**Identification of IgG Autoantibody against Malondialdehyde-Acetaldehyde Adduct (MAA) as Novel Serological Biomarker for Ulcerative Colitis**

**Estimation of circulating serum Anti-MAA immunoglobulin:** A Enzyme-linked immunosorbent assay (ELISA) was used to estimate the levels of anti-MAA Immunoglobulins in the serum from UC, CD, celiac disease, IBS and controls. Briefly, aqueous human serum albumin (HSA) (Talecris Biotherapeutics, Inc., Research Triangle Park, NC) was modified with MAA (2:1 molar ratio). High-affinity binding ELISA plates were incubated with HSA, HSA-MAA and human IgM, IgG, or IgA isotype standard controls for relative antibody concentration (Sigma Chemical Company, St. Louis, MO) overnight at 4oC. Plates were washed with PBS-T, blocked in 2% casein, and incubated with patient serum at a 1:100 dilution.

Following a 1 hour incubation at 37oC, plates were washed in PBS-T and incubated with the secondary antibodies HRP goat anti-human antibody specific for IgM (Fc5u fragment specific), IgG (Fcγ specific) or IgA (α chain particular) (Jackson Immuno-Research, West Grove, PA). Plates were incubated for an additional hour, washed with PBS-T and developed with TMB substrate, reaction stopped with sulfuric acid, and absorbance was determined at 450nm using an EpochPlate reader (BioTek, Winooski, VT) and analyzed using Gene 5 Software (BioTek). A human calibrator sample (specific to MAA antigen) was used as a positive control and to keep consistency between plates, and all data were normalized to this sample. The lowest detectable limit for this assay was five relative units of the antibody. Therefore, anything at or below this level was removed from the analysis.

**Immunofluorescence analysis:** Immunofluorescence method of cellular localization was used to detect MAA expression and localization. Immunofluorescent staining was performed on slide sections from de-identified endoscopic biopsies from IBD patients by using a rabbit polyclonal antibody specific to MAA directly labeled with a Zenon 405 reporter (Molecular Probes, Eugene, OR). Briefly, antigen retrieval in dewaxed slides was performed using citrate buffer (pH-6.0) and heating to 620C, followed by blocking in 2% goat serum. The anti-MAA antibody and control isotype at a concentration of 1:100 was used for overnight incubation. Slides were then washed with PBS three times and mounted with coverslips. Stained tissues were analyzed using a Echo Revolve fluorescent microscope and quantification of images with Image J using the FIJI plugin (National Institutes of Health).