

## ***Studio del Microbioma intestinale nella patogenesi della celiachia nell'adulto***

**TITOLARE DEL GRANT: LUCIA SACCHETTI**

**ISTITUTO: CEINGE-S.C. a R.L. Biotecnologie Avanzate**

**DURATA DEL PROGETTO: 2 anni**

## Background

- ✓ -Celiac disease (CD) is a chronic autoimmune enteropathy caused by gluten in genetically susceptible individuals (HLA-DQ2/DQ8);
- ✓ -Epidemiological evidences support a role for additional genetic and environmental pathogenetic factors (i.e. viral infections, intestinal microbiota) in CD.

## Preliminary Observations

- ✓ -A number of study have identified alterations in the gut microbiota in CD patients but data are inconclusive mainly due to different methodologies and/or biological samples used for the microbiota evaluation.
- ✓ -No studies have assessed whether variations of specific bacteria could influence CD expression by promoting inflammation and impairing epithelial function.

## Aims

**Aim 1:** To characterize by metagenomics the gut microbiome and mycobioma in adults with active or non active CD and controls and to look for signs of dysbiosis associated with the disease.

**Aim 2:** To isolate/identify all cultivable gut bacteria by microbiological methods/mass spectrometry.

**Aim 3:** To test if the isolated bacterial strains found to be significantly altered in CD patients displayed potentially pathogenic features (i.e.inflammation power).

## Methods

### Study Subjects -n=97

{ First Cohort n=41 (20 a-CD, 15 Controls, 6 GFD)  
Second Cohort n=56 (14 a-CD, 20 Controls, 22 GFD)

**Biological Samples** - Duodenal and oropharyngeal samples; intestinal mucosal explants; human and murine dendritic cells.

**Microbiota and Microbiome characterization** - culture methods+mass spectrometry ; metagenomics (DNA sequencing of 16S ribosomal RNA gene, bioinformatics analysis by QIIME v.1.9.1)

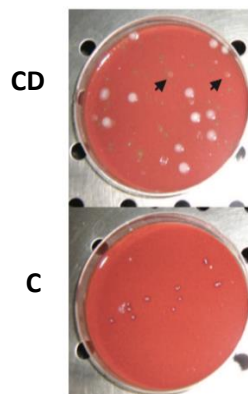
**Mycobioma characterization** - (DNA sequencing of regions ITS-1/2, bioinformatics analysis by QIIME v.1.9.1)

**Statistics** -Analysis of variance with Bonferroni correction; non-parametric two sample *t*-test; non-parametric multivariate analysis of variance (ADONIS) using UNIFRAC distance matrices; between group comparison by Friedman test for paired data. Pearson correlation (Swamp package).

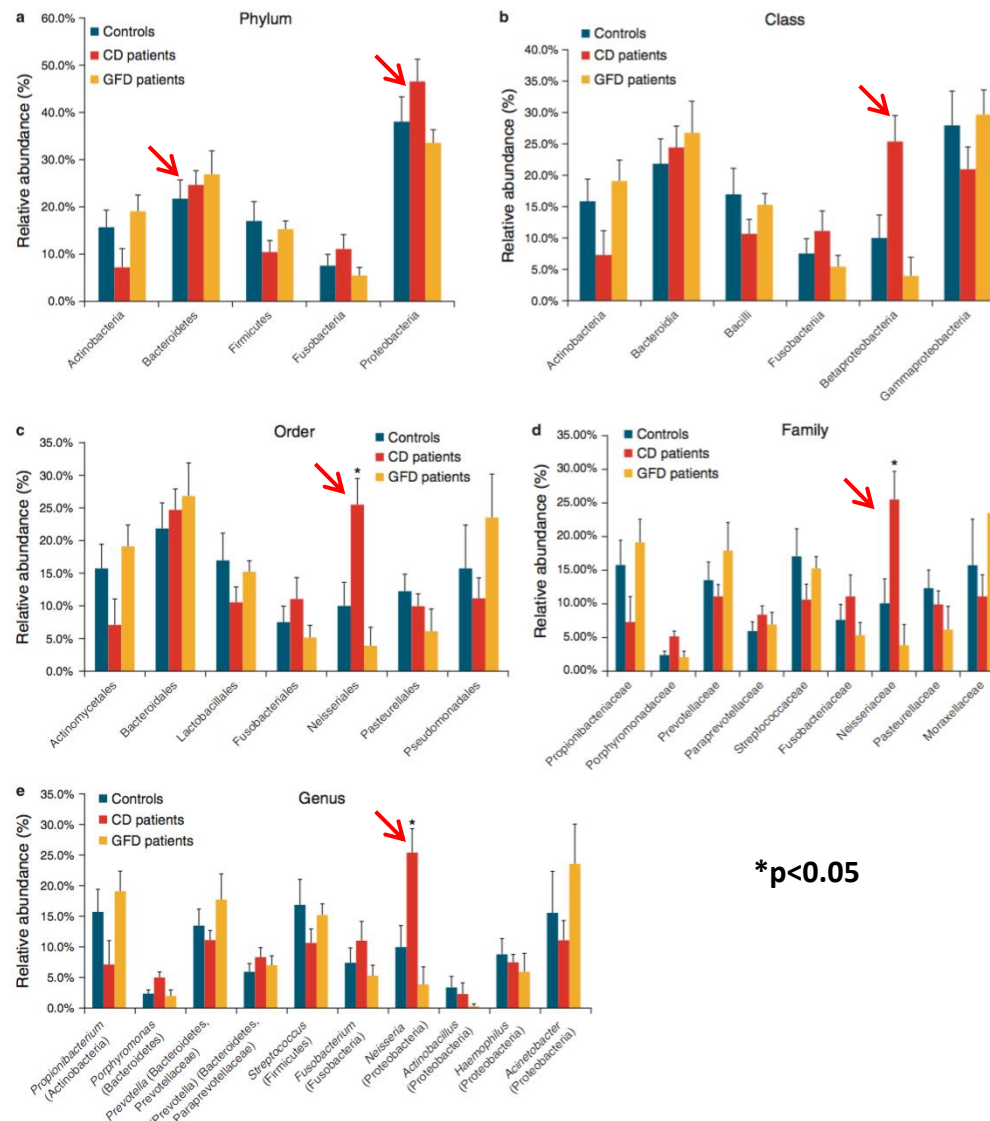
## Duodenal microbiome taxonomic composition in controls, in active and in GFD celiac disease (CD) patients

### Results (1)

#### Viability of *N. flavescens*



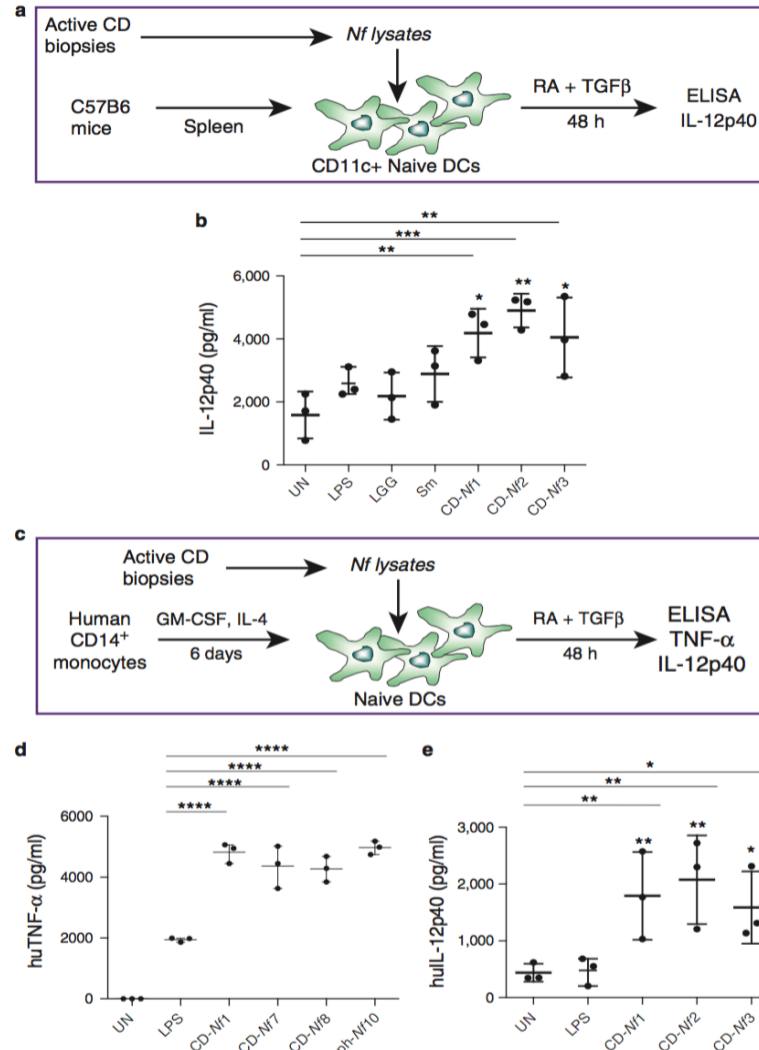
Duodenal cultures



\* $p < 0.05$

*N. flavescens* (Nf) isolated from celiac disease (CD) patients induce a pro-inflammatory phenotype on dendritic cells

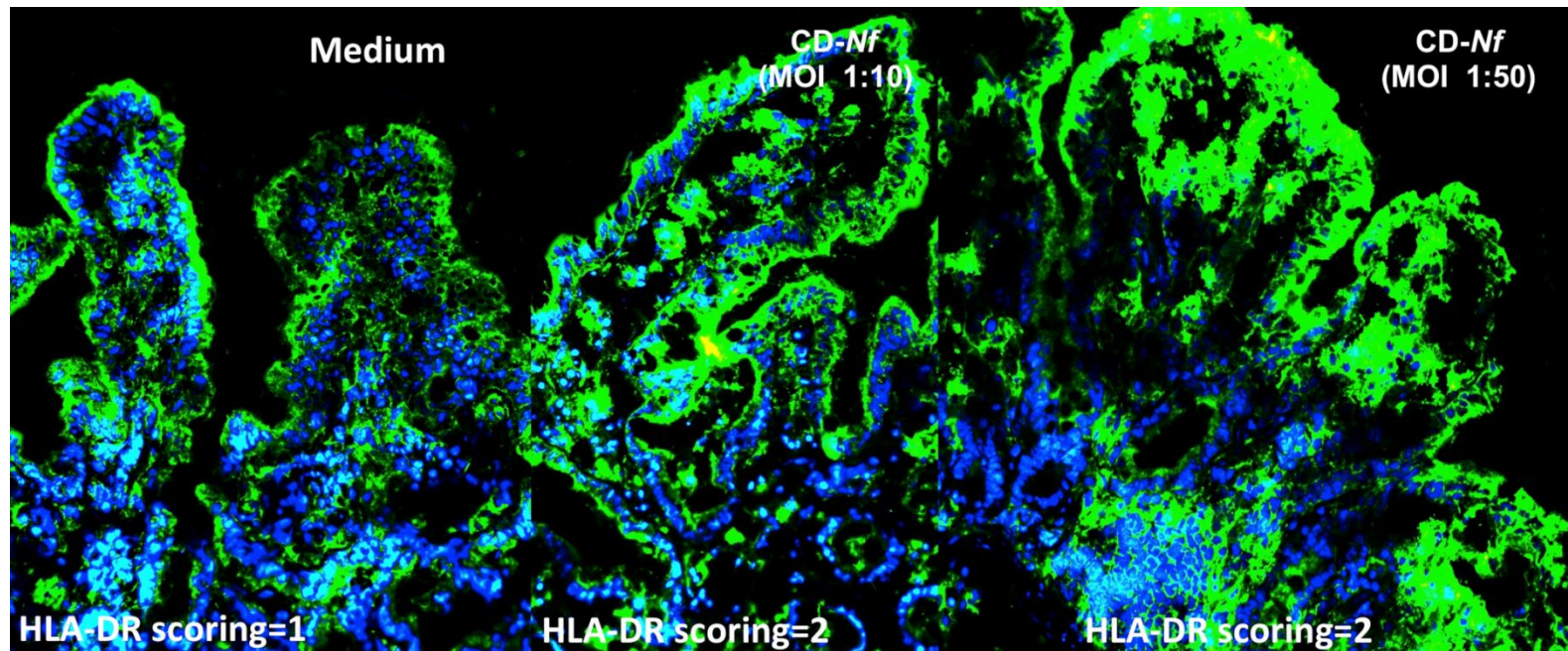
## Results (2)



\*p<0.05  
\*\*p<0.01  
\*\*\*p<0.001  
\*\*\*\*p<0.0001

Immunofluorescence analysis showed that in *ex-vivo* mucosal explants from control subjects the CD-Nf challenge induced the epithelial expression of HLA-DR and COX-2

## Results (2)



COX-2-positive epithelial cells

16/100



25/100

## CD-Nf challenge induces duodenal mucosa from control subjects to secrete pro-inflammatory cytokines in supernatants

| Treatment                       | Levels of cytokines (pg/ml) measured in supernatants mean (s.e.m.) |               |                 |               |              |               |              |
|---------------------------------|--------------------------------------------------------------------|---------------|-----------------|---------------|--------------|---------------|--------------|
|                                 | IFN- $\gamma$                                                      | TNF- $\alpha$ | IL-6            | IL-23         | IL-17A       | IL-17F        | IL-12p70     |
| No treatment                    | 23.44 (9.34)                                                       | 10.10 (0.48)  | 135.29 (72.08)  | 38.69 (8.86)  | 8.42 (5.98)  | 5.17 (4.18)   | 6.43 (1.22)  |
| Challenge with CD-Nf (MOI 1:10) | 59.23 (2.40)                                                       | 14.83 (2.12)  | 253.34 (147.57) | 50.39 (11.84) | 11.16 (7.92) | 7.12 (5.01)   | 10.61 (2.15) |
| Challenge with CD-Nf (MOI 1:50) | 107.94 (2.34)                                                      | 23.24 (2.49)  | 452.66 (19.74)  | 106.01 (3.68) | 25.01 (3.80) | 21.06 (14.15) | 14.71 (2.43) |
| P value <sup>b</sup>            | <b>0.001</b>                                                       | <b>0.011</b>  | 0.249           | <b>0.015</b>  | 0.320        | 0.353         | 0.082        |

IFN- $\gamma$ , interferon- $\gamma$ ; IL-6, interleukin-6; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

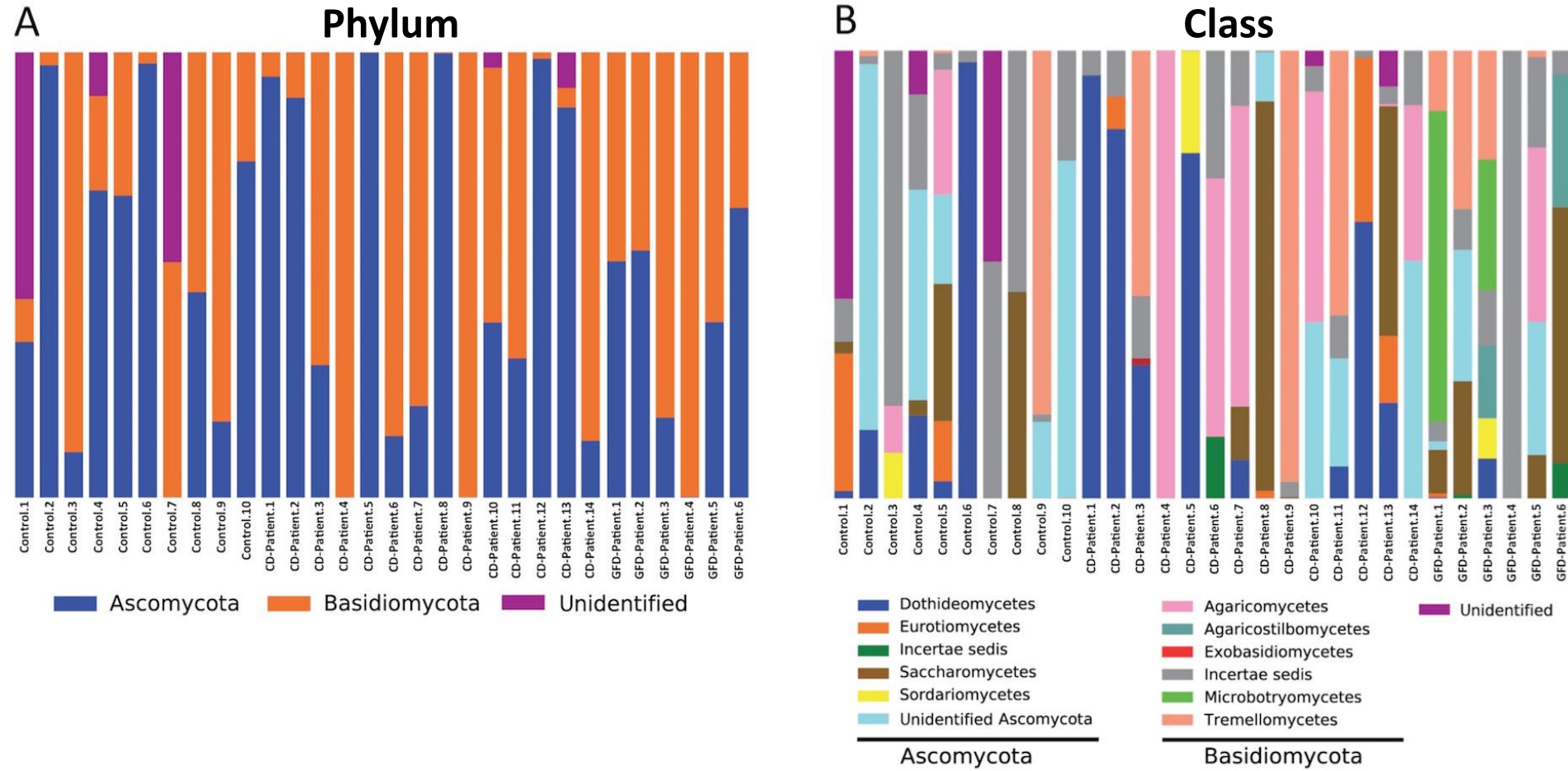
<sup>a</sup>Ex- vivo mucosal explants from three control subjects were treated for 6 h with CD-Nf at a multiplicity of infection (MOI) 1:10 and 1:50, and then levels of inflammatory cytokines were measured in supernatants of those treated with respect to untreated biopsies. Differences between mean levels were tested by the Friedman test with Bonferroni correction, and the *p* value is reported under each column.

<sup>b</sup>Boldface type indicates statistically significant differences among groups (*P*<0.05).

## Conclusions 1

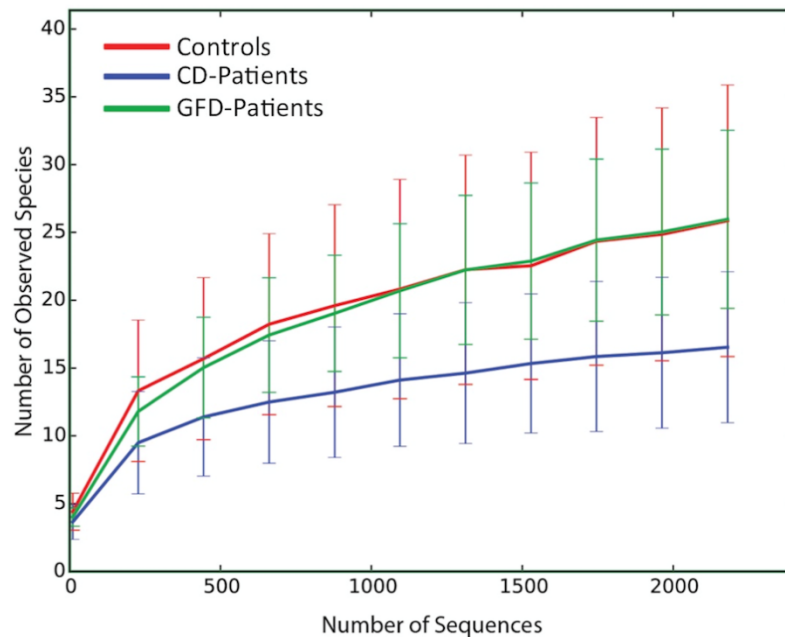
- ✓ Next-generation sequencing characterization of duodenal associated microbiomes revealed increased levels of Proteobacteria and of a *Neisseria flavescens* strain in adult a-CD patients.
- ✓ This CD-associated strain induced inflammation in dendritic cells and in *ex-vivo* mucosal explants suggesting that it may concur to amplify the mucosal derangement in CD patients.

Mycobioma composition did not differ significantly between controls, patients with active celiac disease (CD), and patients on a gluten-free diet (GFD)

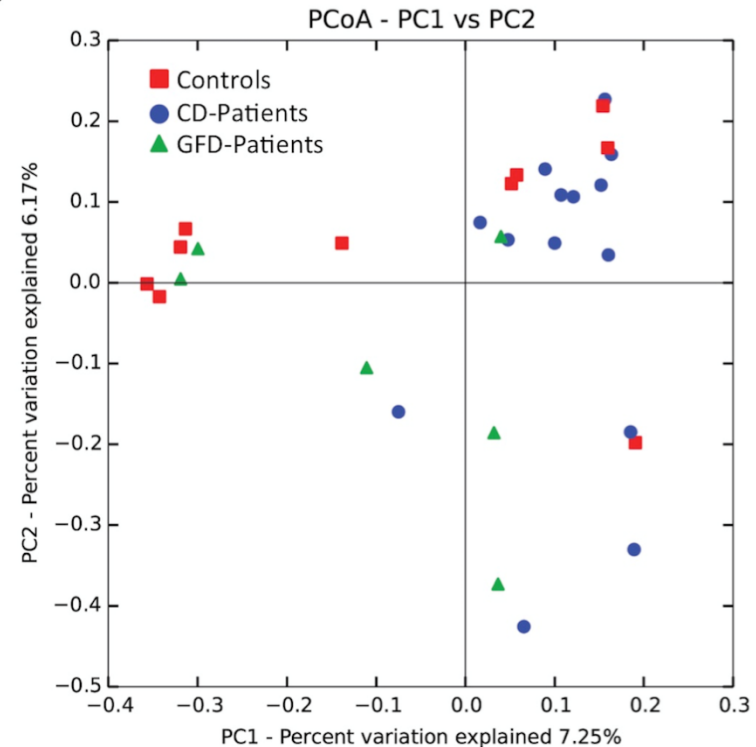


## Mycobioma composition in controls, in patients with active celiac disease (CD), and patients on a gluten-free diet (GFD)

C

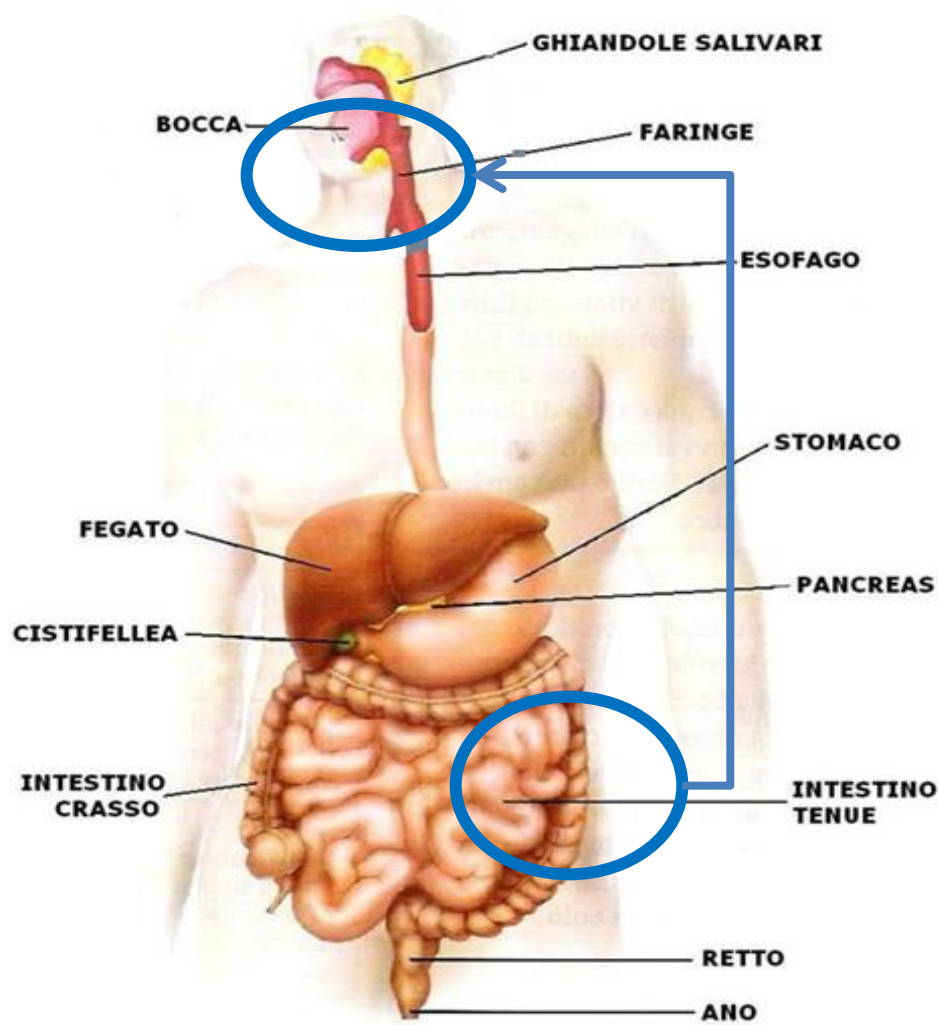


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## Conclusions 2

- ✓ Next-generation sequencing characterization of duodenal associated mycobioma revealed no statistical significant difference in mycobioma profile between the study groups

Question :

Considering the gastrointestinal tract a single ecosystem extending from the oral cavity to the rectum, could duodenum have in common to the oropharynx microbial alterations?

Answer:

The a-CD microbiome profiles in the two niches (oropharynx and duodenum) were very similar.

## Final Conclusions

- ✓ Next-generation sequencing characterization of duodenal associated microbiomes revealed increased levels of Proteobacteria and of a *Neisseria flavescens* strain in adult a-CD patients.
- ✓ This CD-associated strain induced inflammation in dendritic cells and in *ex-vivo* mucosal explants suggesting that it may concur to amplify the mucosal derangement in CD patients.
- ✓ No statistical significant difference in mycobioma profile was observed between the study groups
- ✓ Intriguingly, the a-CD microbiome profiles in the two niches (oropharynx and duodenum) were very similar.
- ✓ CD patients after Gluten free diet showed a control-like oropharyngeal microbiome profile

## Future Perspectives

- ✓ If our data will be confirmed in other cohorts of CD patients, we may speculate that microbiome characterization in the oropharynx, which is a less invasive sampling than the duodenum, could be used to monitor the efficacy of a GFD.

## Pubblicazioni del Grant FC

- (1) V. D'Argenio et al. Characterization of the entire celiac disease intestinal microbiome by next generation sequencing. 16th International Coeliac Disease Symposium, Prague, Czech Republic 21-24 June 2015 (P-147).
- (2) V. D'Argenio et al. Metagenomics Reveals Dysbiosis and a potentially pathogenic *N. flavescens* strain in duodenum of adults celiac patients. The American Journal of Gastroenterology (2016); doi:10.1038/ajg.2016.95.
- (3) V. D'Argenio et al. No change in the mucosal gut mycobioma is associated with celiac disease-specific microbiome alteration in adult patients. The American Journal of Gastroenterology (2016); doi:10.1038/ajg.2016.227.
- (4) L. Iaffaldano et al. The oropharyngeal microbiome diversity in healthy individuals and in celiac disease patients. Biochimica Clinica, 2017,41(3), S30.

## Partecipanti alla ricerca

**Titolare GRANT:** Lucia Sacchetti

**CEINGE-Biotecnologie Avanzate** (Coord. Scientifico **F. Salvatore**)

Facility: High Throughput Sequencing (V. D'Argenio e coll.)

Microscopia Confocale (D. Sarnataro)

Bioinformatica (G. Casaburi, G. Caporaso, Arizona, USA)

(D. S. Horner, M. Chiara, Università di Milano), (G. Pesole, Università di Bari)

(I. Granata, M.R. Guarracino, ICAR, CNR, Napoli)

Valutazione delle proprietà immuni della *Nf*

University of Chicago Celiac Disease Center (**B. Jabrì, S.M. Kim**)

Dip. Sc. Med. Transl./ELFID-Università di Napoli Federico II/CEINGE (**V. Discepolo**)

Microbiologia: (P. Salvatore e coll.)

Dipartimento di Medicina Molecolare e Biotecnologie Mediche

Università degli Studi di Napoli Federico II

Arruolamento casi/controlli

Università degli Studi di Salerno

Dip. Medicina e Chirurgia

(C. Ciacci e coll.)

Università degli Studi di Roma Tor Vergata

Dip. Medicina Interna

(G. Monteleone, G. Del Vecchio Blanco)

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