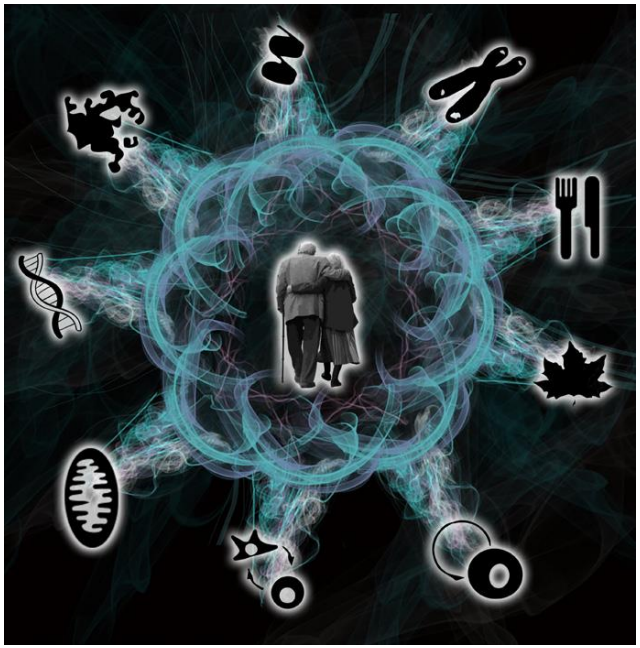
Microbiota studies in cancer



Javier Torres, PhD

Unidad de Investigacion en Enfermedades Infecciosas

Instituto Mexicano del Seguro Social

- ✓ Impact in México
- ✓ Studies in gastric cancer
- ✓ Studies in bile duct cancer

- ✓ **Impact in México**
- ✓ Studies in gastric cancer
- ✓ Studies in bile duct cancer

Gastric and biliary tract cancer

- GC is the second cause of death due to all cancer worldwide.
- GC and BTC are diseases of very poor prognosis, particularly in developing countries.
- The success in treatment rely on an early diagnosis.

Impact in México

- It is estimated that, only during the first year of medical attention, direct cost related to gastric and breast cancer in México sum up approximately 2 thousand million pesos.
- In Mexico cancer is the third leading cause of death, with 128,000 new cases each year.
- Considering the demographic trend in our country, OMS estimate that by year 2020 the number of patients affected by cancer will duplicate.

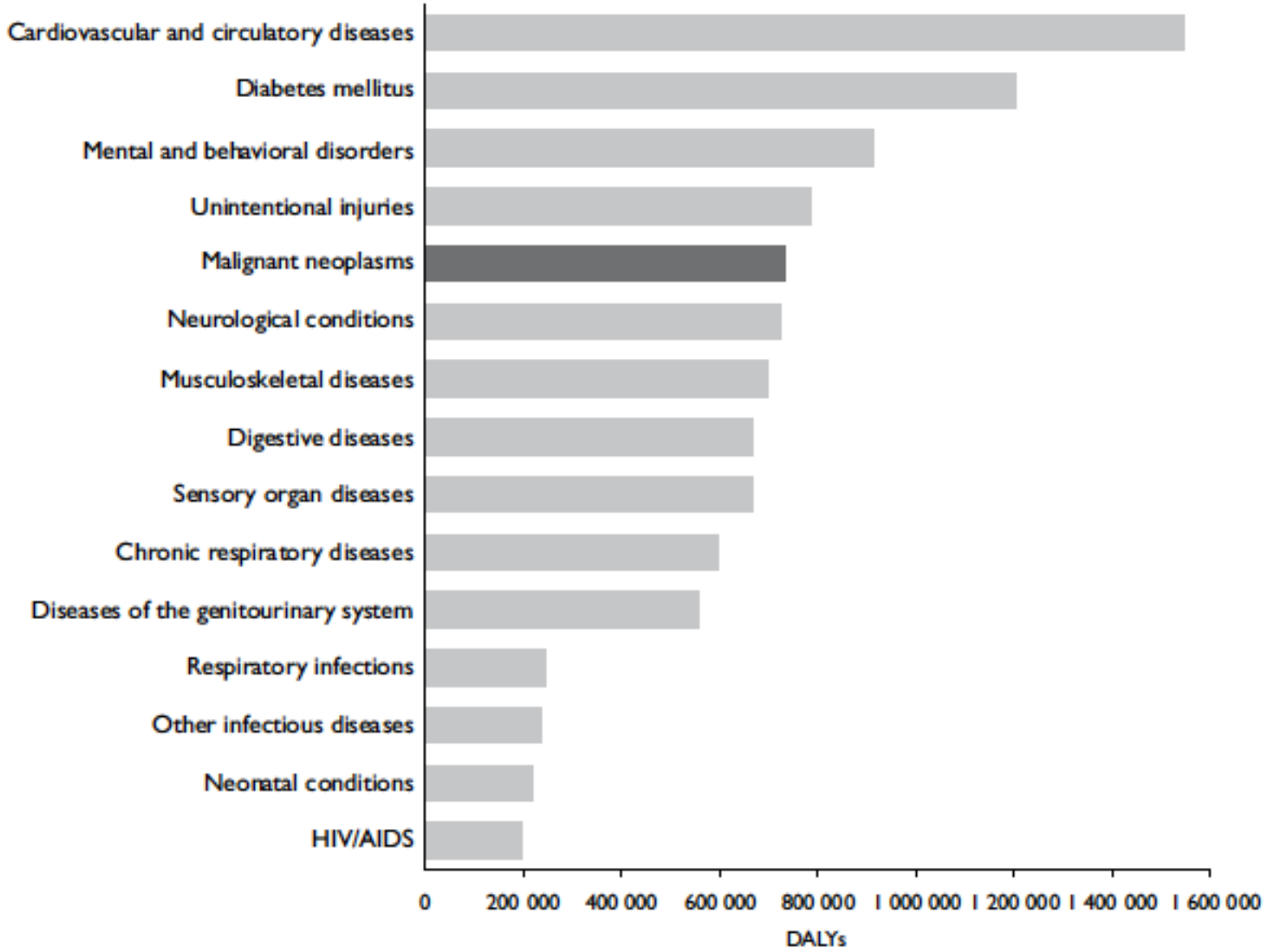
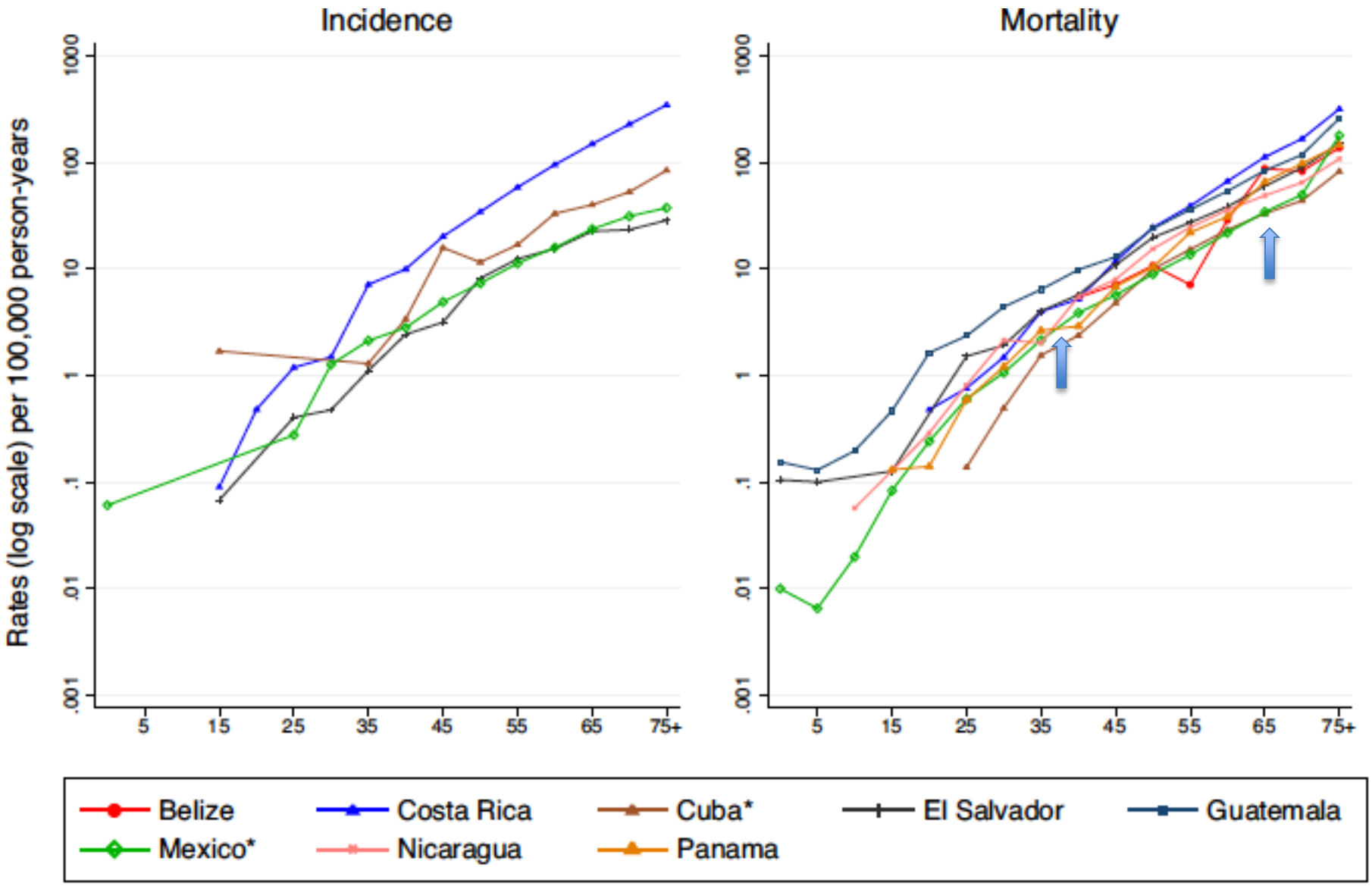


Figure 1. Fifteen major causes of disease burden measured via Disability-Adjusted Life Years (DALYs). Mexico, IMSS 2010



Tasas de incidencia y mortalidad de cáncer gástrico en América central-norte, por edad.

Multivariate logistic regression analysis showing interaction of DQA*04:01 allele with other risk factors

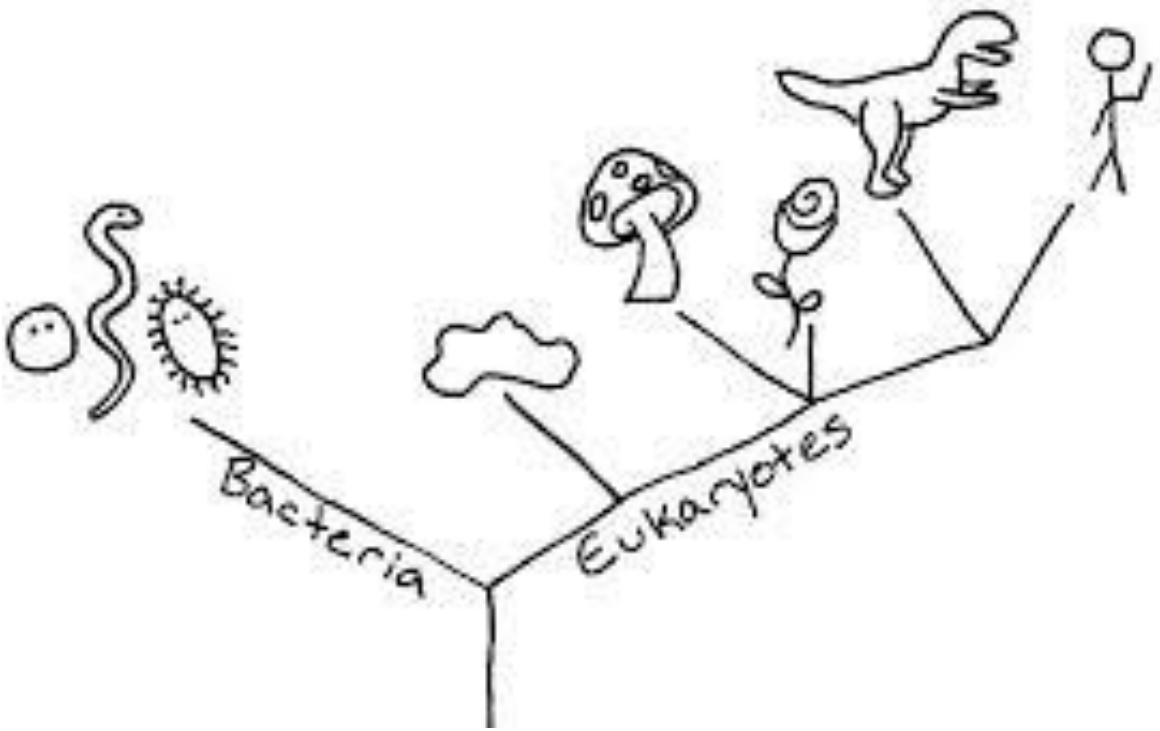
Disease	Variable	β coefficient	Wald X^2	*p*	OR (95% CI)[a]
Non-Atrophic Gastritis	04:01[b]	0.14	0.62	0.430	1.15 (0.81-1.65)
	male	-0.29	2.67	0.102	0.74 (0.53-1.06)
	> 50 years	0.64	12.9	<0.0001	1.89 (1.34-2.69)
	Hp+	0.22	1.36	0.244	1.25 (0.85-1.82)
Intestinal Metaplasia	04:01[b]	0.43	2.14	0.143	1.54 (0.86-2.75)
	male	0.40	1.78	0.182	1.50 (0.83-2.71)
	> 50 years	2.46	65.6	<0.0001	11.7 (6.44-21.2)
	Hp+	1.01	7-07	0.008	2.74 (1.3-5.78)
Gastric Cancer	04:01[b]	0.87	13.7	<0.0001	2.38 (1.5-3.8)
	male	1.04	18.29	<0.0001	2.85 (1.76-4.6)
	> 50 years	2.69	118.1	<0.0001	14.8 (9.1-24.0)
	Hp+	-0.02	0.007	0.932	0.98 (0.59-1.63)

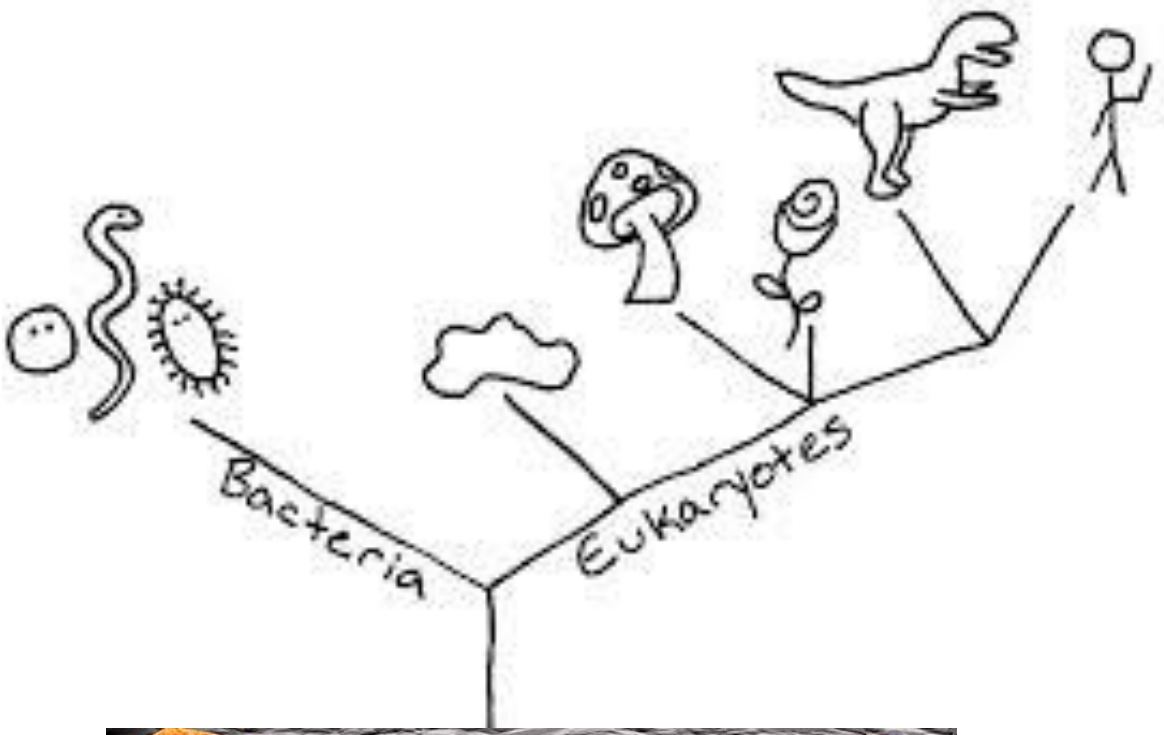
✓Impact in México

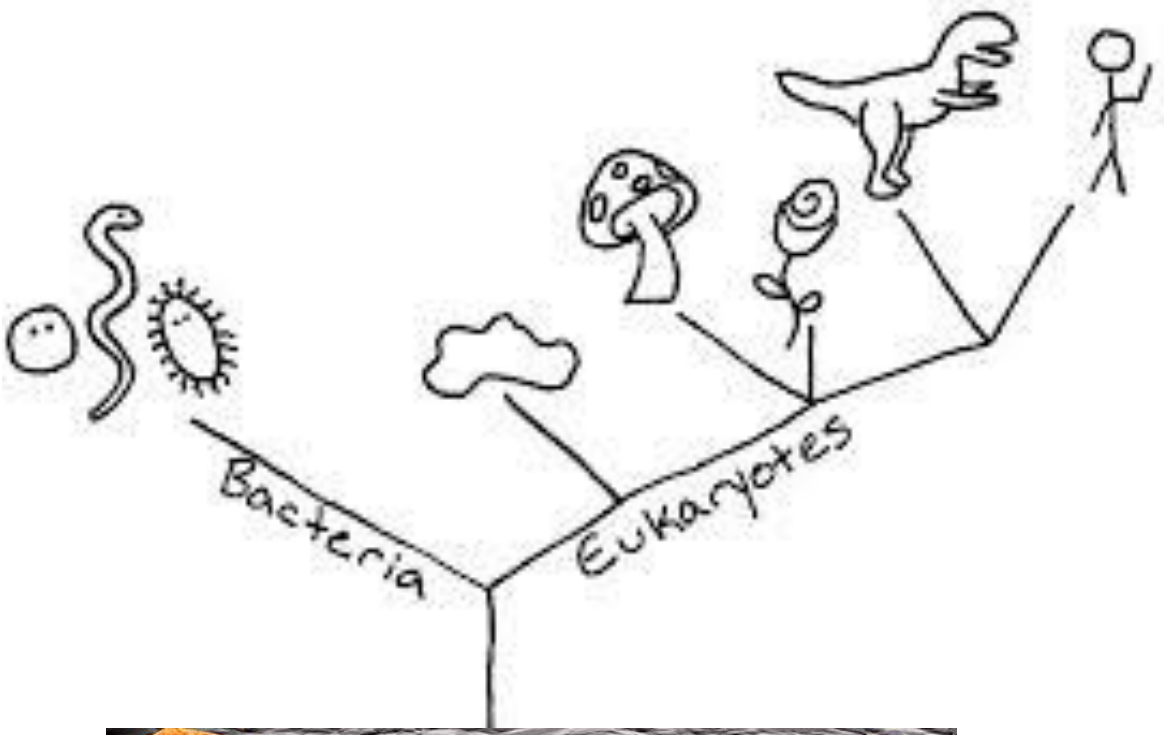
✓**Studies in gastric cancer**

✓Studies in bile duct cancer

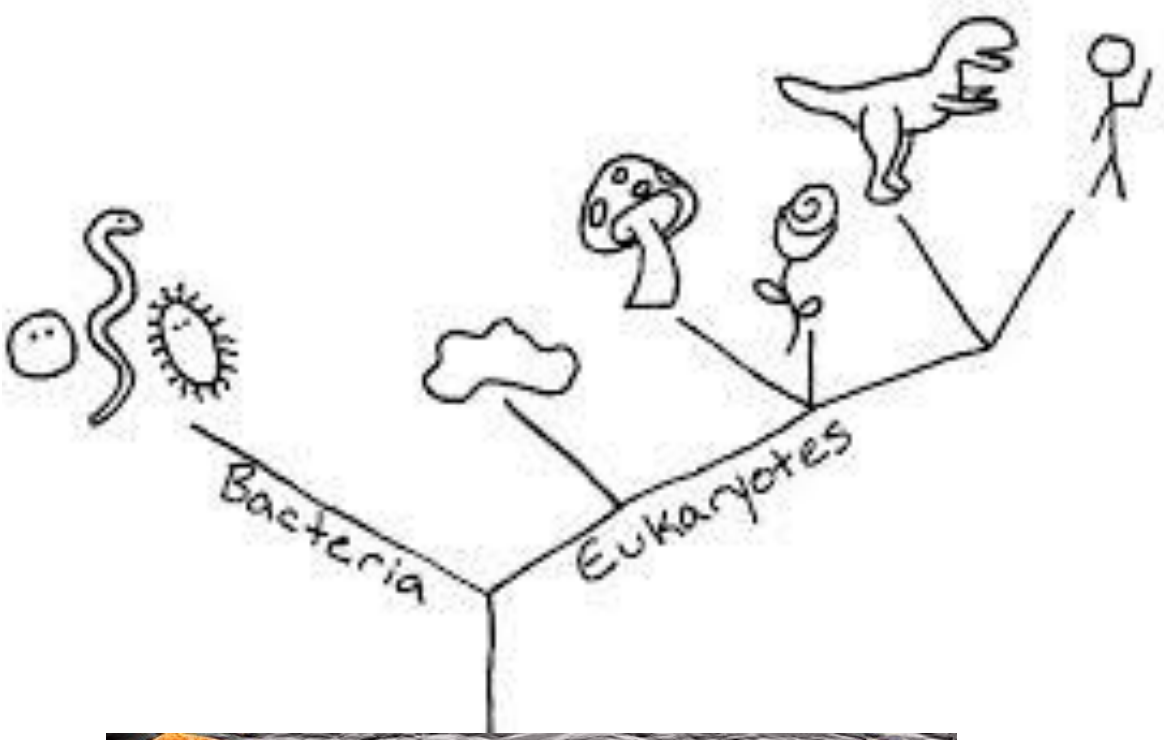

Come on in.
Take a seat.
M. Dawid
Friendly bacteria


Bacteria
Eukaryotes


Bacteria
Eukaryotes

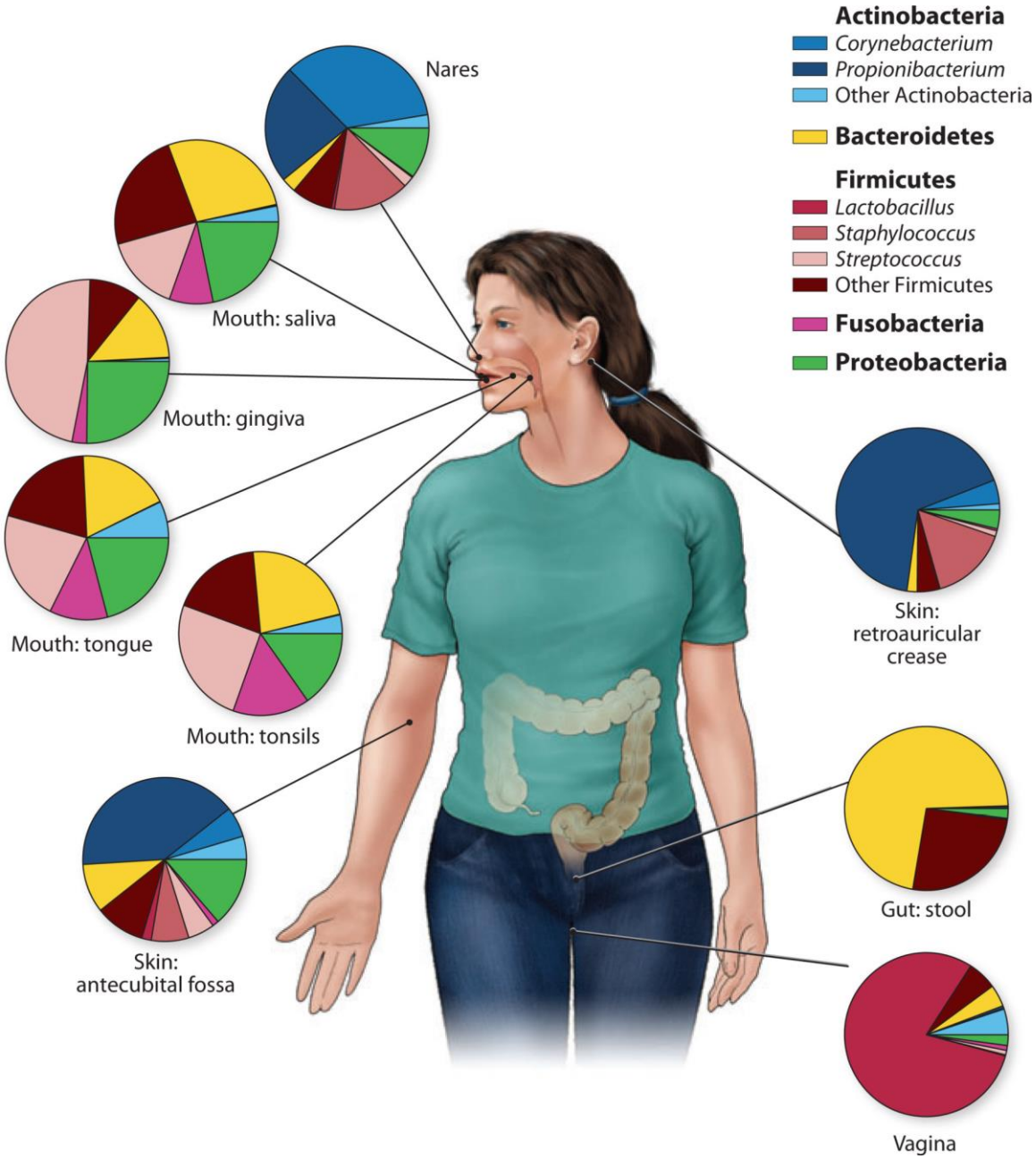


Microbiota

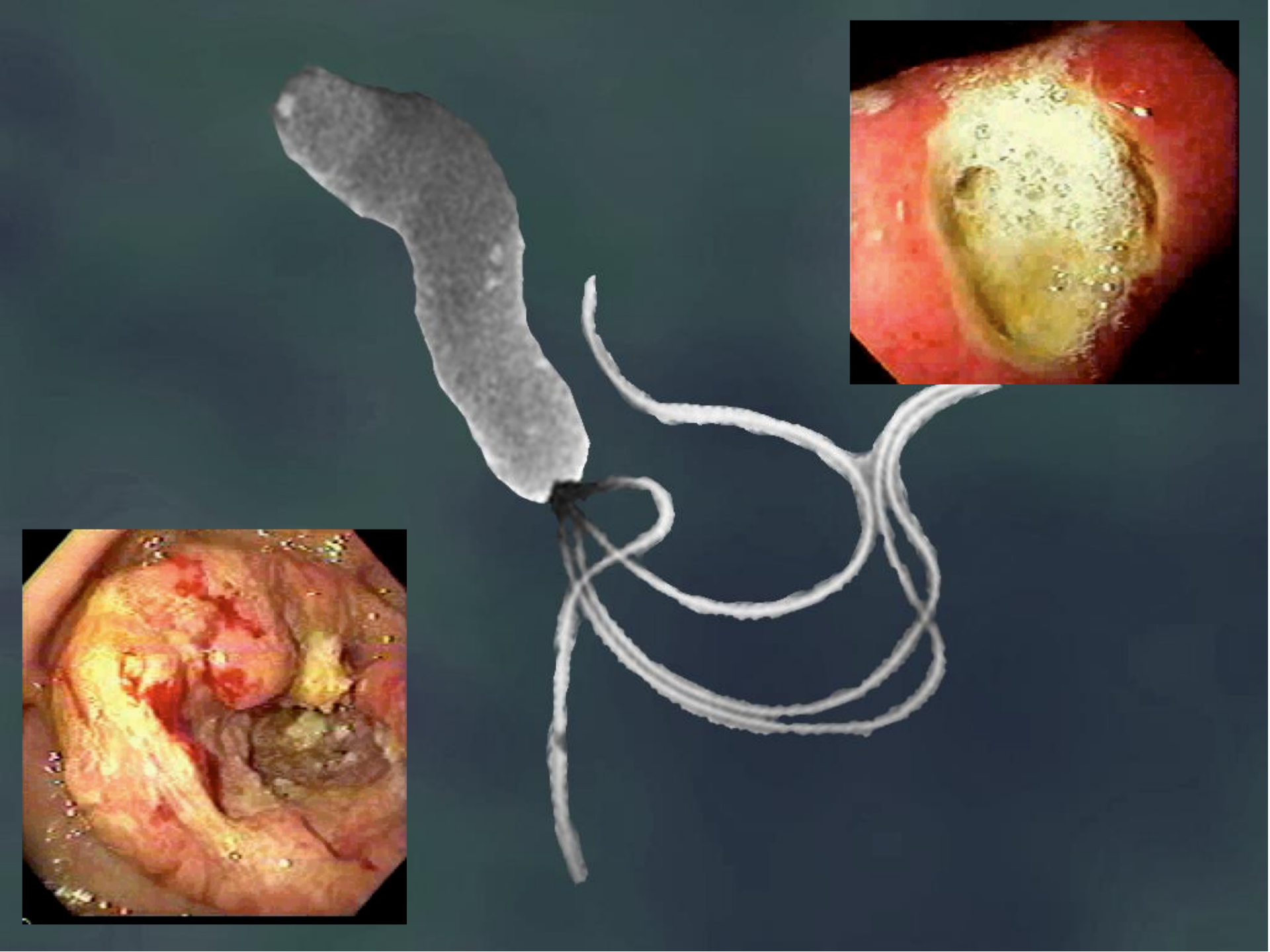


Flora Normal

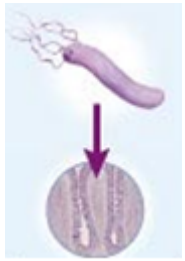
Microbiota



La microbiota humana comprende aprox. 10^{13} bacterias, similar al numero de cel. humanas y 150 veces más el número de genes bacterianos



Natural history of *H. pylori* infection



NORMAL GASTRIC MUCOSA

ACUTE GASTRITIS

CHRONIC GASTRITIS

CHRONIC GASTRITIS PREDOMINANT IN ANTRUM

DUODENAL ULCER

NON-ATROPHIC GASTRITIS

ASYMPTOMATIC

CHRONIC GASTRITIS PREDOMINANT IN CORPUS

GASTRIC ULCER

INTESTINAL METAPLASIA

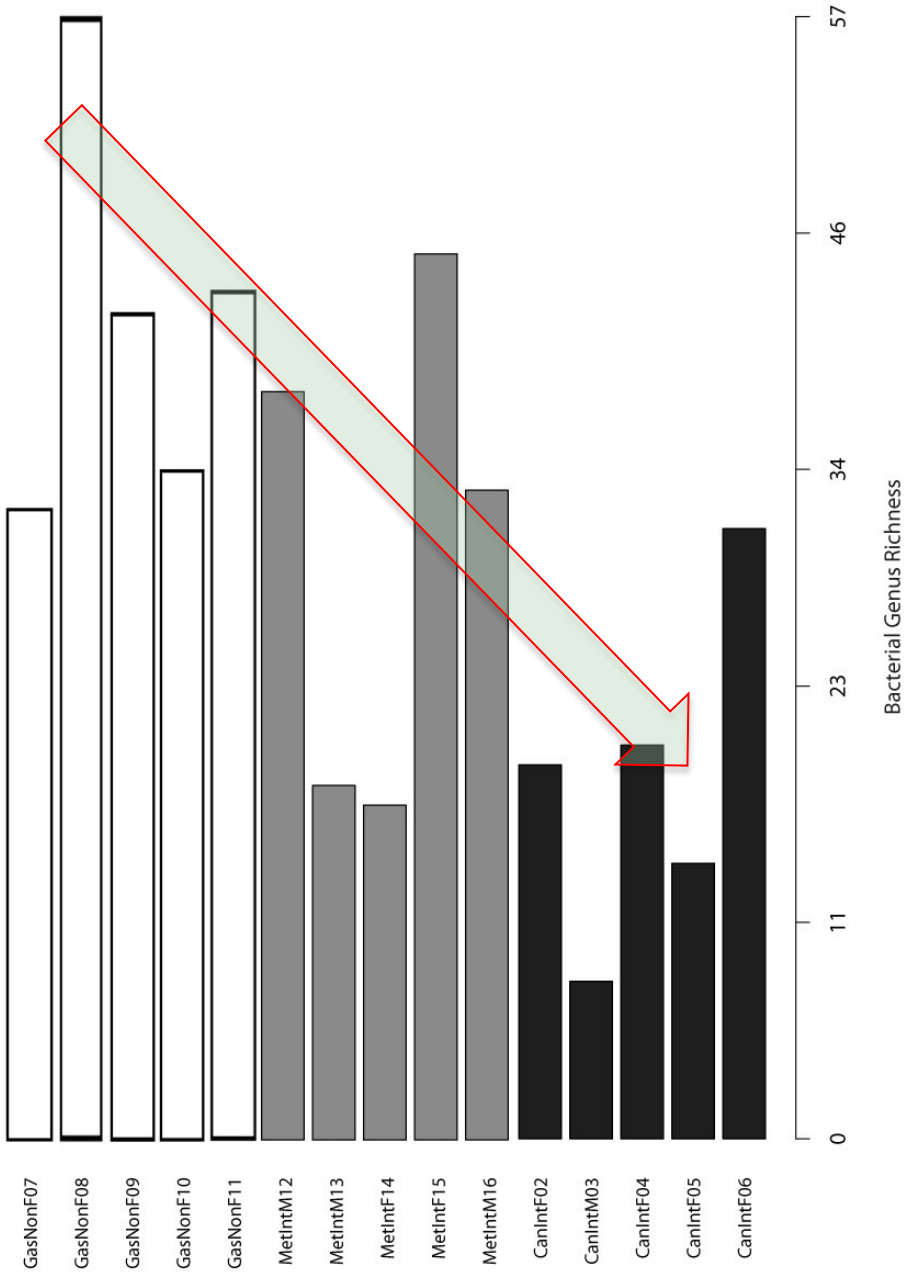
DYSPLASIA

GASTRIC CANCER

INFANCY

ADVANCED AGE

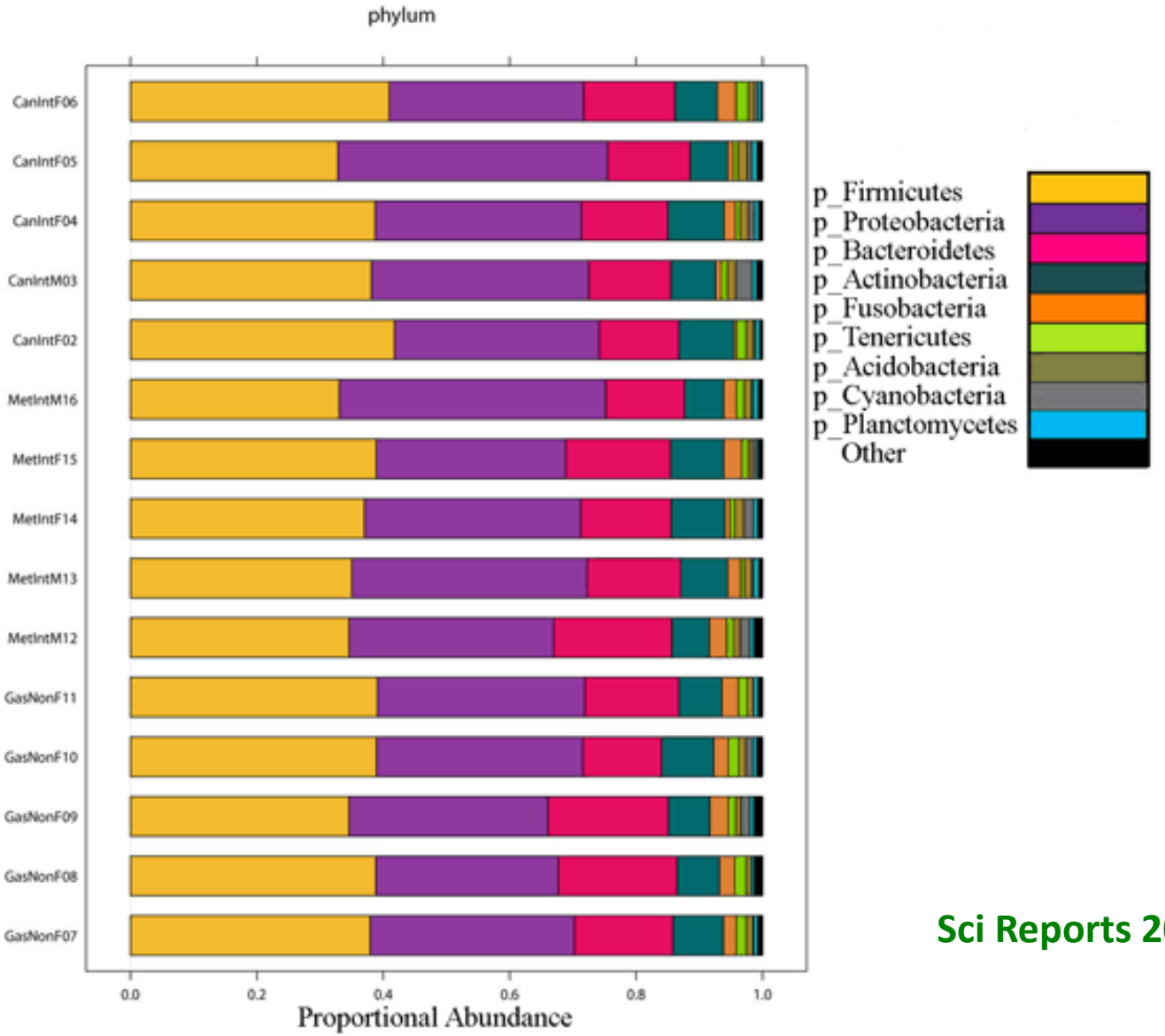
Perfil de la Microbiota con secuencia de V4 16S en la plataforma miSeq



Bacterial diversity tends to decrease in the stomach, from gastritis to metaplasia and to gastric cancer

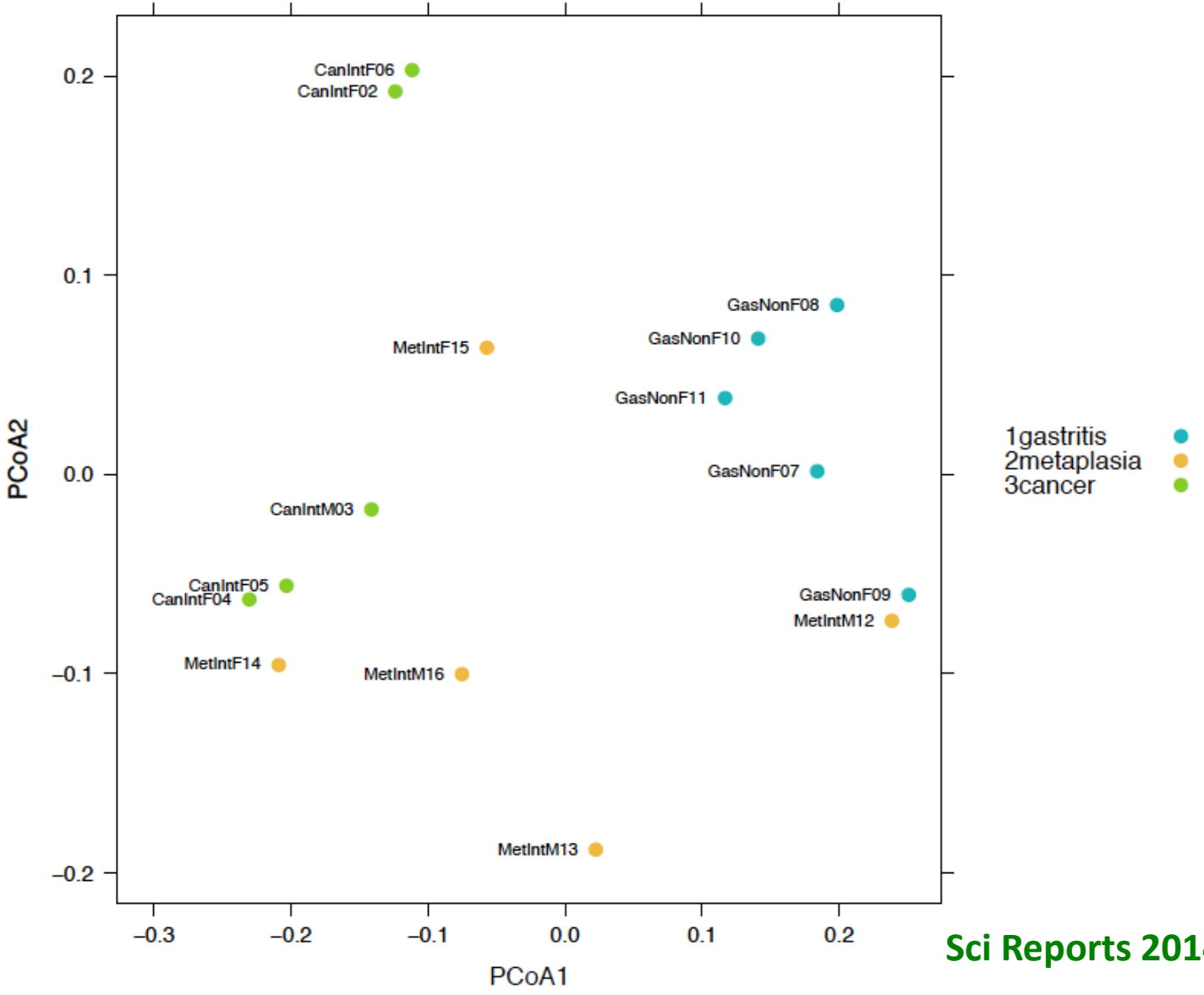
Sci Reports 2014;4:4202

Composition of microbiota of the stomach in pre-neoplasia and cancer

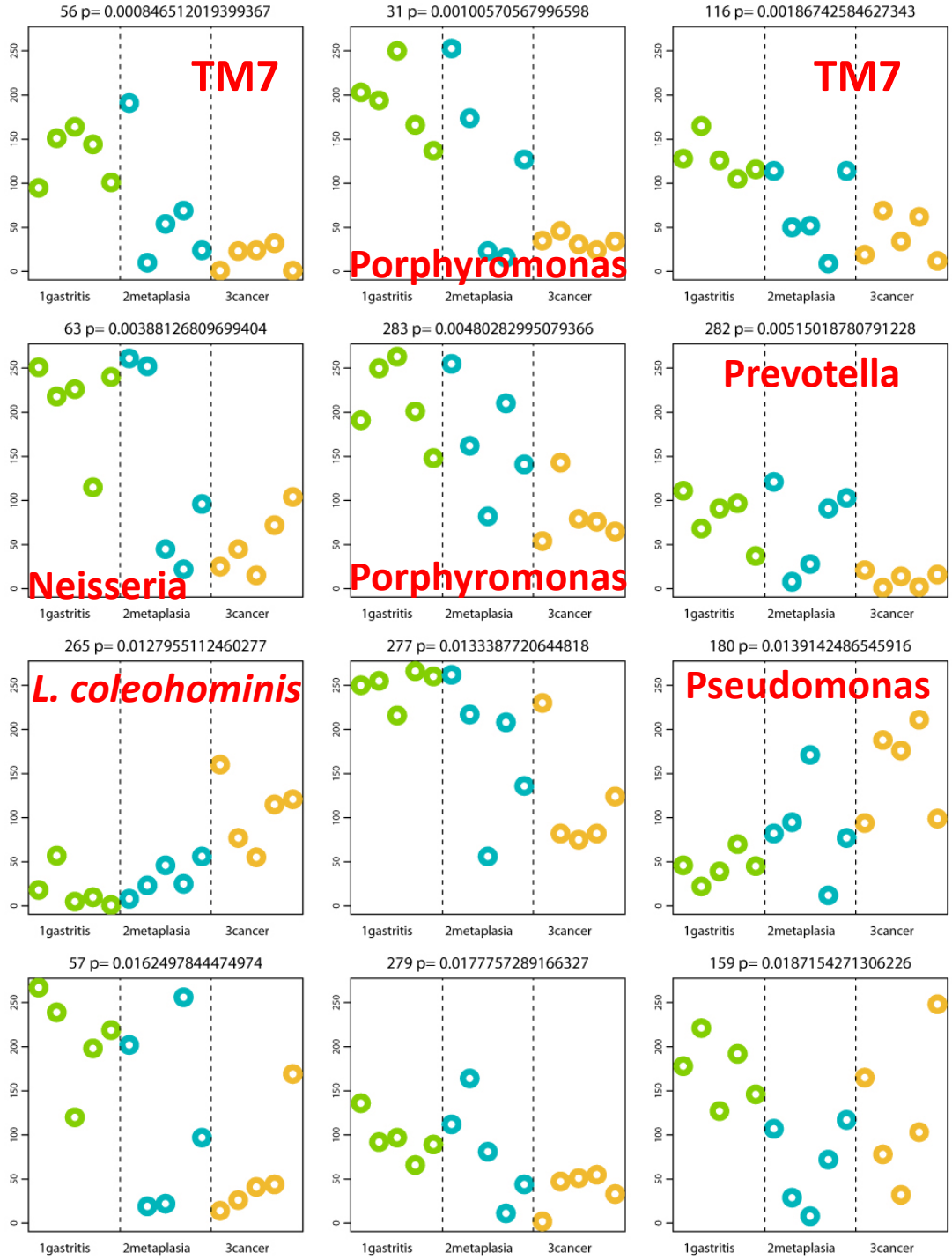


Sci Reports 2014;4:4202

Multicomponent analyses-PCoA shows a trend of microbiota separation from gastritis to metaplasia and to cancer



Sci Reports 2014;4:4202



Profiles of the most significant OTUs

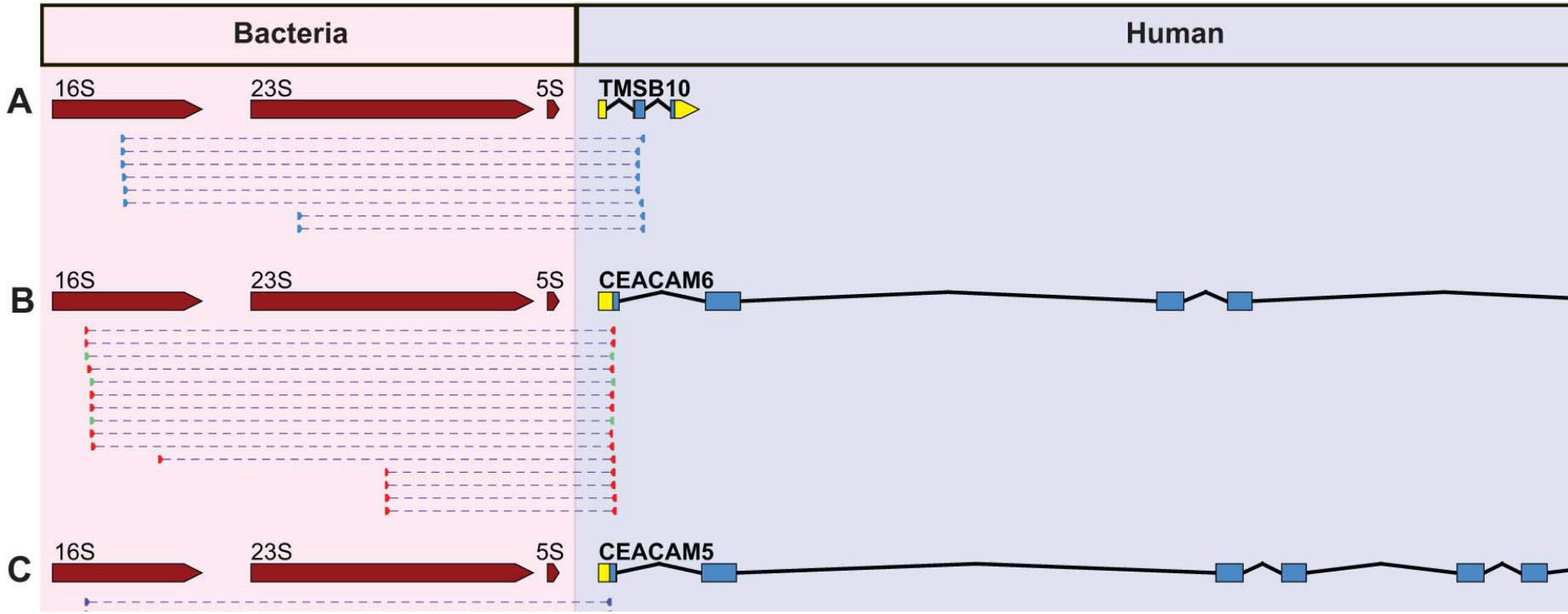
TM7 ↓

Recently described in intestine; code for T-IV, proinflamatory island

Pseudomonas ↑

Sci Reports 2014;4:4202

Evidence of integration of bacterial ribosomal genes of *Pseudomonas* in gastric cancer

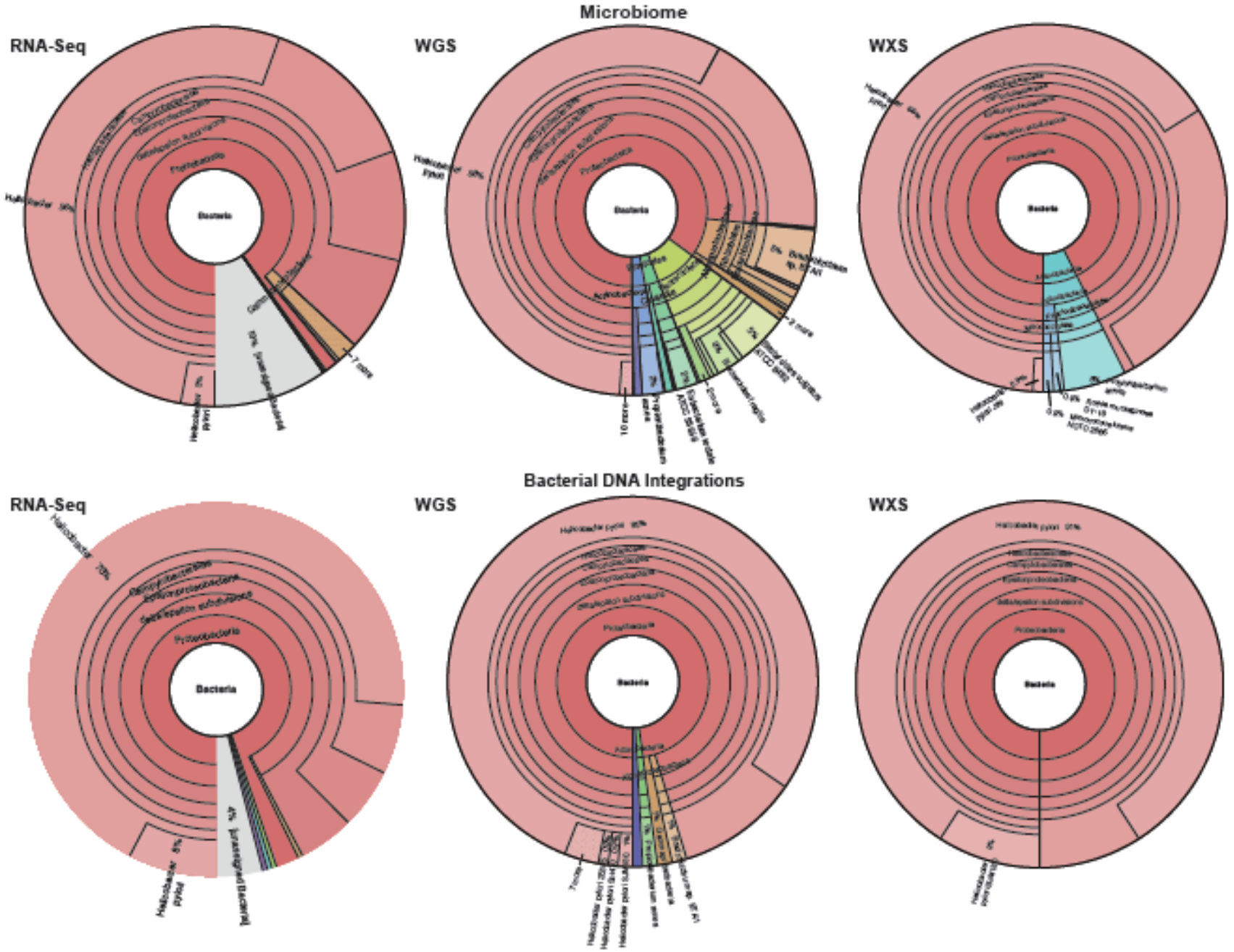


Genes up-regulated in gastric cancer

Julie C. Dunning, Institute for Genome Science, U Maryland

PLoS Comput Biol 9(6): e1003107

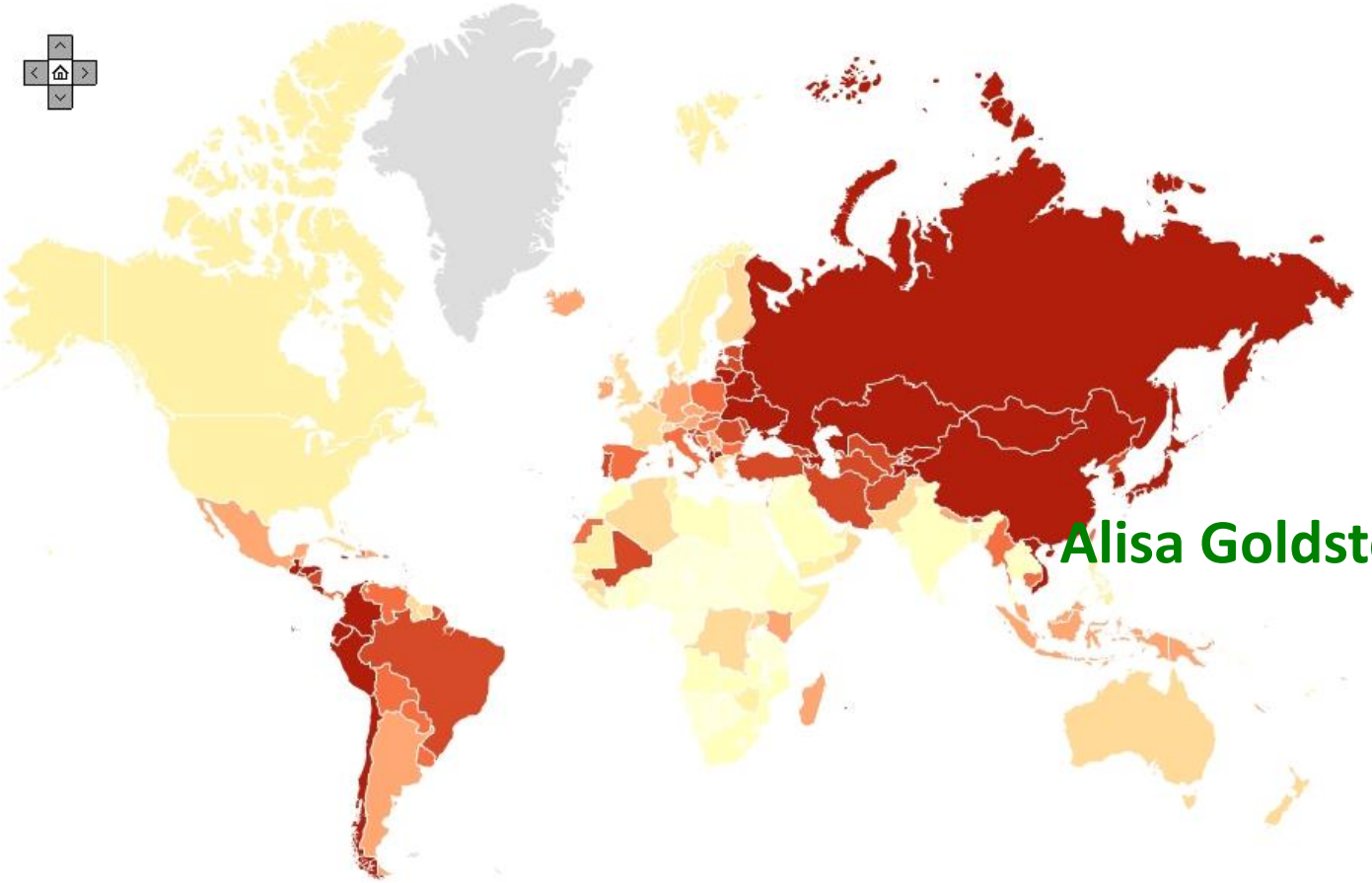
Tumor microbial proportion and bacterial DNA integrations



RNA-Seq Patient 1 genes with BDIs

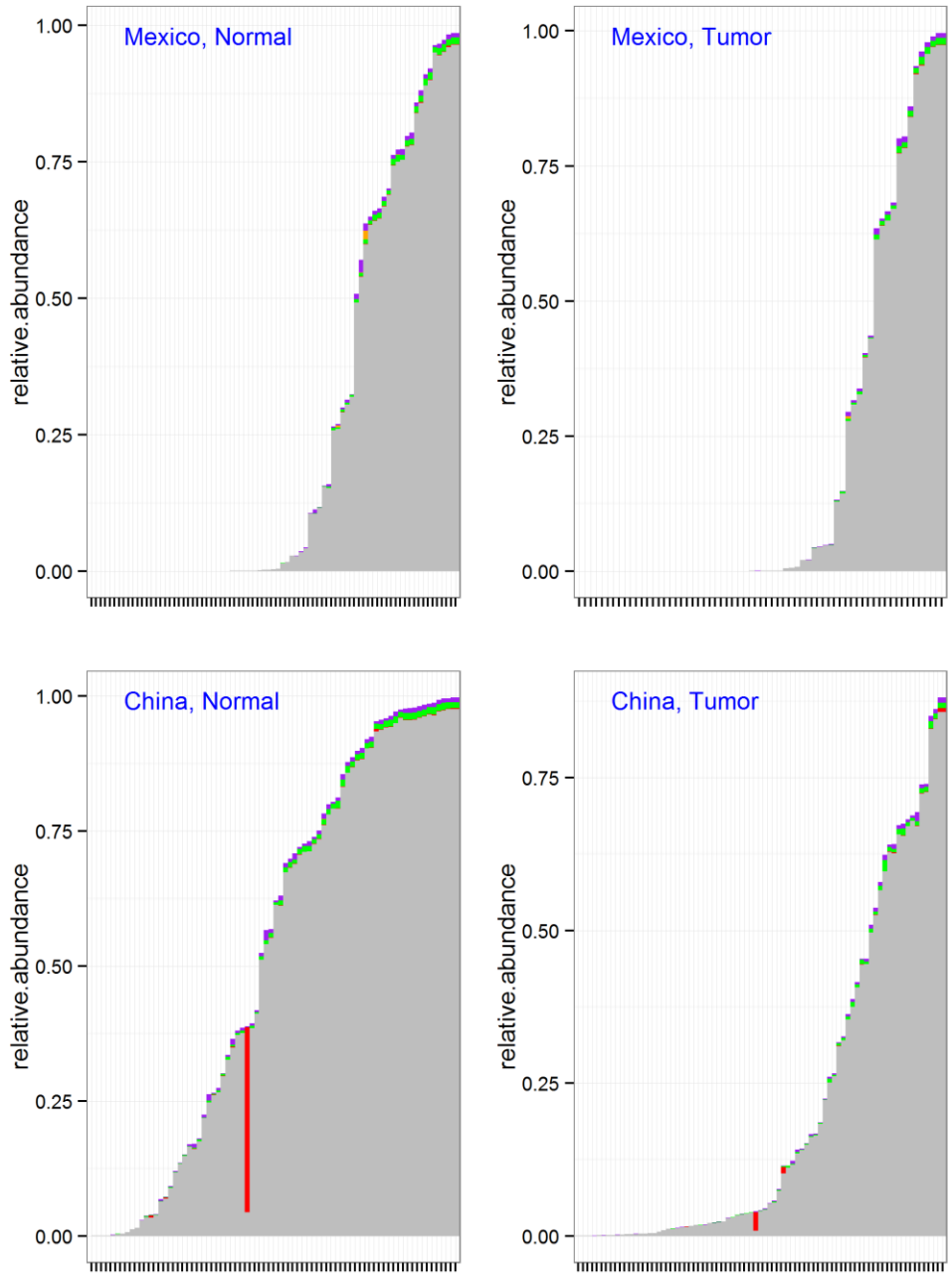
Chromosome	Gene	Sample
chr7	*AGR2*	T1
chr11	*PGA3*	A1
chr14	*IgG*	T1, A1
chr21	unknown	T1
chr22	*IGLL5*	T1
chrM	unknown	T1, A1
chrUn	*RNA45S5*, unknown	T1, A1

Asia and Latin America are the regions with the highest mortality rates for GC



Alisa Goldstein, NIH/NCI

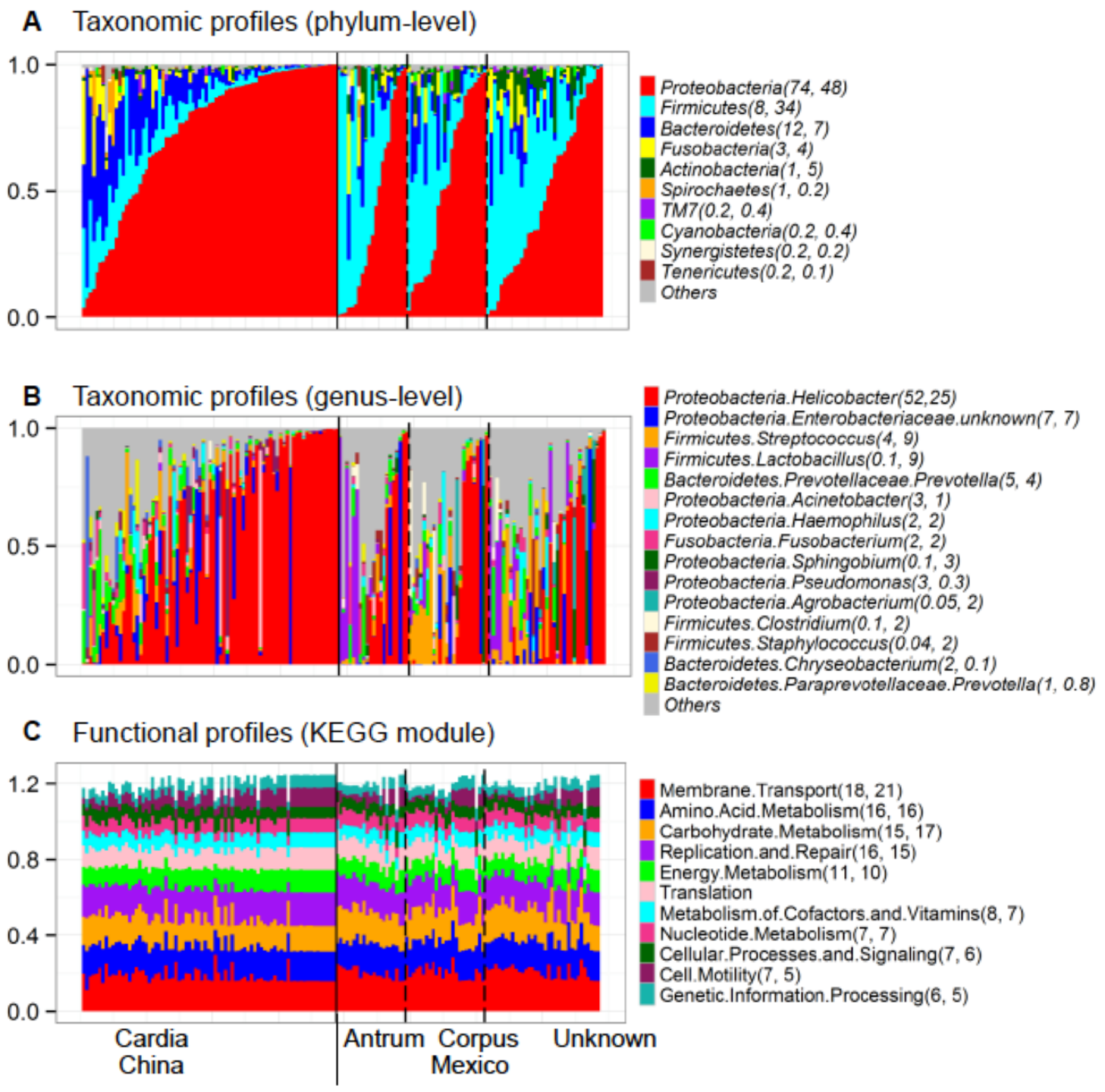
Globocan 2010

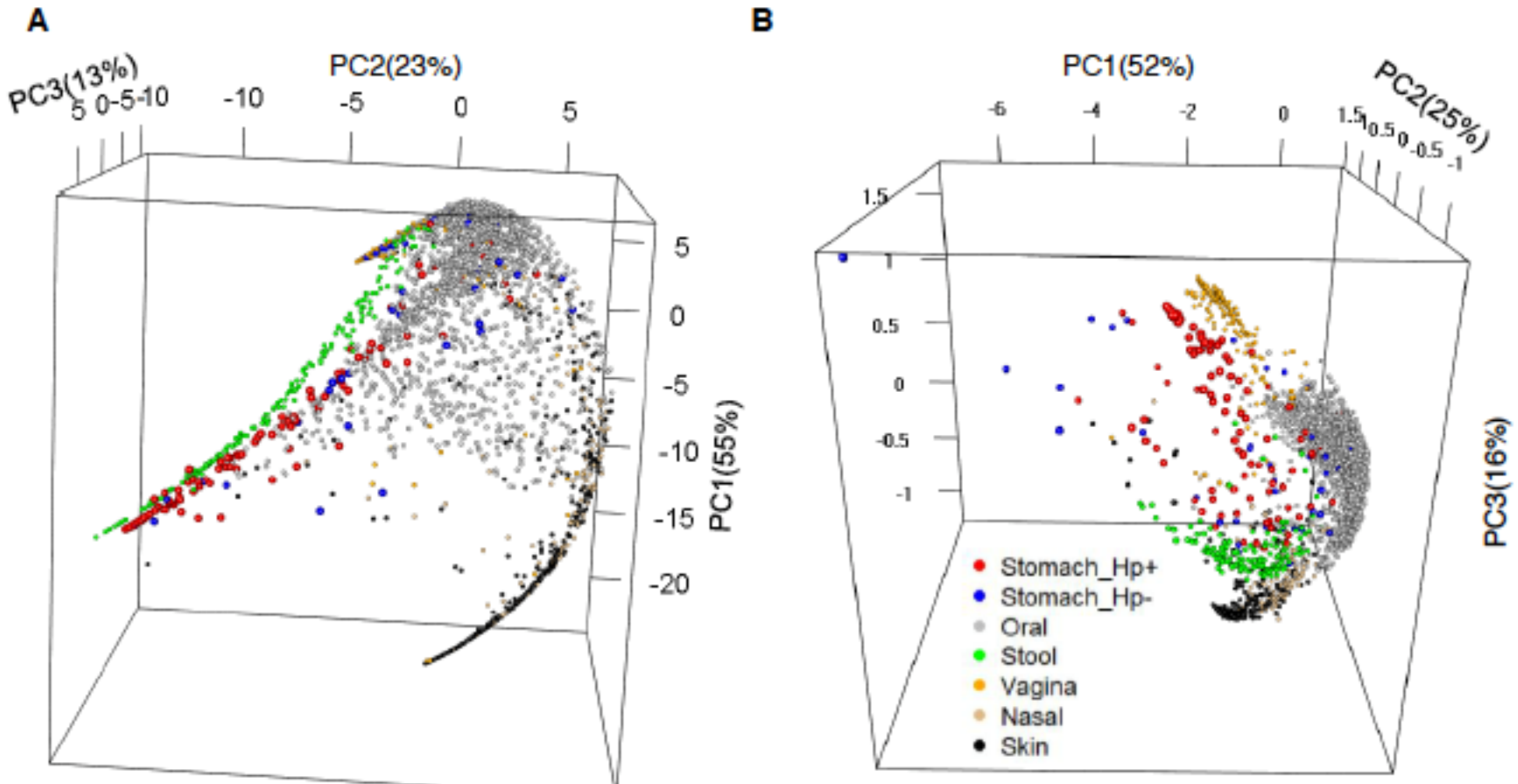


***H. pylori* is highly frequent in tumor and adjacent tissue of Mexican and Chinese patients with gastric cancer**

***H. pylori* OTUs**

Dr. Alisa Goldstein, U Maryland


A Taxonomic profiles (phylum-level)
1.0
0.5
0.0
Proteobacteria(74, 48)
Firmicutes(8, 34)
Bacteroidetes(12, 7)
Fusobacteria(3, 4)
Actinobacteria(1, 5)
Spirochaetes(1, 0.2)
TM7(0.2, 0.4)
Cyanobacteria(0.2, 0.4)
Synergistetes(0.2, 0.2)
Tenericutes(0.2, 0.1)
Others
B Taxonomic profiles (genus-level)
1.0
0.5
0.0
Proteobacteria.Helicobacter(52,25)
Proteobacteria.Enterobacteriaceae.unknown(7, 7)
Firmicutes.Streptococcus(4, 9)
Firmicutes.Lactobacillus(0.1, 9)
Bacteroidetes.Prevotellaceae.Prevotella(5, 4)
Proteobacteria.Acinetobacter(3, 1)
Proteobacteria.Haemophilus(2, 2)
Fusobacteria.Fusobacterium(2, 2)
Proteobacteria.Sphingobium(0.1, 3)
Proteobacteria.Pseudomonas(3, 0.3)
Proteobacteria.Agrobacterium(0.05, 2)
Firmicutes.Clostridium(0.1, 2)
Firmicutes.Staphylococcus(0.04, 2)
Bacteroidetes.Chryseobacterium(2, 0.1)
Bacteroidetes.Paraprevotellaceae.Prevotella(1, 0.8)
Others
C Functional profiles (KEGG module)
1.2
0.8
0.4
0.0
Membrane.Transport(18, 21)
Amino.Acid.Metabolism(16, 16)
Carbohydrate.Metabolism(15, 17)
Replication.and.Repair(16, 15)
Energy.Metabolism(11, 10)
Translation
Metabolism.of.Cofactors.and.Vitamins(8, 7)
Nucleotide.Metabolism(7, 7)
Cellular.Processes.and.Signaling(7, 6)
Cell.Motility(7, 5)
Genetic.Information.Processing(6, 5)
Cardia
China
Antrum
Corpus
Mexico
Unknown



Principal coordinates plots showing variation among samples based on Bray-Curtis distance of phylum-level taxonomic profiles (A); and KEGG module-level functional profiles (B)

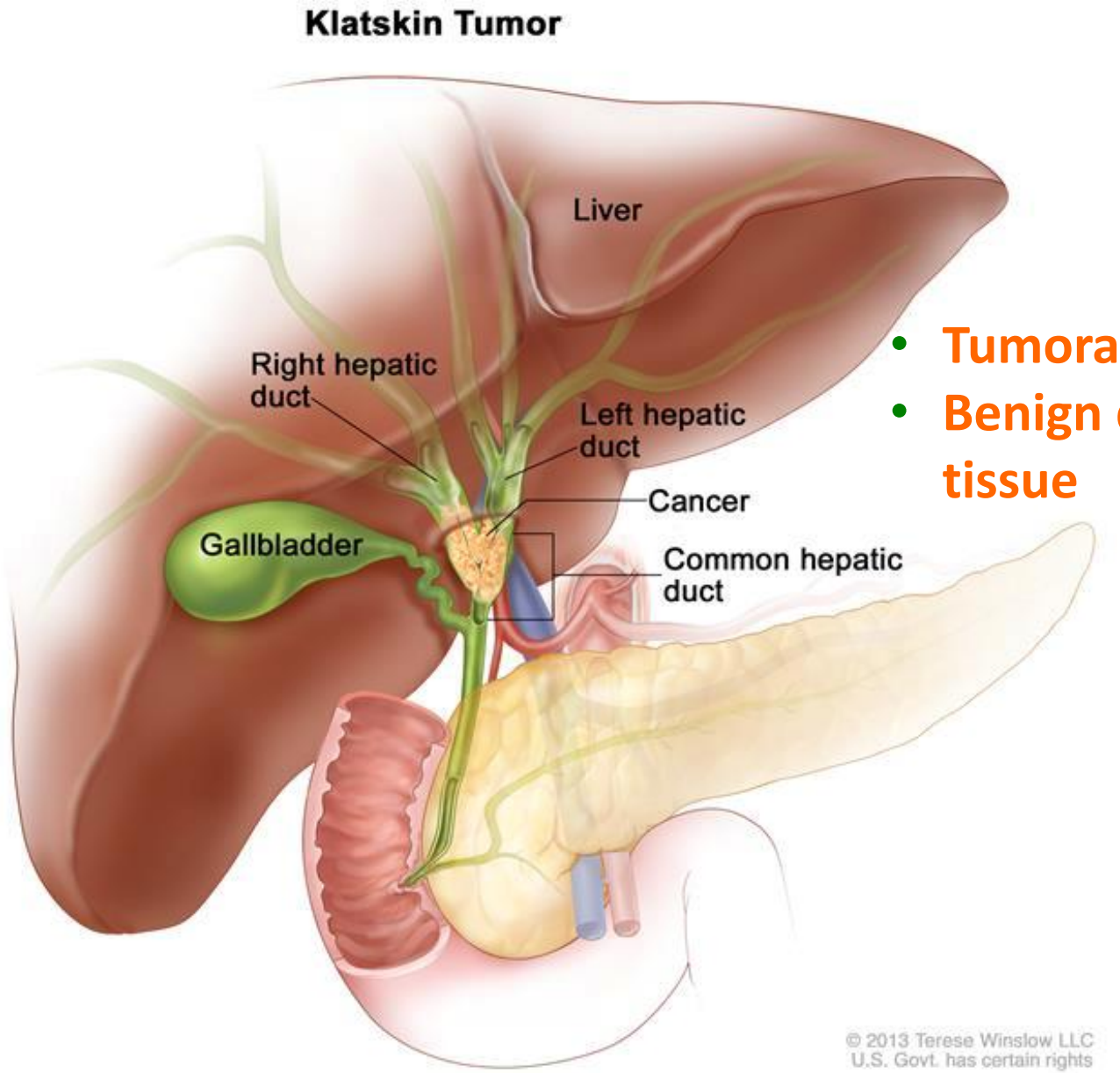
Conclusions

- ✓ **Bacterial diversity in the stomach decrease as the severity of lesions advance from NAG to IM and to GC**
- ✓ **Microbiota studies indicate that *H. pylori* is frequent and abundant in the stomach of patients with gastric cancer.**
- ✓ **Preliminary evidence suggests integration of *H. pylori* ribosomal genes in human genome.**
- ✓ **Comparative analyses showed higher variation of microbiota and functional profiles in the stomach than in other body sites.**

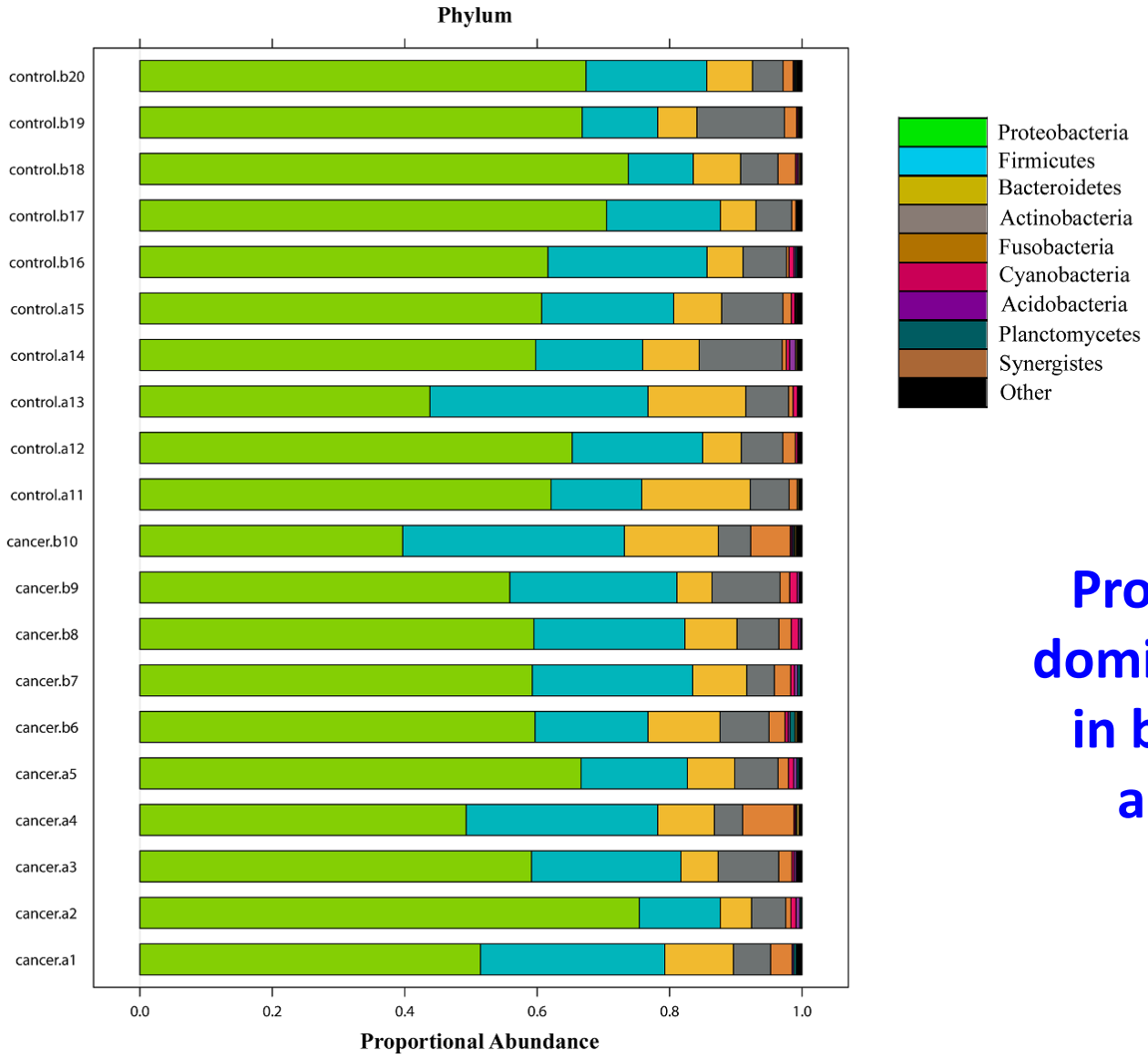
✓Impact in México

✓Studies in gastric cancer

✓**Studies in bile duct cancer**

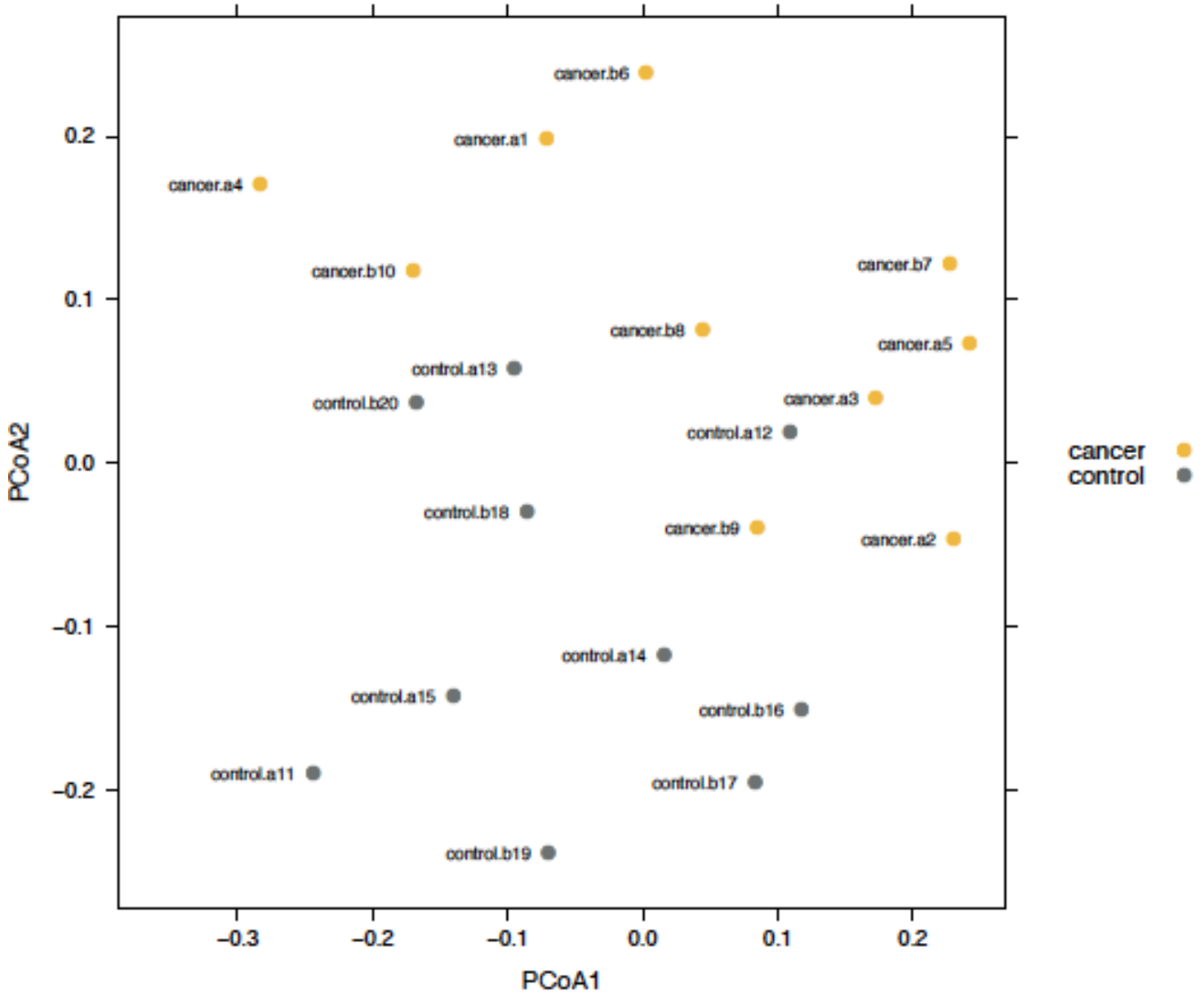


- Tumoral tissue
- Benign disease tissue



Proteobacteria is the dominant phylum (60%) in bile duct of cancer and benign cases

Multi-component analyses-PCoA of 4,002 taxa shows separation of bile duct cancer and benign disease microbiota



The 12 OTUs with the most significant *p*-values for abundance in one of the studied groups

TAXA ID	PHYLUM	CLASS	ORDER	FAMILY	GENUS	SPECIES	TREND
809814	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	unclassified	Unclassified	Increase
75585	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Nesterenkonia	97otu9179	Decrease
102924	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	Mesorhizobium	Unclassified	Decrease
621279	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Nesterenkonia	unclassified	Decrease
845354	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	unclassified	unclassified	Decrease
4451302	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	unclassified	unclassified	Increase
2802748	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	unclassified	unclassified	Decrease
343239	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	unclassified	unclassified	Increase
903426	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	mucilaginosa	Decrease
955102	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	97otu84559	Increase
3091248	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	unclassified	unclassified	Increase
2283862	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Dialister	unclassified	Increase

- **Rothia decreases also in skin cancer**
- **Nesterenkonia no previously reported in humans**
- **Novosphingobium**

Frequency of *Nesterenkonia sp.* colonization in the biliary tract of patients with benign or malignant disease

Disease group	No. tested	No. Positive (%)	p-value	OR (95% CI)
Benign biliary disease	90	22 (24.4)		
Biliary tract cancer	100	11 (11)	0.0244	0.38 (0.17-0.84)

Nesterenkonia lacusekhoensis strain EL-30 16S ribosomal RNA gene, partial sequence
Sequence ID: ref|NR_028928.1|Length: 1467Number of Matches: 1
Range 1: 272 to 1161GenBankGraphics

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1640 bits(888)	0.0	889/890(99%)	0/890(0%)	Plus/Plus

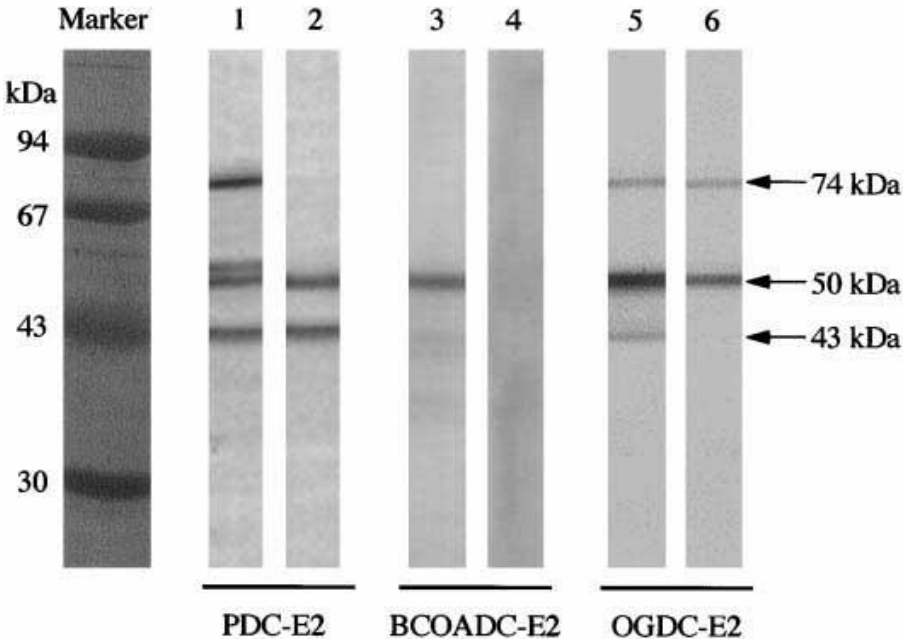
```
Query  1    CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  272  CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT  331

Query  61   TGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGTGGGATGACGGCCTTCGGGTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  332  TGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGTGGGATGACGGCCTTCGGGTT  391

Query  121  GTAAACCACTTTCAGCAGGGAAGAAGCTTTTTGTGACGGTACCTGCAGAAGAAGCGCCGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  392  GTAAACCACTTTCAGCAGGGAAGAAGCTTTTTGTGACGGTACCTGCAGAAGAAGCGCCGG  451

Query  181  CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTATCCGGAATTATTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  452  CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTATCCGGAATTATTG  511
```

American Journal of Gastroenterology
© 2004 by Am. Coll. of Gastroenterology
Published by Blackwell Publishing

ISSN 0002-9270
doi: 10.1111/j.1572-0241.2004.41121.x

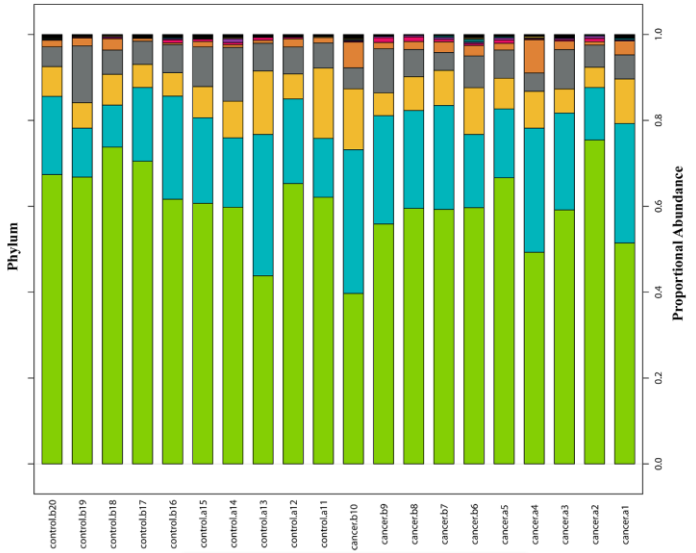
EDITORIAL

Novosphingobium Aromaticivorans: A Potential Initiator of Primary Biliary Cirrhosis

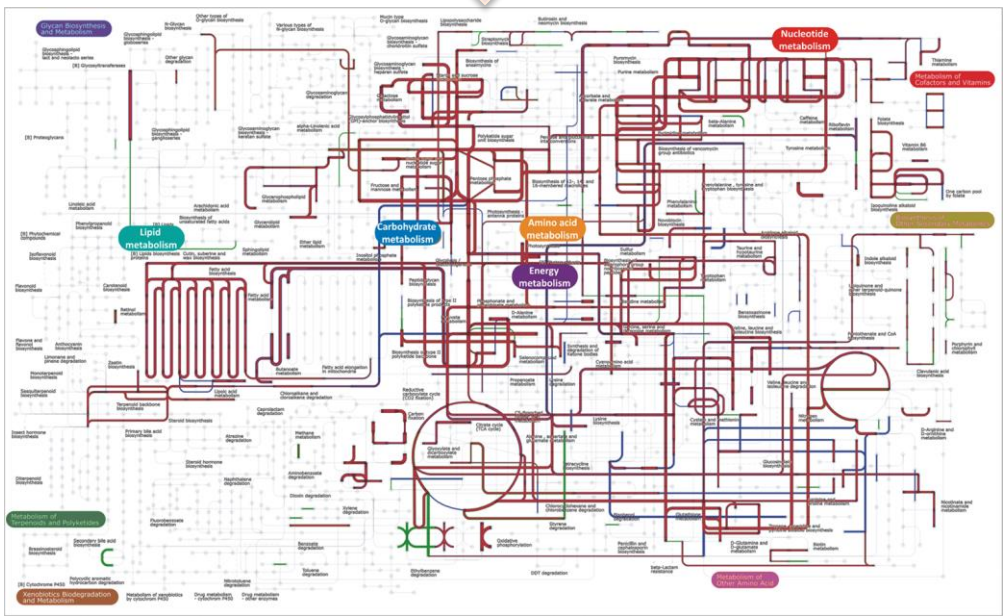


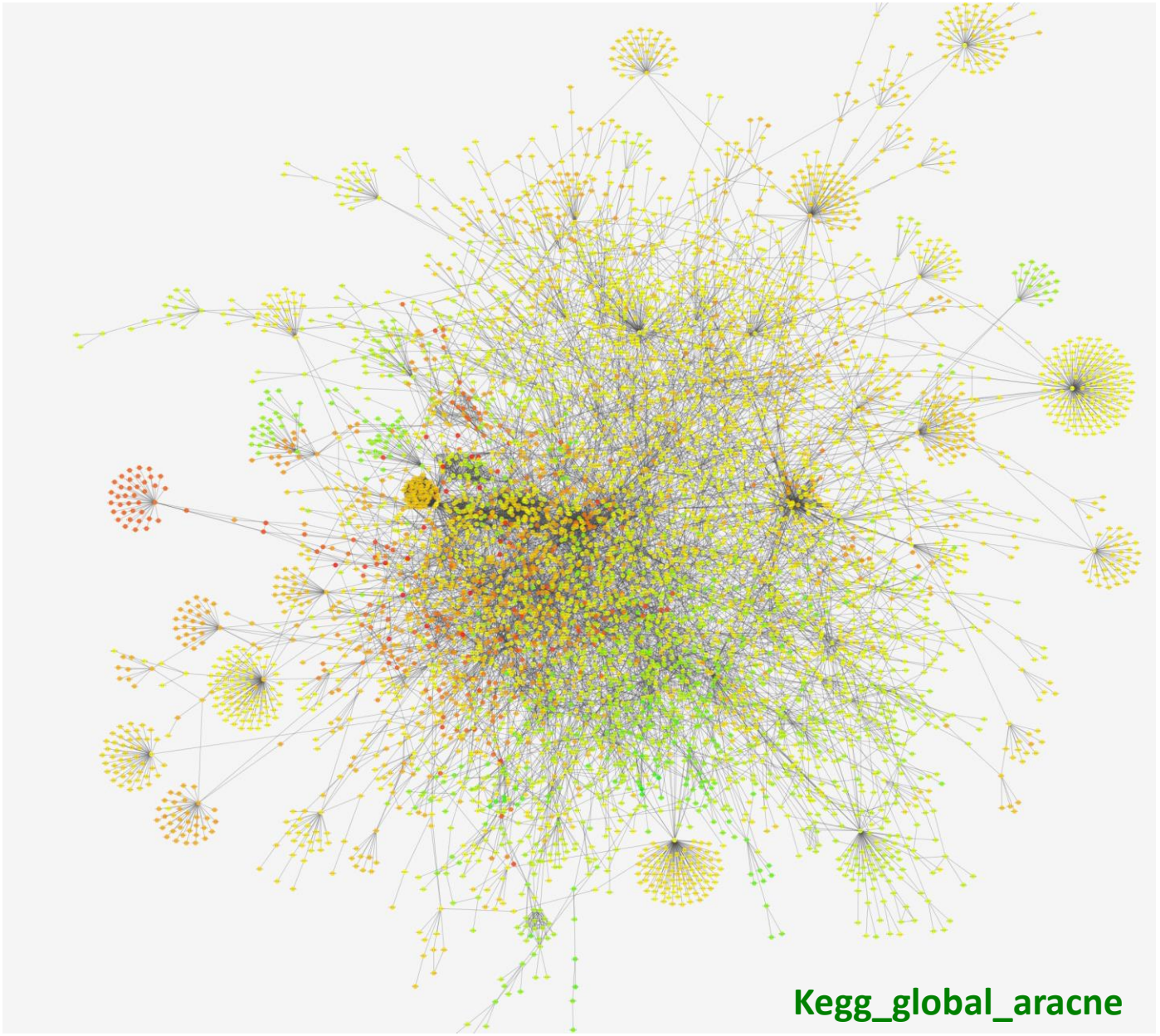
Enzymes expressed by epithelial cells in the bile duct

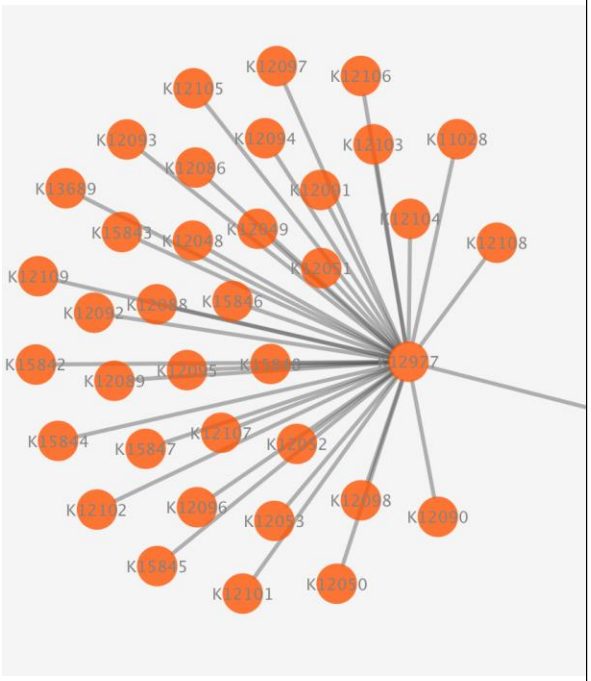
Eric Gershwin, University of California, Davis

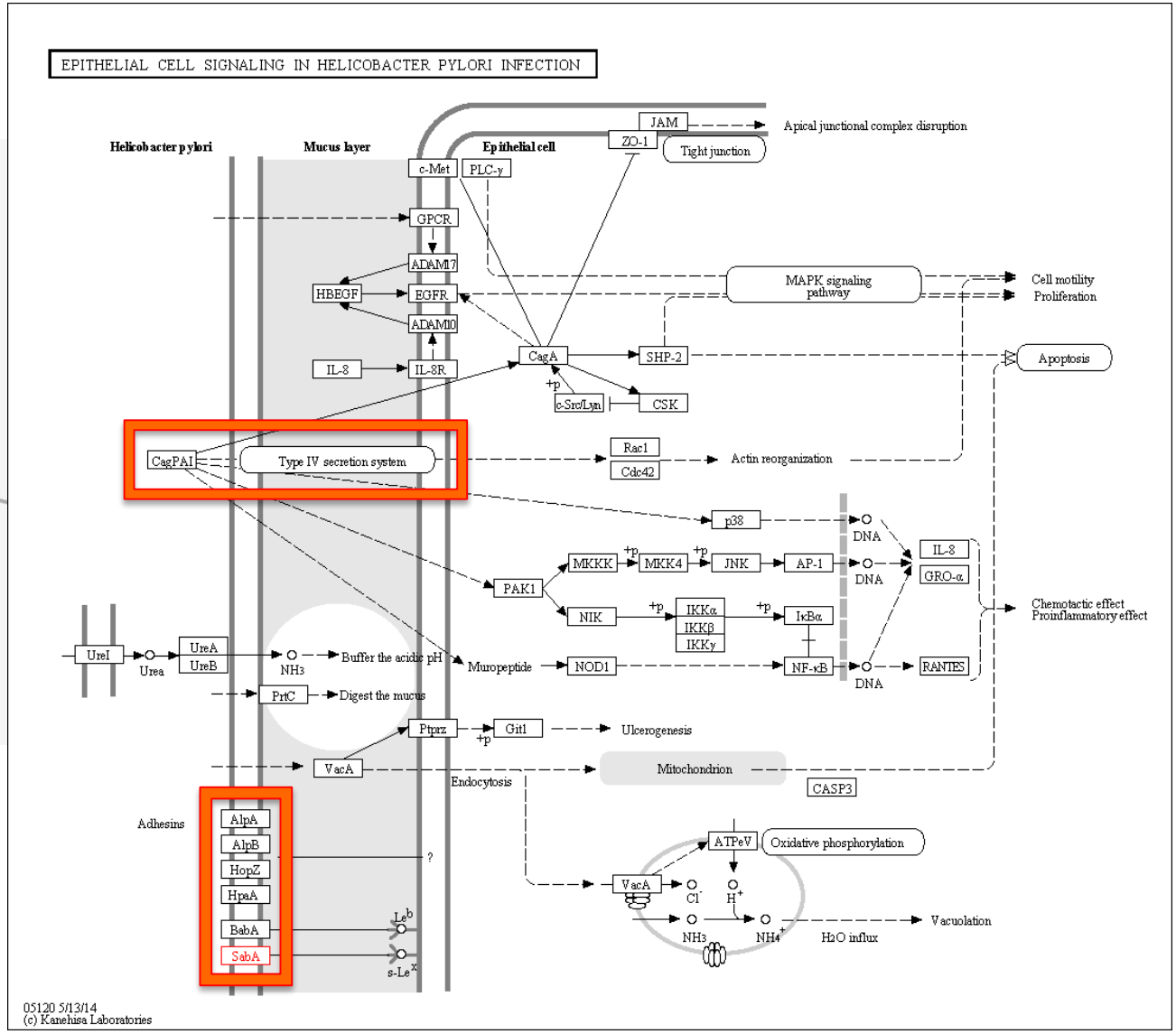

Phylum
Proportional Abundance
0.0
0.2
0.4
0.6
0.8
1.0
control.b20
control.b19
control.b18
control.b17
control.b16
control.a15
control.a14
control.a13
control.a12
control.a11
cancer.b10
cancer.b9
cancer.b8
cancer.b7
cancer.b6
cancer.a5
cancer.a4
cancer.a3
cancer.a2
cancer.a1

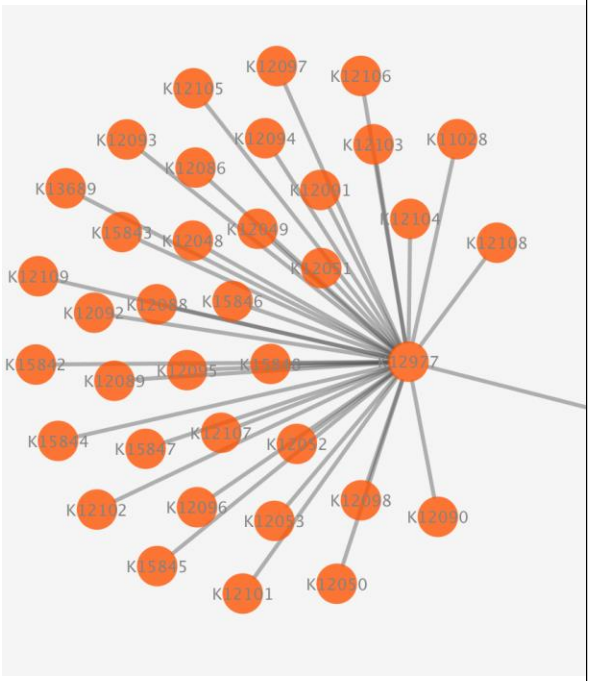
PICRUSt

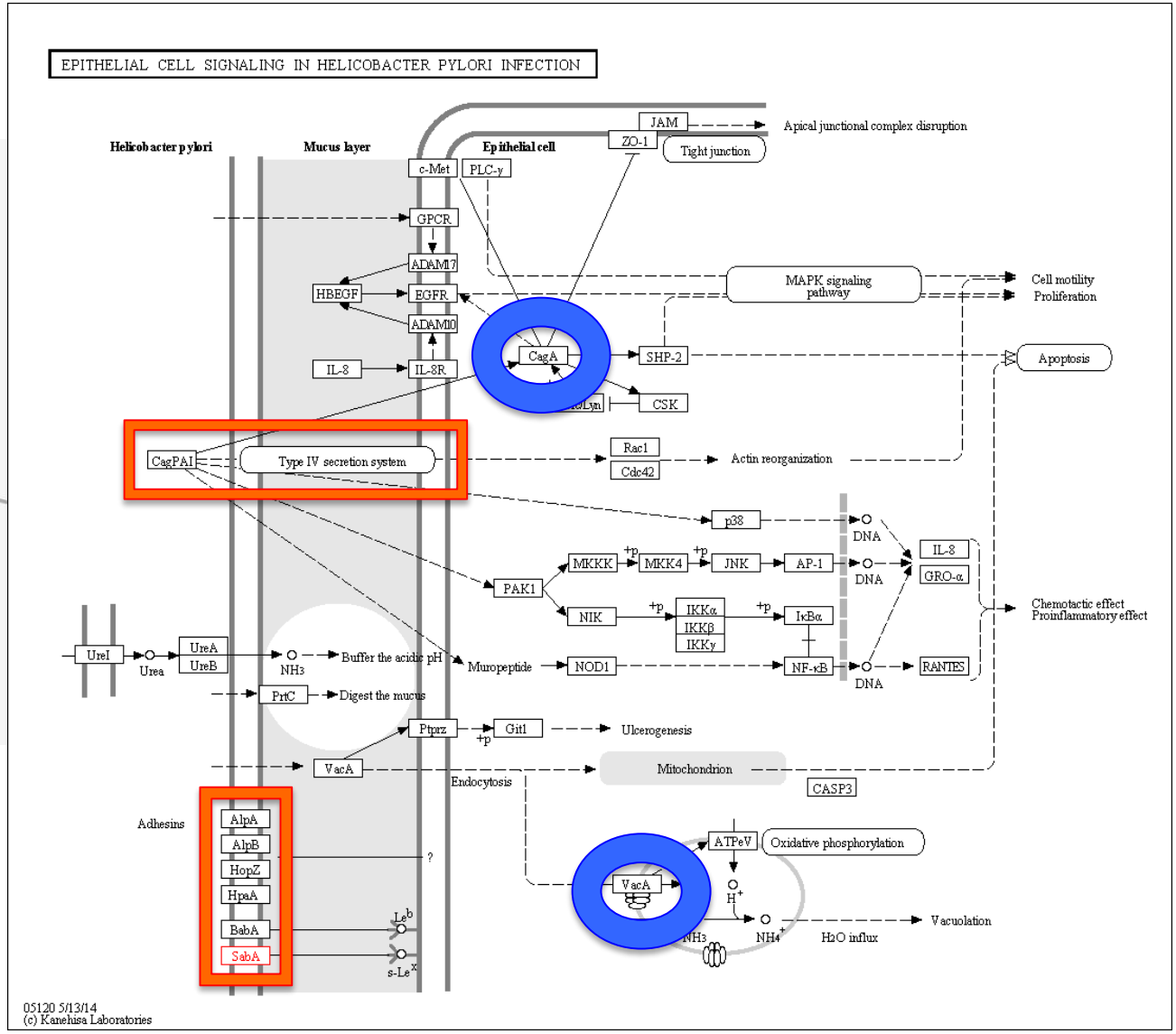

Glycan Biosynthesis and Metabolism
Nucleotide metabolism
Metabolism of Cofactors and Vitamins
Lipid metabolism
Carbohydrate metabolism
Amino acid metabolism
Energy metabolism
Metabolism of Terpenoids and Polyketides
Xenobiotics Biodegradation and Metabolism
Metabolism of Other Amino Acid


Kegg_global_aracne


K12097
K12106
K12105
K12093
K12094
K12103
K11028
K12086
K13689
K12091
K12104
K15843
K12098
K12849
K12108
K12109
K12051
K12092
K12088
K15846
K15842
K12095
K15848
K12977
K12089
K15844
K15847
K12107
K12092
K12102
K12096
K12093
K12098
K12090
K15845
K12101
K12090


EPITHELIAL CELL SIGNALING IN HELICOBACTER PYLORI INFECTION
Helicobacter pylori
Mucus layer
Epithelial cell
JAM
ZO-1
Tight junction
Apical junctional complex disruption
c-Met
PLC-γ
GPCR
ADAM17
HBEGF
EGFR
ADAM10
MAPK signaling pathway
Cell motility
Proliferation
IL-8
IL-8R
CagA
SHP-2
Apoptosis
c-Src/Lyn
CSK
CagPAI
Type IV secretion system
Rac1
Cdc42
Actin reorganization
p38
DNA
MKKK
MKK4
JNK
AP-1
IL-8
GRO-α
PAK1
NIK
IKKα
IKKβ
IKKγ
IκBα
NF-κB
RANTES
Chemotactic effect
Proinflammatory effect
UreI
Urea
UreA
UreB
NH3
Buffer the acidic pH
Muropeptide
NOD1
PrtC
Digest the mucus
Ptprz
Git1
Ulcerogenesis
VacA
Endocytosis
Mitochondrion
CASP3
Adhesins
AlpA
AlpB
HopZ
HpaA
BabA
SabA
Le^b
s-Le^x
ATPeV
Oxidative phosphorylation
Cl-
H+
NH3
NH4+
H2O influx
Vacuolation
05120 5/13/14
(c) Kanehisa Laboratories


K12097
K12105
K12106
K12093
K12094
K12103
K11028
K12086
K13689
K12091
K12104
K15843
K12098
K12849
K12108
K12109
K12051
K12092
K12088
K15846
K15842
K12095
K15848
K12977
K12089
K15844
K15847
K12107
K12092
K12102
K12096
K12093
K12098
K12090
K15845
K12101
K12090

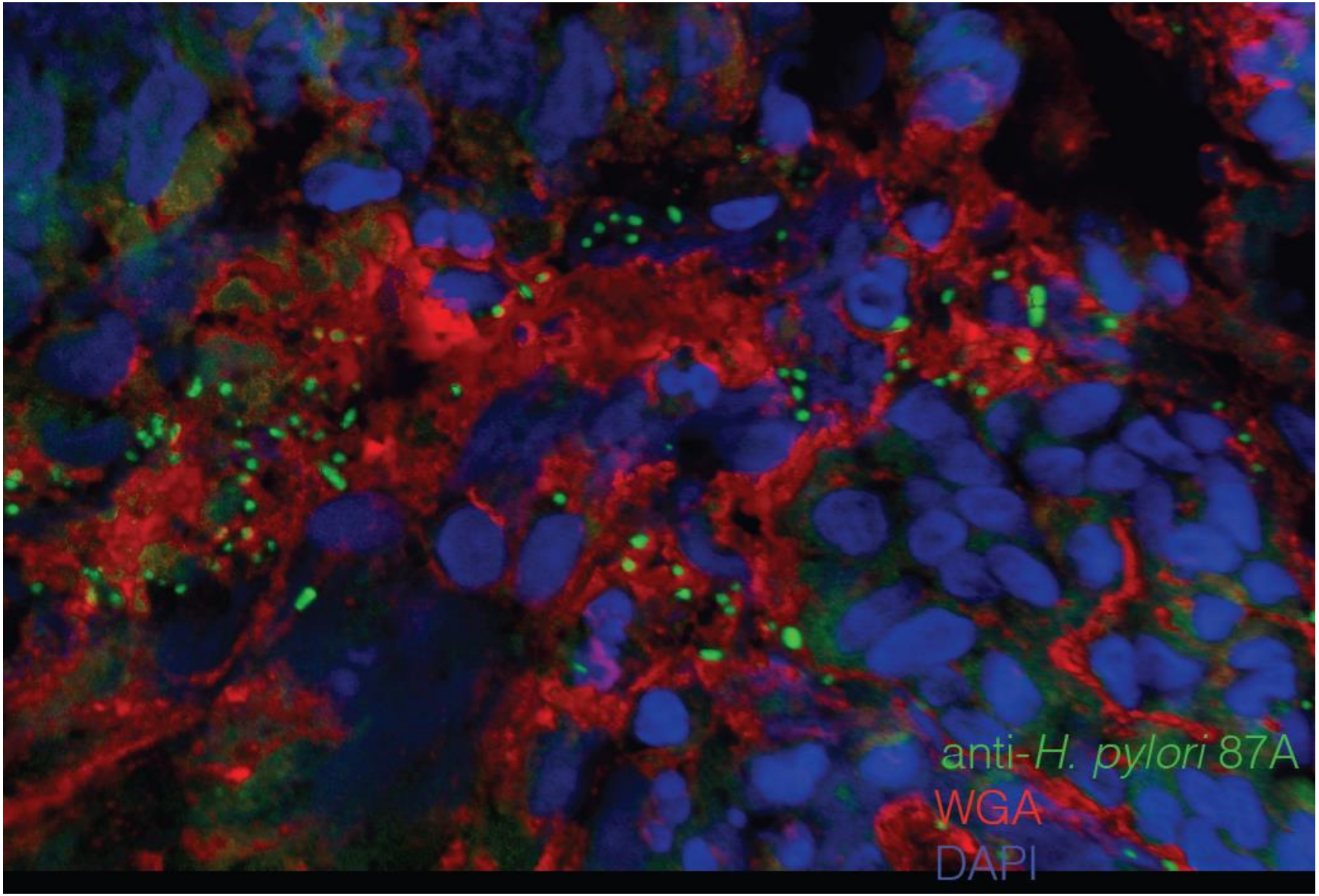

EPITHELIAL CELL SIGNALING IN HELICOBACTER PYLORI INFECTION
Helicobacter pylori
Mucus layer
Epithelial cell
JAM
ZO-1
Tight junction
Apical junctional complex disruption
c-Met
PLC-γ
GPCR
ADAM17
HBEGF
EGFR
ADAM10
MAPK signaling pathway
Cell motility
Proliferation
IL-8
IL-8R
CagA
SHP-2
Apoptosis
CSK
CagPAI
Type IV secretion system
Rac1
Cdc42
Actin reorganization
p38
DNA
MKKK
MKK4
JNK
AP-1
IL-8
GRO-α
PAK1
NIK
IKKα
IKKβ
IKKγ
IκBα
NF-κB
RANTES
Chemotactic effect
Proinflammatory effect
UreI
Urea
UreA
UreB
NH3
Buffer the acidic pH
Muropeptide
NOD1
PrtC
Digest the mucus
Ptprz
Git1
Ulcerogenesis
VacA
Endocytosis
Mitochondrion
CASP3
Adhesins
AlpA
AlpB
HopZ
HpaA
BabA
SabA
Le^b
s-Le^x
ATPeV
Oxidative phosphorylation
H+
NH3
NH4+
H2O influx
Vacuolation
05120 5/13/14
(c) Kanehisa Laboratories

PCR detection of *vacA* and *cagA* in DNA from cells of the bile duct

	vacA + (%)*	*cagA* + (%)	*vacA* and/or *cagA* + (%)
Cases	50/97 (51.5)	46/100 (46.0)	75/100 (75.0)
Controls	21/86 (24.4)	39/92 (42.4)	52/92 (56.5)

*OR 3.29 (C.I. 95% 1.754521-6.176529); p-value=0.0003

Presence of *H. pylori* in the bile duct of patients with cancer



Conclusions

- ✓ **Microbiota studies reveal the presence of extremo-philic bacteria reported in the sea sediments and desserts, in the bile duct of humans**
- ✓ **Microbiota and PCR studies suggest *H. pylori* might be present in bile duct.**
- ✓ **Presence of these bacteria might be associated with BD cancer.**

Francisco Aviles-Jimenez
Unidad de Investigación en
Enfermedades Infecciosas, IMSS

Alfonso Mendez-Tenorio
Laboratorio de Biotecnología y
Bioinformática Genómica, ENCB, IPN

Rafael Medrano, Alejandra Mantilla
UMAE Oncologia, CMN SXXI, IMSS

Alfredo Guitron, Fany Lopez-Segura
UMAE 71 IMSS, Coahuila

US Democrats

Shoko Iwai
Second Genome, San Francisco

Alisa Goldstein, Yu Guoqin
National Cancer Institute, NIH

Julie C. Dunning, Kelly M. Robinson
Institute for Genome Science, U Maryland

Manuel Amieva
Stanford University