved tumor control, augmented T cell responses, and greater efficacy of anti-PD-L1 therapy. Our results suggest that the commensal microbiome may have a mechanistic impact on antitumor immunity in human cancer patients.



## Translocation of a gut pathobiont drives autoimmunity in mice and humans.

Manfredo Vieira S et al. *Science*, 359, 1156-1161, 2018.

- Translocation of *Enterococcus gallinarum* to the liver and other systemic tissues triggers autoimmune responses in a genetic background predisposing to autoimmunity.
- *E. gallinarum*-specific DNA was recovered from liver biopsies of autoimmune patients.

## *Klebsiella pneumoniae* strains enriched in primary sclerosing cholangitis (PSC) trigger bacterial translocation and pathologic Th17 priming in the liver

Under revision

Collaboration with Kanai lab, Keio Univ.

\*PSC is a rare, chronic liver disease highly associated with ulcerative colitis (UC).

Feces of UC patients → Gnotobiotic mice → no liver inflammatory response  
 Feces of PSC patients → Gnotobiotic mice → **liver inflammatory response (Th17↑)**

### PSC-fecal gnotobiotic mice

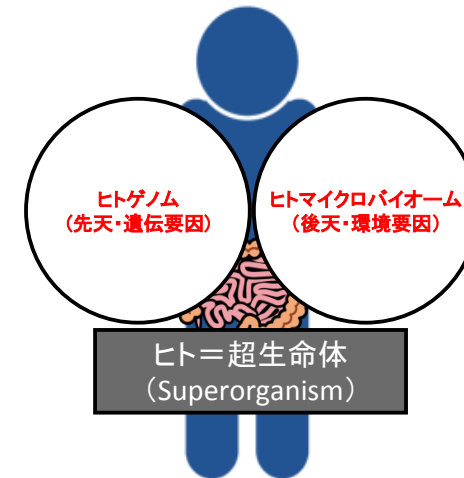
- Cultured bacterial in the mesenteric lymph nodes (腸間膜リンパ節)
- Isolated *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus gallinarum*.

\*The epithelial-damaging effect of KP was strain-specific and associated with the presence of Type VI secretion system.

## 超生命体としての「ヒト」

ヒトはヒトゲノムとヒトマイクロバイオーームからなる(Lederberg J, 2000)

- \* ヒトゲノム研究はヒト遺伝子を解析して『ヒト』を理解する科学
- \* ヒトマイクロバイオーーム研究はヒト微生物を解析して『ヒト』を理解する科学



## Precision Medicine Initiative (PMI)

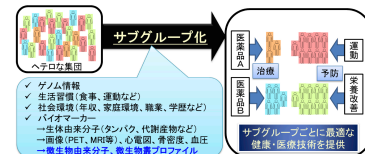
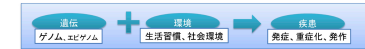


PMI announced by President Obama  
 January 30, 2015

<https://www.whitehouse.gov/the-press-office/2015/01/30/fact-sheet-president-obama-s-precision-medicine-initiative>

日本: AMED-CREST/LEAP/PRIME  
 2016.10~

緻密・最適な健康・医療へ



3つの重要な情報

### 宿主データ

- ・ゲノム多型
- ・血液/尿マーカー等

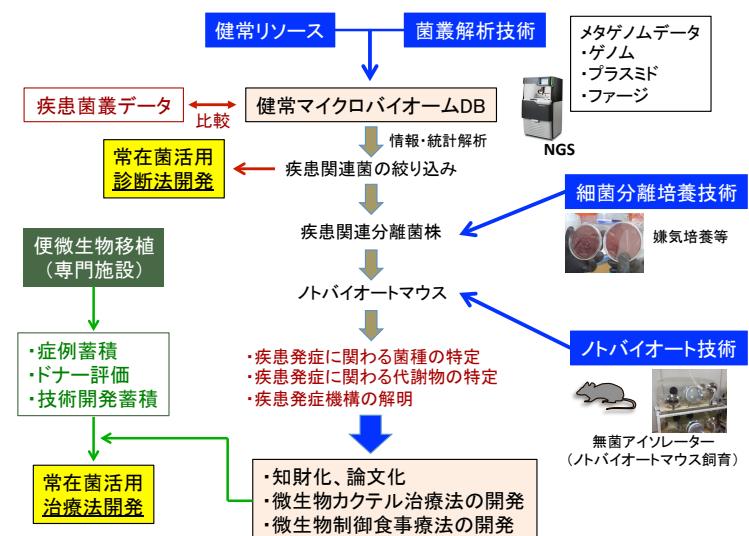
### 環境データ

- ・食事
- ・生活環境等

### マイクロバイオーームデータ

- ・メタゲノム
- ・メタボローム等

## マイクロバイオーーム研究から医療技術の開発



# Thank you for your attention

All in a day's catch !

The collage features several scientific publications and a central diagram illustrating metagenomics. The publications include:

- The Economist**: "Microbes maketh man" with sub-headlines: "The Catholic church's unlikely mess", "Paul Ryan: the man with the plan", "Generation Xtra", "China, victim of the Omicron?", and "On the origin of zero".
- 実験医学** (Experimental Medicine): "常在細菌叢が操るヒトの健康と疾患" (The Health and Disease of Humans Controlled by the Normal Bacterial Flora).
- MISSING MICROBIOME**: A book cover with a human silhouette and colorful dots representing the microbiome.
- NGSアプリケーション** (NGS Applications): "メタゲノム解析 実験プロトコール" (Metagenome Analysis Experimental Protocol).

The central diagram illustrates the metagenomics workflow:

- 環境DNA** (Environmental DNA) is collected from a sample.
- DNA抽出** (DNA Extraction) is performed.
- シーケンシング** (Sequencing) is conducted using a sequencer.
- 解析** (Analysis) is performed on the resulting data.

Additional labels in the diagram include: "細菌叢のDNA", "宿主のDNA", "メタゲノム", "シーケンシング", "解析", "環境DNA", "DNA抽出", "シーケンシング", "解析".

Newton 2013