



# Claudin-4 expression in lamina propria mononuclear cells of patients with ulcerative colitis

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## BACKGROUND

Disruption of the intestinal barrier is associated with the development of inflammatory bowel disease (IBD), and recent studies suggest that claudins, tight junction proteins, may play a key role in its pathogenesis. Given the multifactorial nature of the disease and evidence that claudins have diverse functions beyond their role as transmembrane proteins, their involvement in IBD development has become an important research topic. Čužić et al. investigated claudin expression in mucosal biopsies from the rectum and sigmoid colon of a small cohort of ulcerative colitis (UC) patients, revealing their presence in cells derived from the mesoderm.

## AIM

To investigate claudin-4 expression across different cells population within lamina propria mononuclear cells (LPMCs) of patients with UC and healthy controls.

## MATERIALS AND METHODS

Mucosal samples from four colonic segments of 20 UC patients and 20 healthy controls were collected.

In UC patients, biopsies were taken from inflamed mucosa of the rectum and sigmoid colon, the border region (1 cm from the inflamed segment toward healthy mucosa), and non-inflamed intestinal areas. In patients without IBD, biopsies were collected from the rectum, sigmoid colon, transverse colon, and ascending colon.

LPMCs were isolated, stained with LIVE/DEAD dye to distinguish live from dead cells, and labeled with an antibody cocktail for immunophenotyping and claudin-4 expression analysis. Subsequently, the cells were analyzed by flow cytometry (Attune NxT) using FlowJo v10.10 software. Statistical analyses were performed using the Mann–Whitney and ANOVA test followed by Tukey's multiple comparisons test, with GraphPad Prism software (San Diego, CA, USA). Statistical significance was determined at level  $p < 0.05$ .

## RESULTS

Claudin-4 was detected in all samples from UC patients and healthy controls. Specific cell subpopulations, including macrophages, plasma cells, and lymphocytes (CD4<sup>+</sup>, CD8<sup>+</sup> and B cells), showed differential Claudin-4 expression not only between corresponding tissue segments of UC patients and healthy individuals, but also between some colon segments of UC patients, as well as healthy controls. For example, plasma cells showed consistently higher claudin-4 expression across all segments in UC patients compared with healthy controls, while within the UC cohort, significant differences were observed between transverse colon and border region compared to the ascending colon (Figure 1).

A similar trend was observed in other cell populations; however, the number of segments showing significant changes varied. In resident macrophages, claudin-4 expression was higher in the transverse and ascending colon of patients with UC,

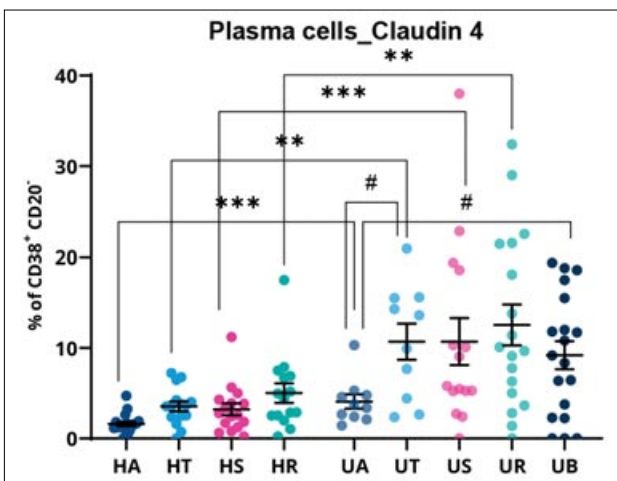


Figure 1. Claudin-4 expression in plasma cells in UC and healthy controls across regions:

HA (healthy ascending colon), HT (healthy transverse colon), HS (healthy sigmoid colon), HR (healthy rectum), UA (UC ascending colon), UT (UC transverse colon), US (UC sigmoid colon), UR (UC rectum), UB (UC Border region).

Data are expressed as mean  $\pm$  SEM,  $p < 0.05$ .

\*indicate statistical significance when comparing UC colon segment with the corresponding segment in the control group,

#indicate statistical significance when comparing within colon segments of the UC group.

while in inflammatory macrophages increased expression was observed only in the transverse colon. CD4<sup>+</sup> lymphocytes have higher expression in the transverse and ascending colon of patients with UC and CD8<sup>+</sup> lymphocytes have higher expression in transverse colon in UC. B lymphocytes showed higher claudin-4 expression across all segments (rectum, sigmoid colon, ascending colon, border region) in UC compared with healthy controls.

Regarding differential claudin-4 expression among colon segments within the UC cohort, CD4<sup>+</sup> lymphocytes exhibited higher expression of claudin-4 in transverse colon and rectum compared with ascending colon of UC patient. Similarly, CD8<sup>+</sup> lymphocytes showed higher expression in the rectum, sigmoid, transverse colon and border region compared with the ascending colon.

## CONCLUSIONS

Claudin-4 is expressed in LPMCs isolated from all mucosal segments of both UC patients and healthy controls. Its expression varies among specific mononuclear cell populations and colonic regions in both, healthy individuals and UC patients. Increased expression in macrophages, plasma cells, T and B lymphocytes in UC patients compared to healthy controls in certain segments may indicate a localized immune cell response. These results suggest that claudin-4 might play a role in regulating immune responses and contribute to mucosal pathophysiology in ulcerative colitis.

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