

## ALTERED GUT MICROBIOME COMPOSITION AND FUNCTION ARE ASSOCIATED WITH GUT BARRIER DYSFUNCTION IN HEALTHY RELATIVES OF CROHN'S DISEASE PATIENTS

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**Background:** The gut microbiome may play a role in gut barrier homeostasis including epithelial barrier function, but data are scarce and limited to animal studies

**Aims:** To assess if alterations in gut microbiome are associated with gut barrier function

**Methods:** We utilized the Genetic Environmental Microbial (CCC GEM) cohort of healthy first-degree relatives (FDRs) of Crohn's disease (CD) patients. Gut barrier function was assessed using the ratio of urinary fractional excretion of lactulose to mannitol (LMR). Stool bacterial DNA was extracted and sequenced for the V4 hypervariable region of the 16S rRNA gene using MiSeq and processed using QIIME2. Microbial functions were imputed using PICRUSt2. The cohort was divided into a North American discovery cohort (n=2,472) and non-North American external validation cohort (n=655). LMR>0.025 was defined as abnormal. LMR-microbiome associations were assessed using multivariable regression model and Random Forest (RF) classifier algorithm. q<0.05 was considered significant when multiple tests were performed

**Results:** The median age of the entire cohort was 17.0 years [IQR 12.0; 24.0], 52.6% were females and 25.4% had LMR>0.025. In the discovery cohort, subjects with LMR>0.025 had markedly reduced alpha diversity (Chao1 index, estimate= -0.0037, p=4.0e-04) and altered beta diversity (Bray-Curtis dissimilarity index, PERMANOVA: pseudo-F statistic = 2.99, p=1.0e-03). We identified eight bacterial genera and 52 microbial pathways associated with LMR>0.025 (q<0.05). Four genera (decreased *Adlercreutzia* [odds ratio(OR)=0.74, 95% confidence interval (CI) 0.6-0.91], *Clostridia-UCG-014* [OR=0.71, 95%CI 0.59-0.86], and *Clostridium-sensu-stricto-1* [OR=0.75, 95%CI 0.61-0.92] and increased *Colidextribacter* [OR=1.65, 95%CI 1.2-2.26]) and eight pathways (including decreased biosynthesis of glutamate [OR=0.4, 95%CI 0.21-0.74], tryptophan [OR=0.06, 95%CI 0.01-0.27] and threonine [OR=0.038, 95%CI 0.003-0.41]) were replicated. Bacterial community composition was associated with gut barrier homeostasis

as defined by the RF analysis ( $p= 1.4e-6$ )

**Conclusions:** Gut microbiome community and pathways are associated with gut barrier function. These findings may identify potential microbial targets to modulate barrier function

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