

Title: Unraveling Transcriptomic Landscapes in Overt and Potential Celiac Disease Using Spatial Transcriptomics**Authors:**

Emilia Siukola, Helka Kaunisto, Esko Kempainen, Alina Popp, Antonio Federico, Dario Greco, Kalle Kurppa, Katri Lindfors

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Abstract

While overt celiac disease (CeD) is characterized by TG2 autoantibodies and small bowel mucosal damage, subjects with potential CeD (PCeD) display elevated TG2 and endomysium (EMA) autoantibodies in the absence of mucosal lesions, representing an early disease stage. Transcriptomics has advanced our understanding of CeD pathology, conventional approaches lack spatial and cellular resolution. We applied spatial transcriptomics to characterize compartment-specific transcriptional changes across CeD, PCeD, and controls.

Duodenal biopsies from CeD (n=10), PCeD (n=2), and control (n=5) patients were analyzed using GeoMx Digital Spatial Profiling. Regions of interest were selected from crypt and villus compartments, further divided into epithelial and lamina propria areas.

In overt CeD, antigen presentation pathways were strongly enriched, with upregulation of both HLA class II and class I genes, along with increased expression of immune activation markers such as *IL-32* and *CD74*, primarily in the villus epithelium and crypt cells. In contrast, PCeD exhibited intermediate transcriptional profiles, characterized by upregulation of HLA class I and stress-response genes but lacking the full antigen presentation signature seen in overt disease. In situ hybridization (RNAscope) confirmed *IL32* expression in the villus epithelium, predominantly in CeD.

Spatial transcriptomics reveals compartment-specific immune activation in CeD and identifies PCeD as a molecular transition state characterized by early epithelial changes. These findings highlight the value of spatially resolved approaches for detecting early immune signals, with potential implications for diagnosis, disease monitoring, and therapeutic intervention in CeD.