

LIFESTYLE-ASSOCIATED ALTERATIONS OF INTESTINAL MICROBIOTA AND THEIR POTENTIAL ROLE IN ETIOLOGY OF IBD: A METAGENOMIC ANALYSIS

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**Clinic for Internal Medicine I
UK S-H Campus Kiel**

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – **Major Objectives**

→ **Background:**

Epidemiologic studies point to the critical role of environmental factors in the pathogenesis of chronic inflammatory barrier diseases, e.g. the chronic inflammatory bowel diseases (IBD) or asthma (‘civilization diseases’). Lifestyle factors, e.g. nutrition, hygiene, and improved medical services, were accounted for the raising incidence of these disorders over the last decades in the industrialized countries (‘hygiene hypothesis’). Intestinal microbiota as an integral part of the enteric mucosal barrier reflects the evolution of lifestyle factors.

→ **Aim of the study:**

The aim of this study is to compare the composition of the intestinal microbiota of rural areas with low incidence of civilization diseases (representing the “ancient” lifestyle of the pre-industrialization period) and a region in an urban area, which is over decades influenced by multiple environmental factors (“modern” lifestyle).

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – **Study Design**

Patients N=90

→ Lithuania

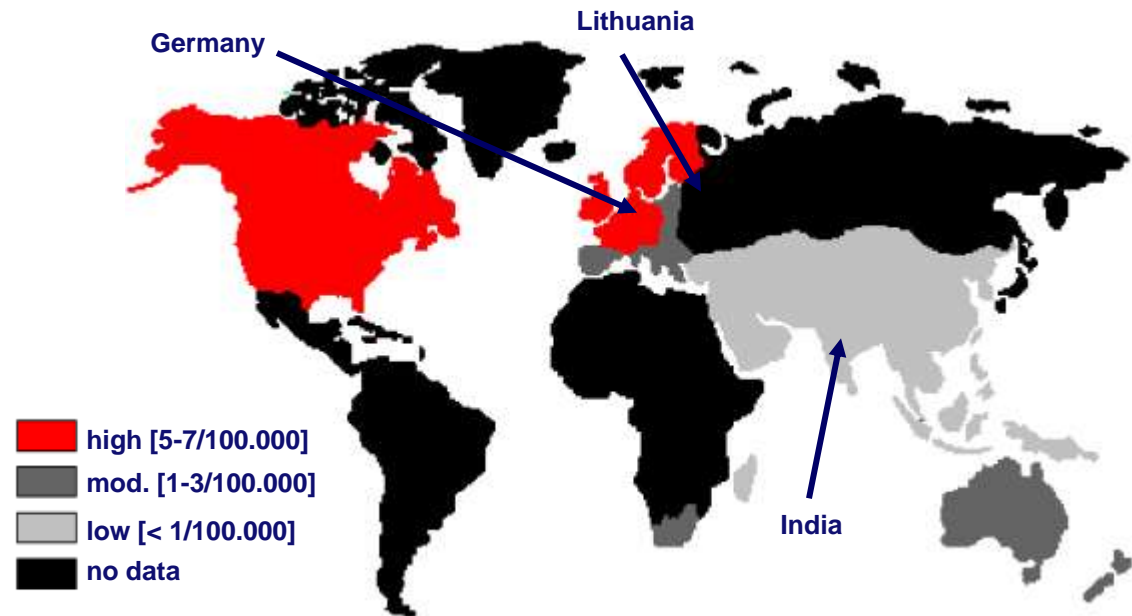
Healthy Controls N=10
Ulcerative Colitis N=10
Crohn Disease N=10

→ Germany

Healthy Controls N=10
Ulcerative Colitis N=10
Crohn Disease N=10

→ India

Healthy Controls N=10
Ulcerative Colitis N=10
Crohn Disease N=10



→ Source Material

Sigma Biopsies

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→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – **Status of the Project**

→ Patient Recruitment

Complete set of samples from all 90 individuals

→ DNA/RNA Extraction

Completed for all 90 samples.

→ Microbial Molecular Investigation

Completed for all cohorts, RNA and DNA, > 600.000 sequences.

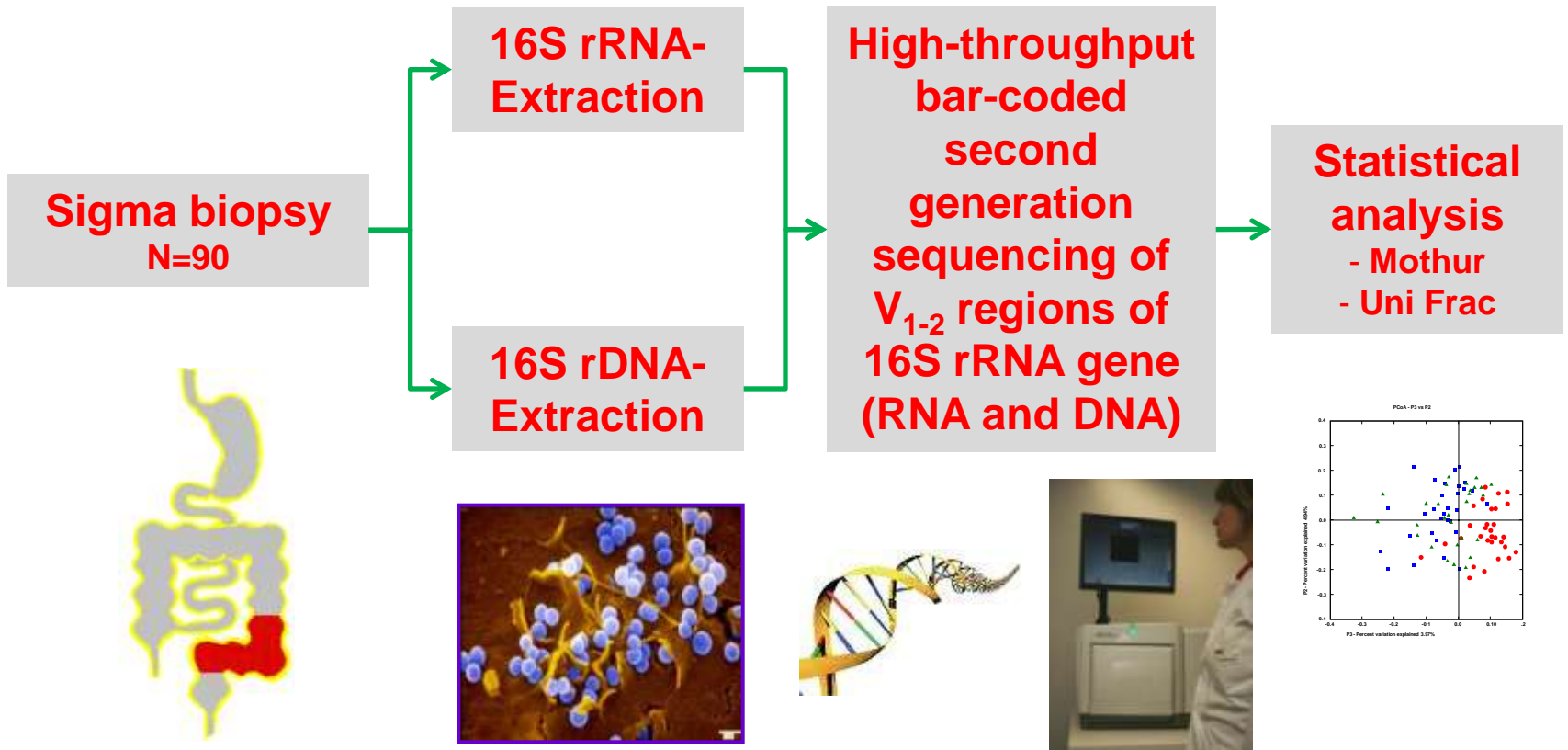
→ Statistical Analysis

Analysis on species level for DNA, complete RNA work (transcriptional level), most of the comparative analysis to be done.

→ Publication

In preparation.

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Experimental Setup



→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – **Statistical Analysis**

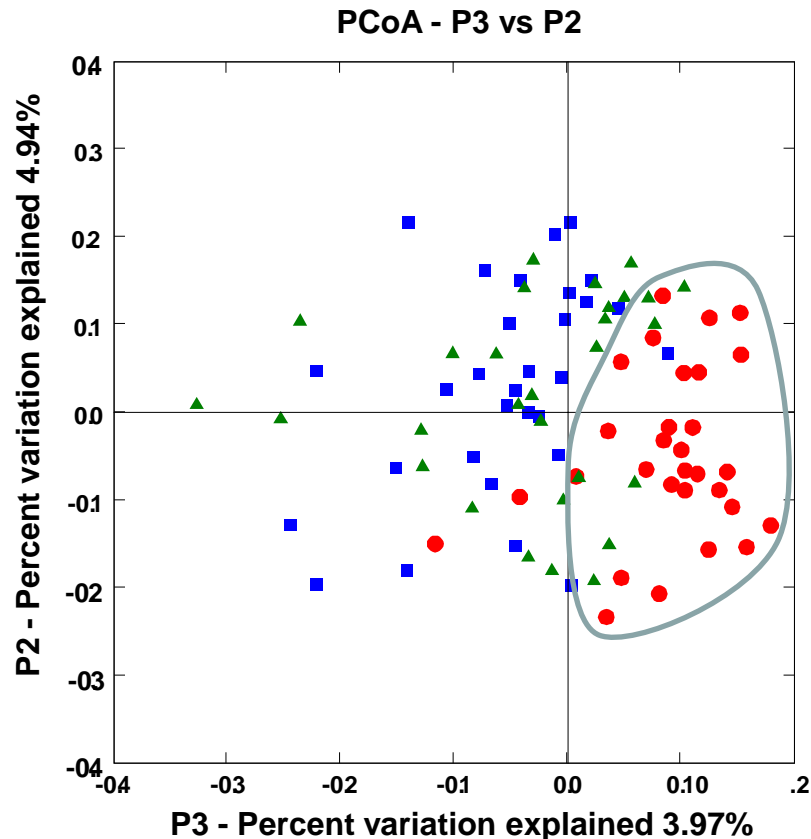
- sequence reads were screened and filtered for quality and length using perl script (Giongo *et al.*, 2010)
- sequences were trimmed and binned by samples using the specific MIDs (barcode)
- bad quality sequences were excluded from further analysis
Criteria:
 - no perfect match with MID sequences or specific primers
 - less than 200 base long reads
 - quality score less than 20,
 - ambiguous bases
 - sequences with more than 6 homopolymers
- chimeric sequences were removed by MOTHUR chimera slayer command
- sequences were classified by using RDP multiclassifier

Giongo, A. et al., 2010. PANGEA: pipeline for analysis of next generation amplicons. *ISME J*, 4(7), 852-861.

Pruesse, E. et al., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucl. Acids Res.*, 35(21), 7188-7196.

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Unifrac Analysis of All Samples

Unweighted fast unifrac analysis



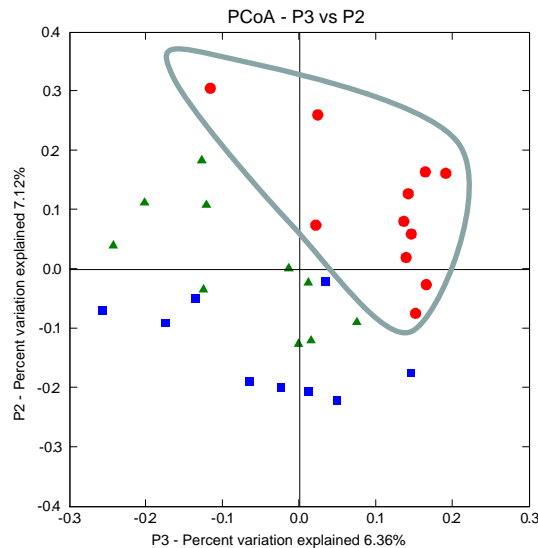
- Indian
- ▲ Lithuanian
- German

Total of ~ 620.000 sequences

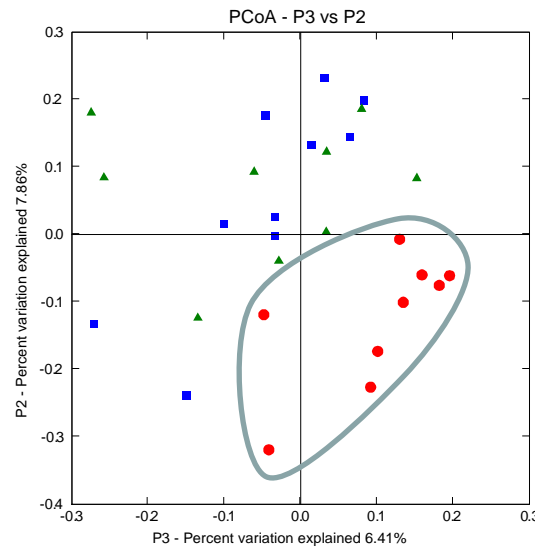
Regardless of disease status
Indian cohort clusters distinctly

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – **Unifrac Analysis Healthy vs. Disease**

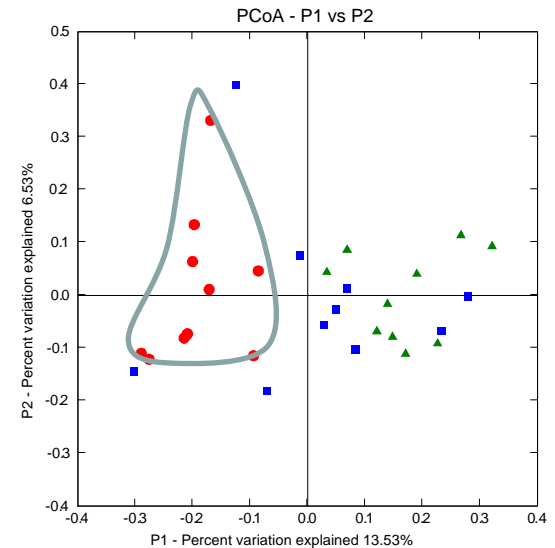
Unweighted fast unifrac analysis



Healthy individuals



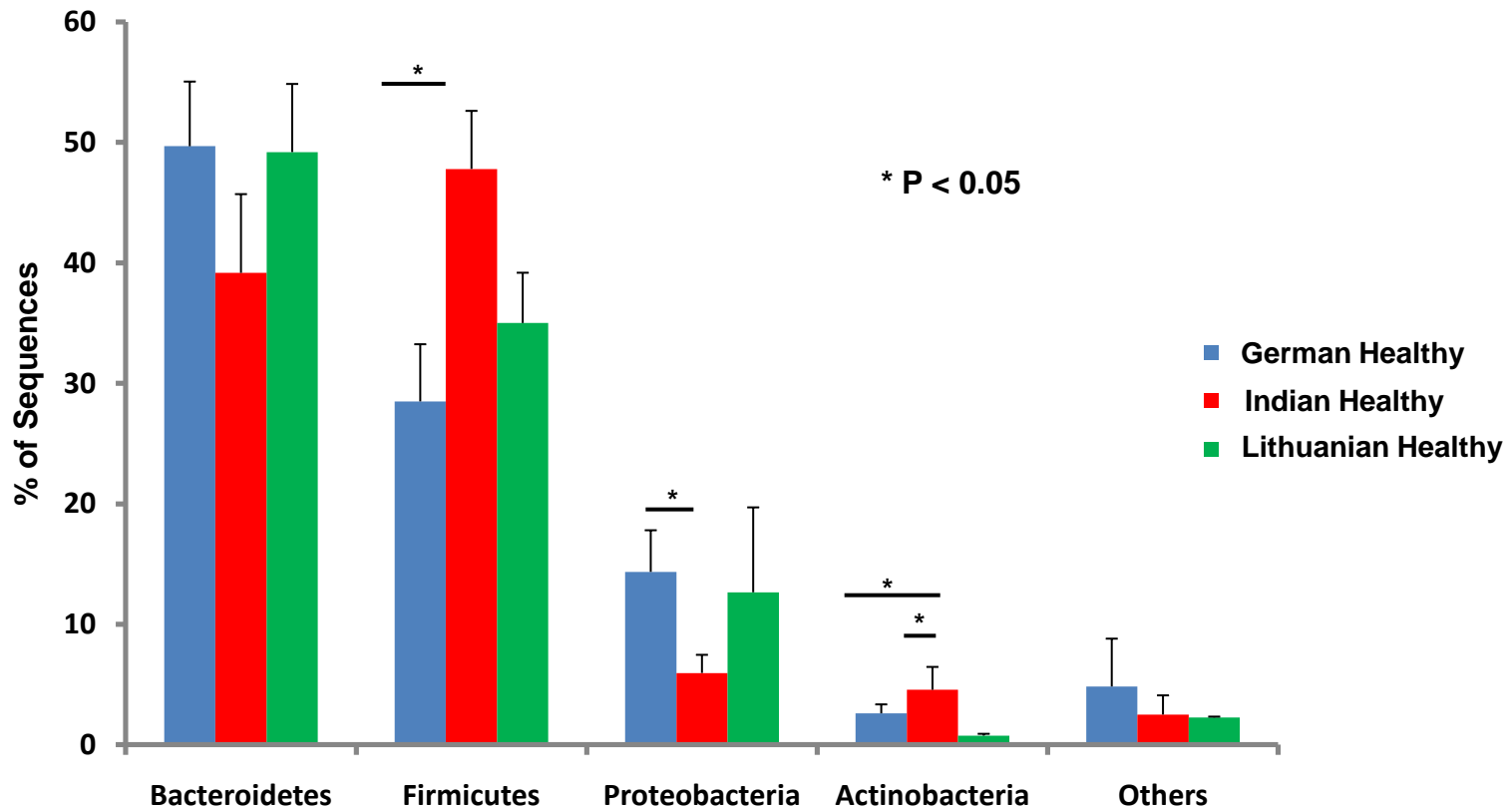
Crohn's disease



Ulcerative colitis

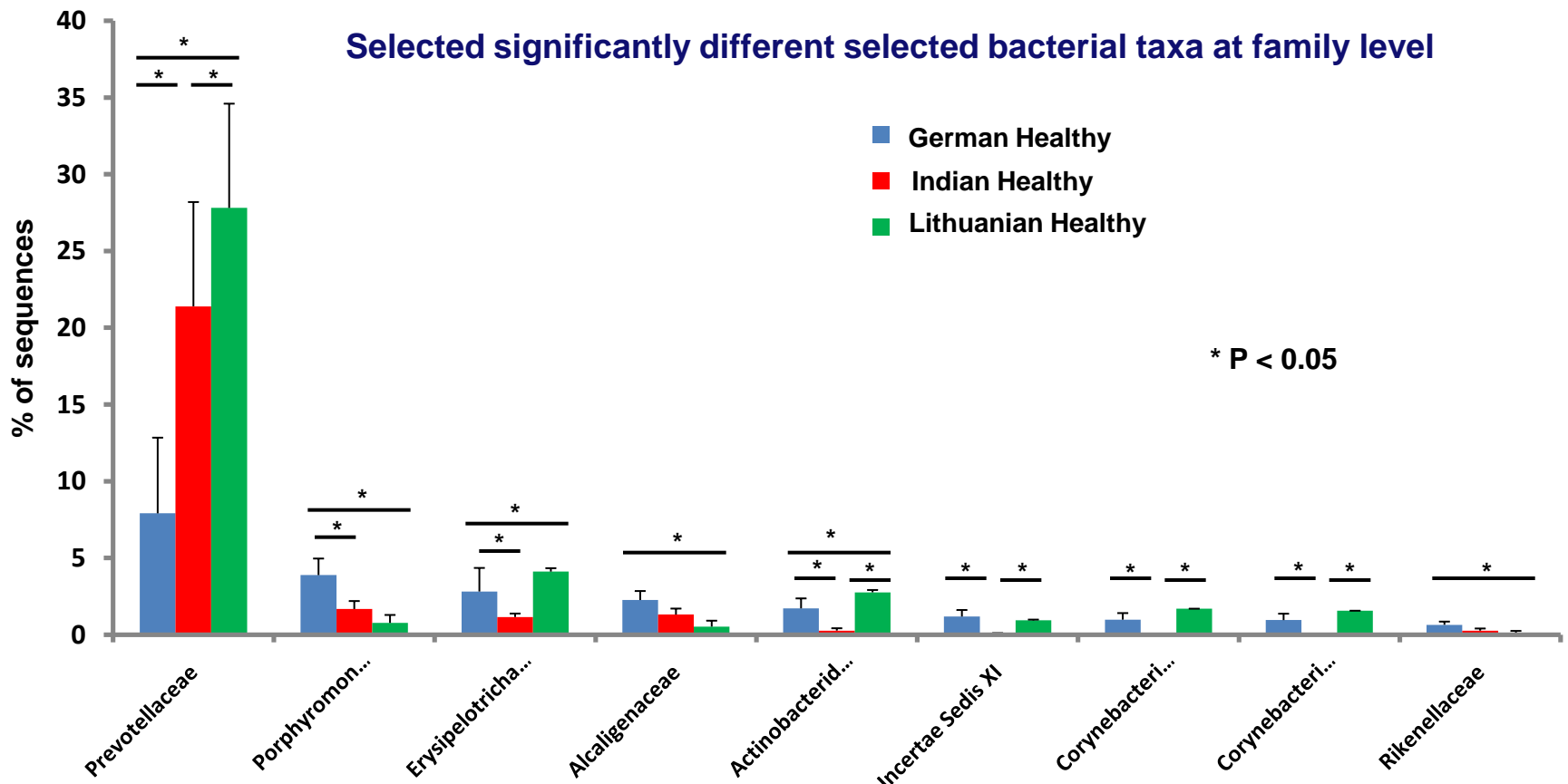
- Indian
- ▲ Lithuanian
- German

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Major Phyla in Healthy Individuals

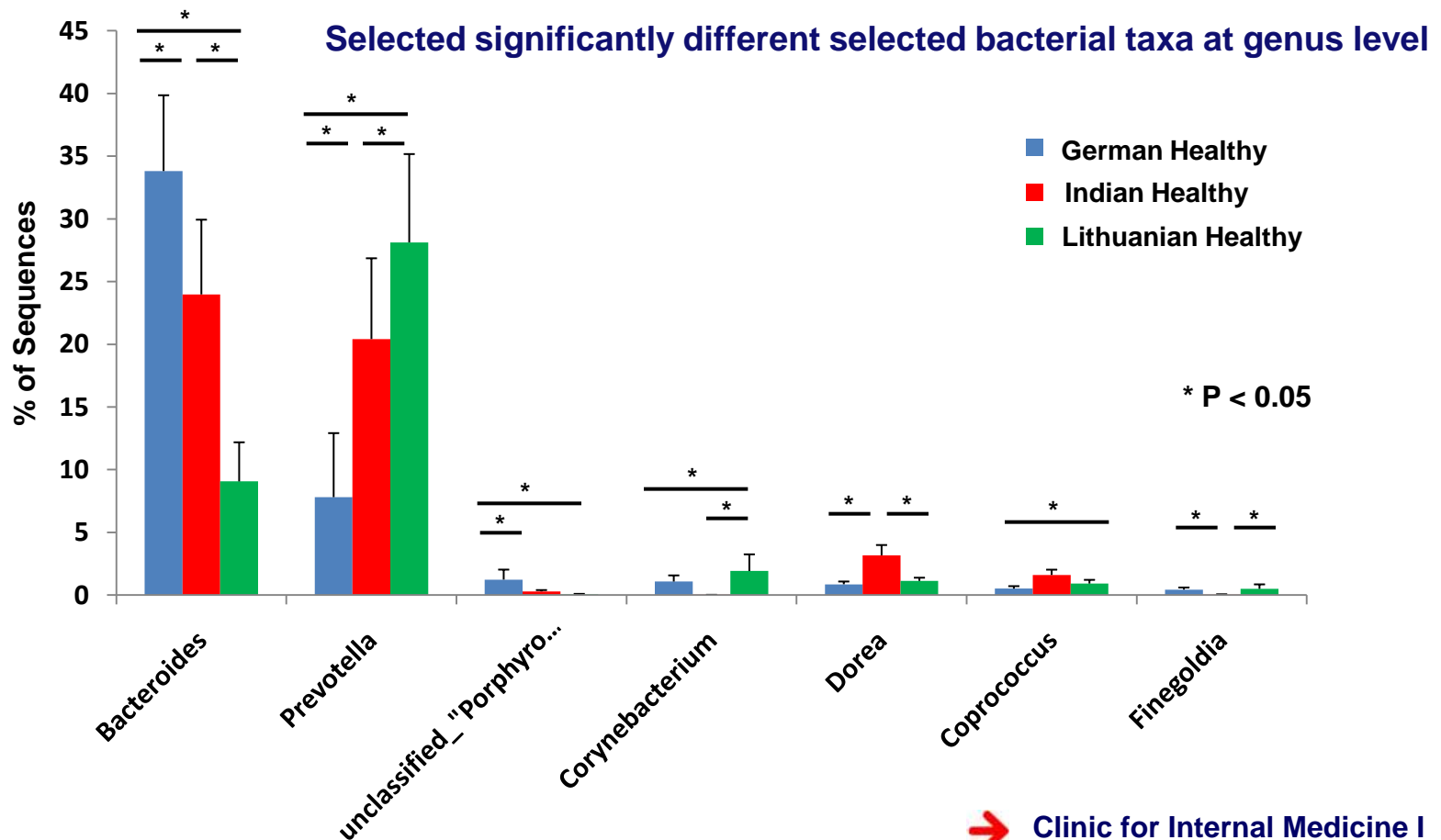


Major phyla in healthy individuals from three Cohorts.

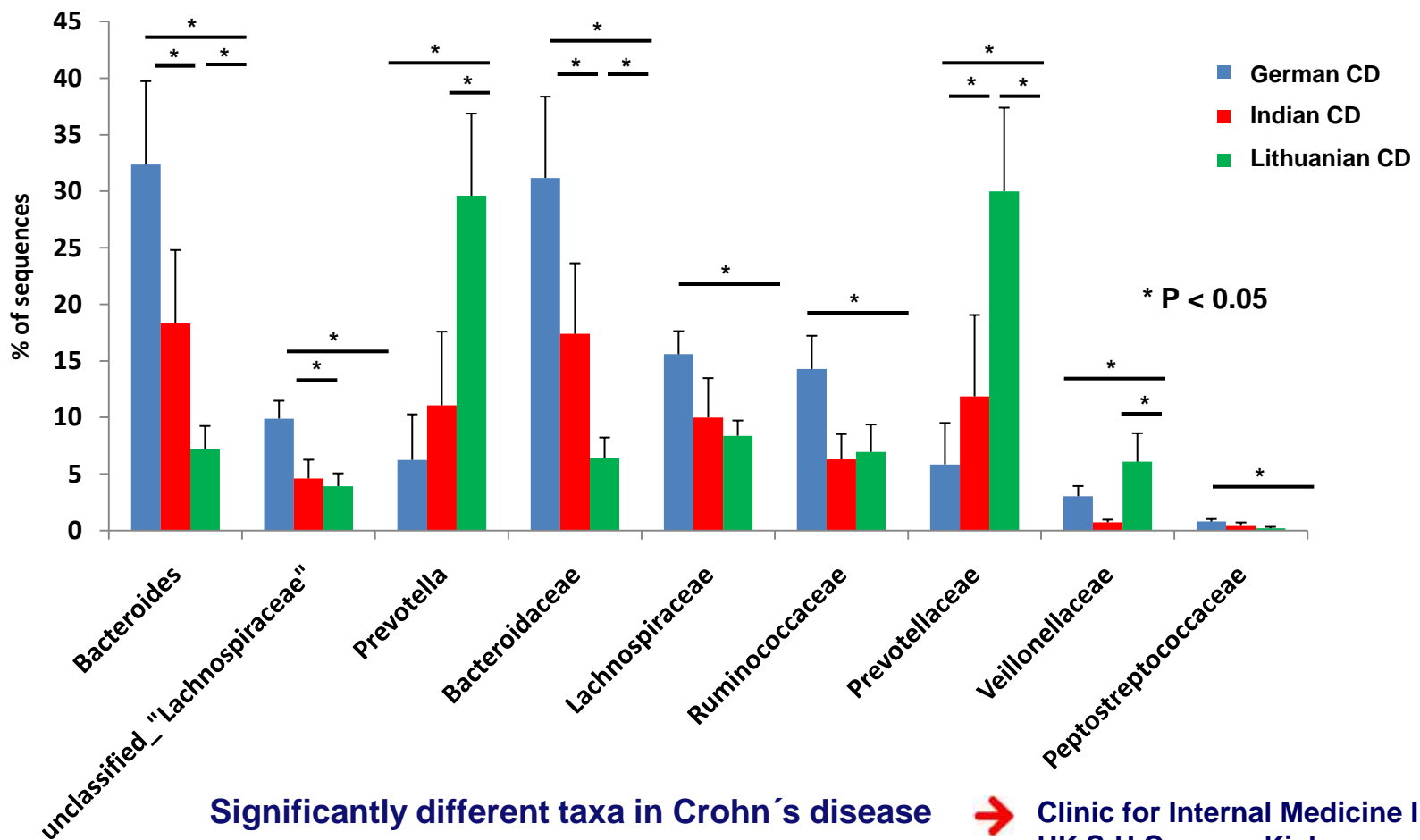
→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Differences on Bacterial Family Level



→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Differences on Bacterial Genus Level



→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Differences in Crohn’s Disease

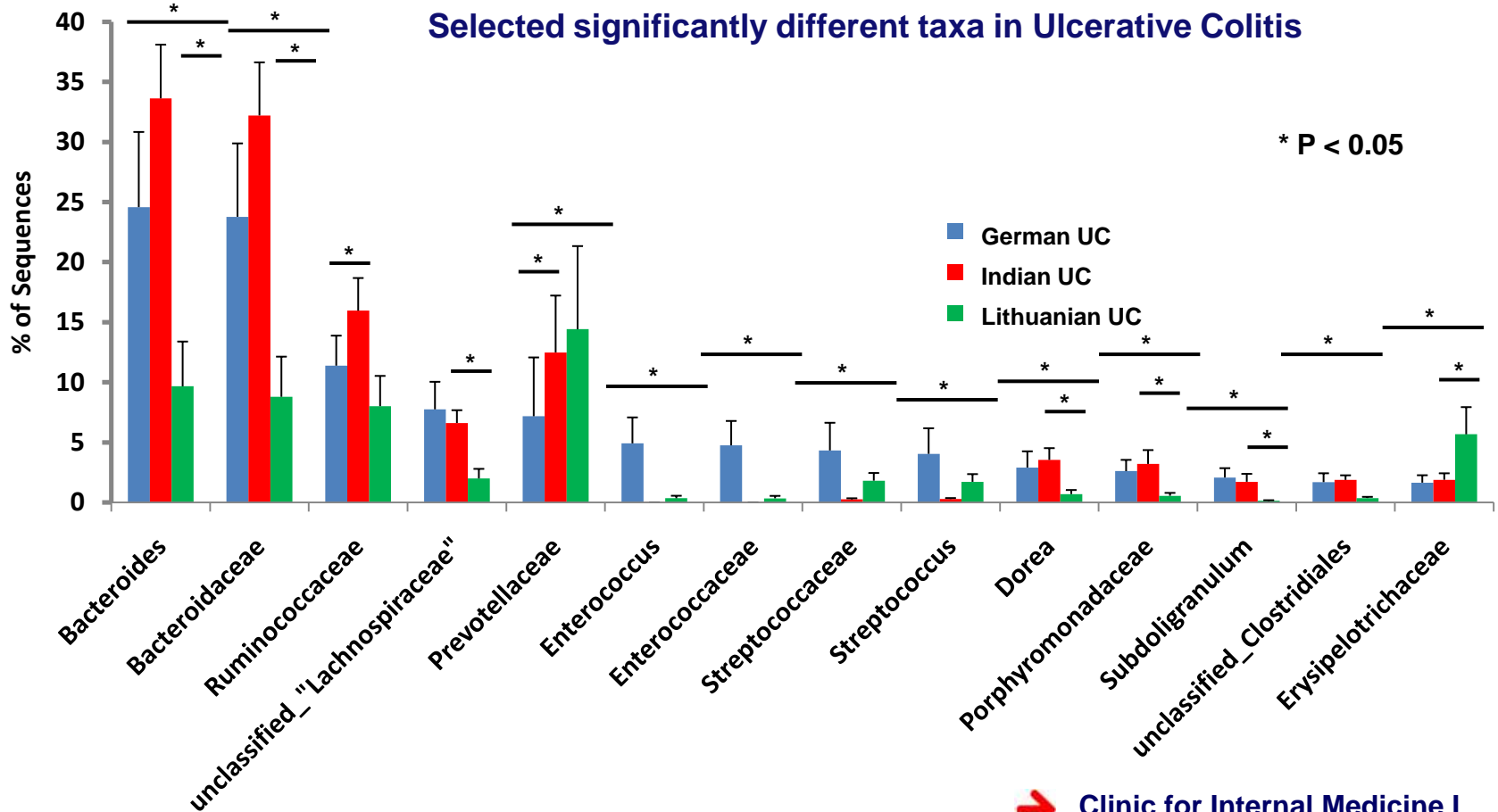


Significantly different taxa in Crohn’s disease

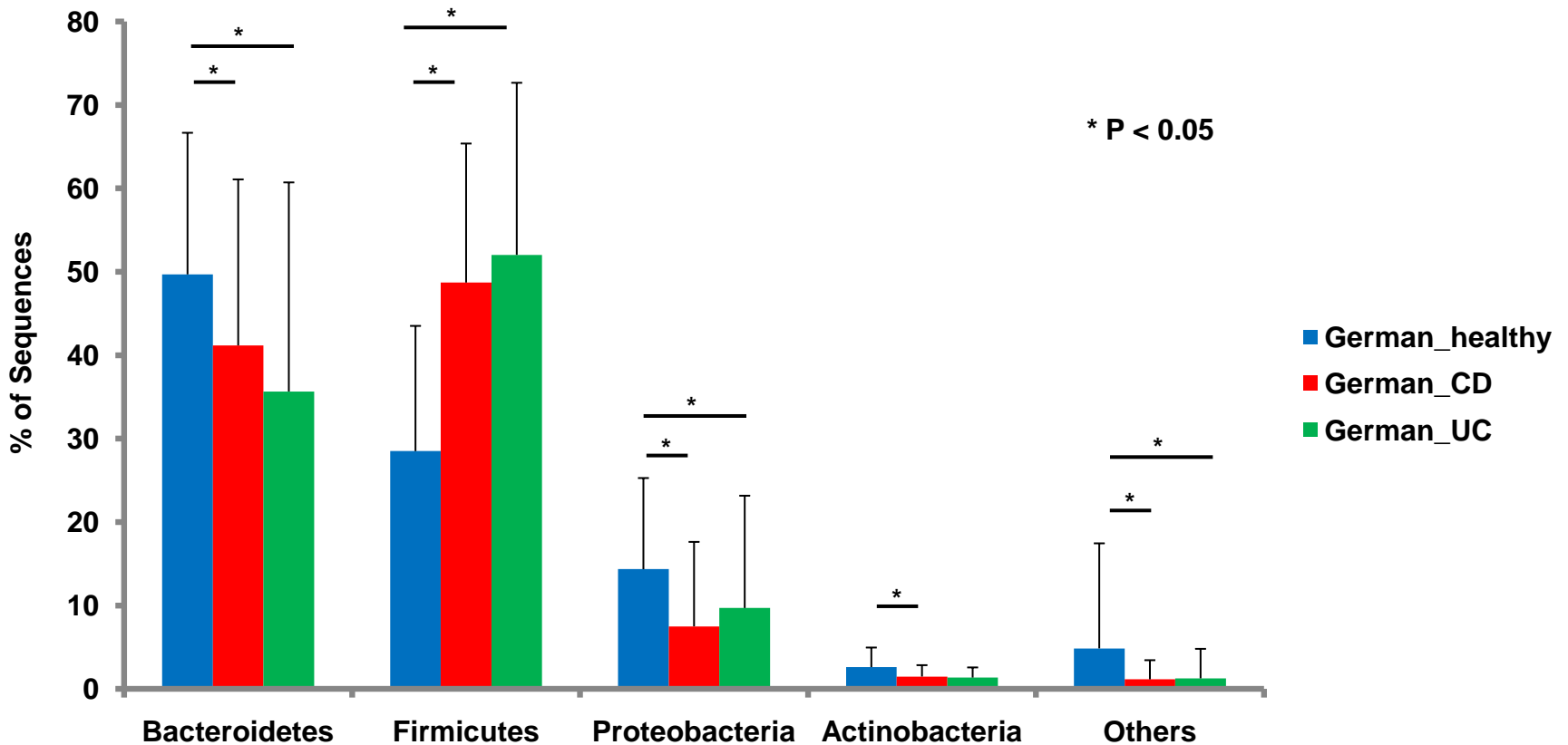


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→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Differences in Ulcerative Colitis

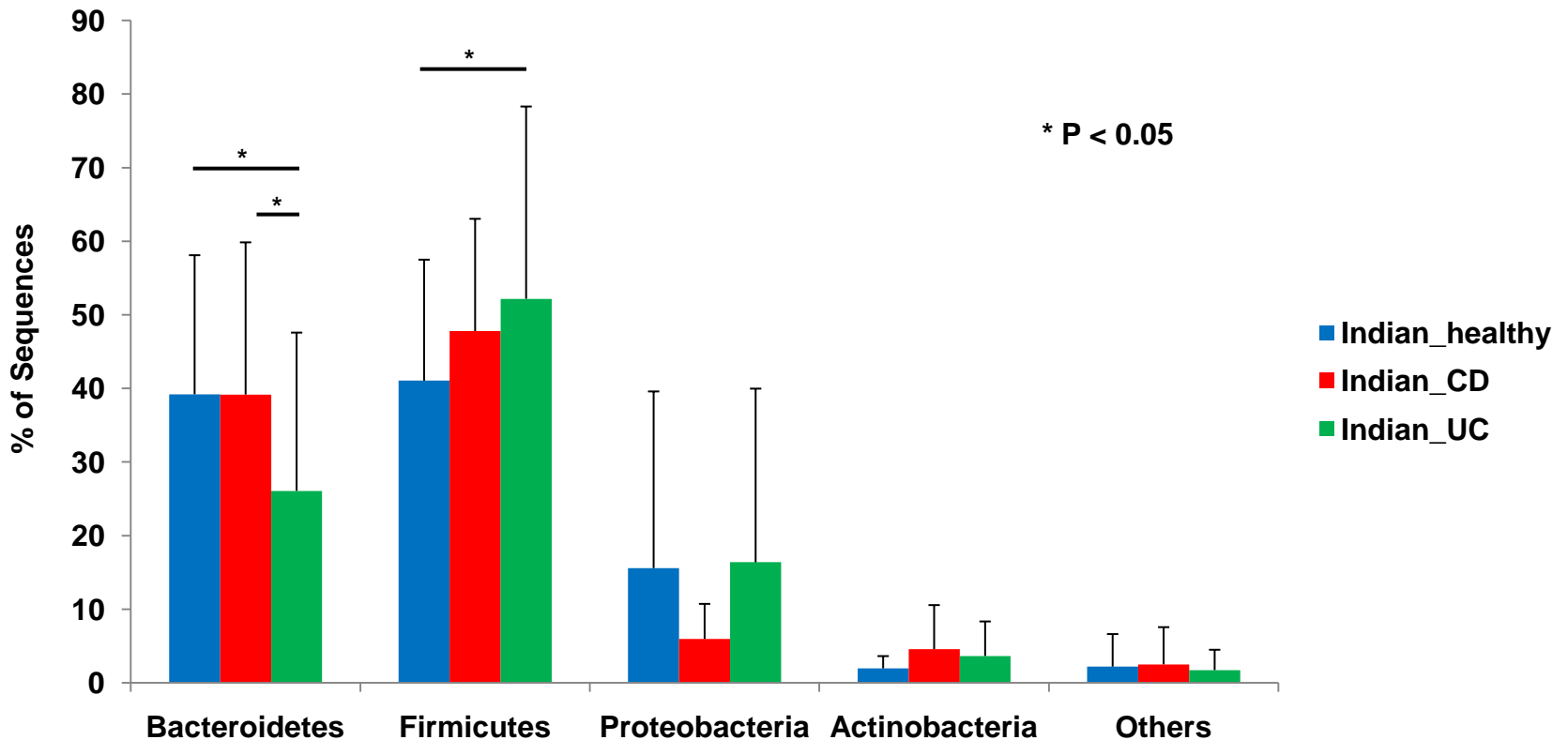


→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Differences Healthy vs. Disease



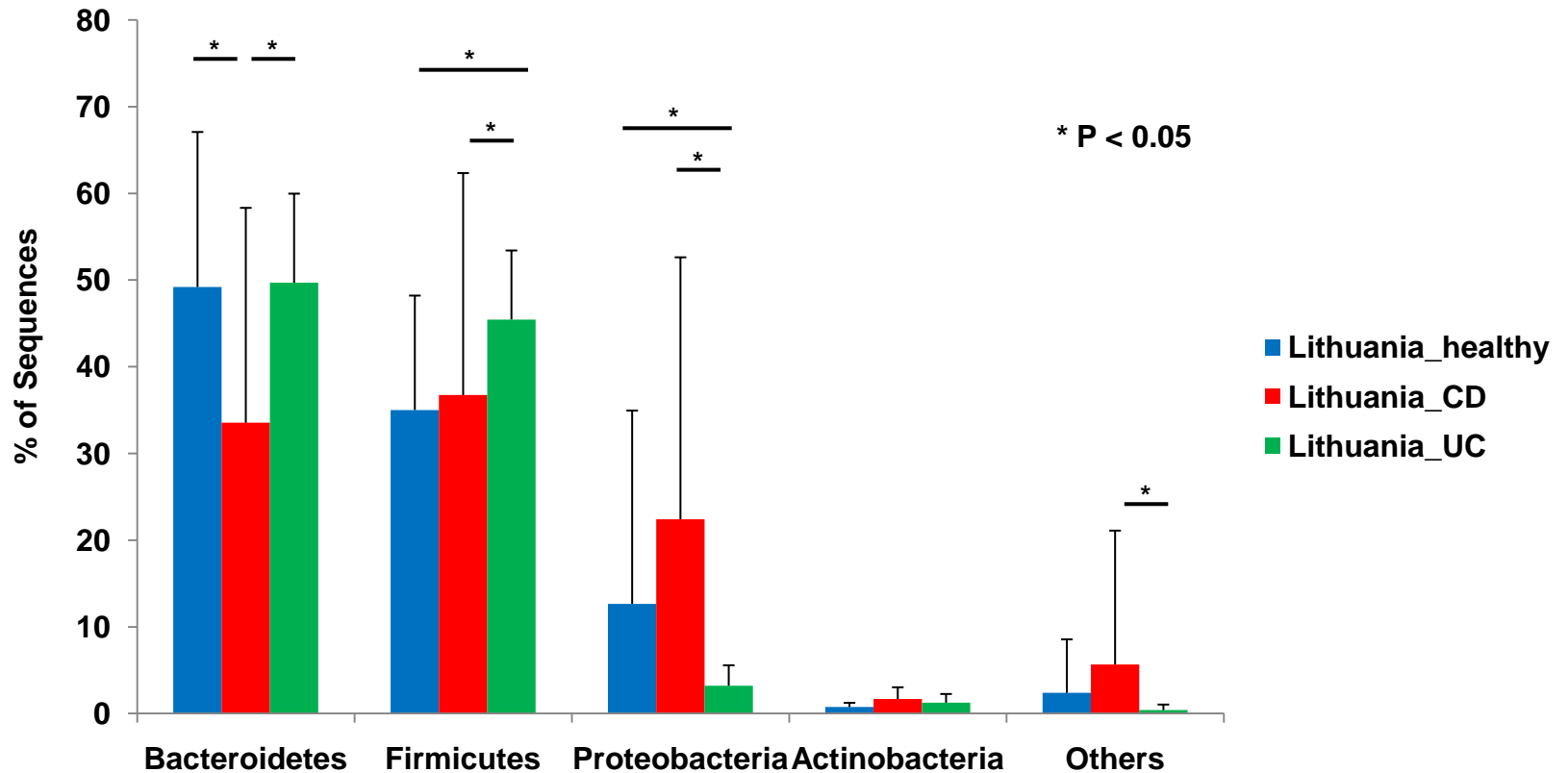
Major phyla healthy vs. disease in Germans

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Differences Healthy vs. Disease



Major phyla healthy vs. disease in Indians

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – Differences Healthy vs. Disease



Major phyla healthy vs. disease in Lithuanians

→ Influence of Environmental Factors on the Intestinal Microbiome – Comparison of Different Cultures and Lifestyles – **Conclusions so far**

- **The Indian individuals show significant differences in their intestinal bacterial composition:**
 - analysis of questionnaires might reveal environmental or nutritional (vegetarians) factors to be responsible
- **The intestinal bacterial microbiota is significantly different between the different populations and the disease groups:**
 - further analysis of microbiota on species level will provide more detailed information
- **Future perspectives:**

To identify characteristics of a healthy, ancient intestinal microbiota, i.e. species that might be protective or harmful for the host as a basis for specific manipulation (e.g. new probiotics).

→ Many Thanks!

**Kiel - Institute for Clinical
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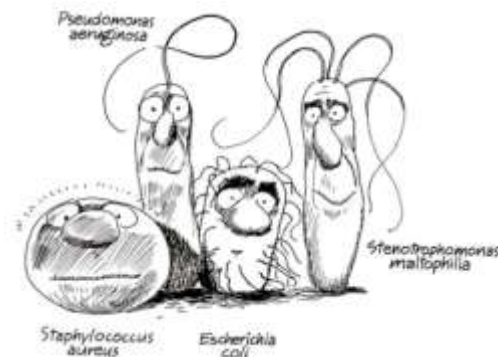
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Deepak Amarapurkar



**→ I. Medizinische Klinik,
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