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# A Molecular Investigation into a Potential Signalling Pathway Underlying Increased Chymase-containing Mast Cells in Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a common and debilitating syndrome, with deficits in epithelial permeability and tight junction protein expression a common pathophysiological feature. Mast cells in particular have been implicated in intestinal permeability dysfunction in IBS. Specifically, we recently reported an increase in colonic chymase-containing mast cells (MCc) in IBS patient biopsies, in addition to decreased colonic barrier function (1), and therefore we speculate that MCc may contribute to permeability deficits in IBS. While pharmacologic strategies that serve to inhibit chymase function may prove to be useful, few, if any, have progressed clinically. Therefore, our focus was to investigate a potential signalling pathway involved in the regulation of MCc which may be of therapeutically relevant in IBS. Both stem cell factor (SCF) and interleukin-6 (IL-6) increase the frequency of MCc in culture (2), the latter previously reported by our group to be elevated in IBS (3, 4), and by others to influence permeability (5).

**Aim** To evaluate the IL-6, SCF and chymase 1 mast cell (CMA1) expression profile of colonic biopsies from IBS and healthy patients to investigate a potential signalling pathway underlying the increased MCc in IBS.

**Method** Mucosal biopsies were obtained from the rectum and sigmoid colon of healthy and IBS patients, as approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Quantitative PCR (Q-PCR) was carried out using probes (FAM) for IL-6, SCF and CMA1 (Applied Biosystems) and β-Actin as an endogenous control. Q-PCR was carried out on an ABI7300 Real Time PCR machine Experimental samples were run in triplicate. Data was normalised using β-Actin and transformed using the DeltaCt method (6). Statistical analysis was performed using Graphpad Prism 4 statistical software. Data was presented as the mean +/- SEM. Statistical differences were determined using Student's t-tests.

**Results** We demonstrated that IL-6 (H, 1.00 ± 0.04 v IBS, 1.48 ±0.02), SCF (H, 1.00 ± 0.03 v IBS, 1.02 ± 0.03) and CMA1 (H, 1.00 ± 0.04 v IBS, 1.07 ± 0.11) were not significantly altered in IBS colonic biopsy tissue compared to healthy controls. In this same patient population we previously reported that MCc were significantly increased in IBS patients relative to healthy control subjects (H, 0.54 ± 0.16 v IBS, 1.56 ± 0.36, $P < 0.05$) (1).

**Conclusion** At the gene level, we did not identify a confluence between SCF, IL-6 and CMA1 that would account for increased MCc observed in IBS patients. Further studies are required to fully understand the mechanisms underlying increased chymase mast cells in IBS including its association with increased intestinal

permeability.

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