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(54) **Title:** DIAGNOSTIC METHOD

(57) **Abstract:** The invention relates to a method of diagnosing or assessing an inflammatory bowel disease in a subject, comprising comparing the level of one or more markers in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the marker is selected from the group consisting of Secretogranin-1 or a fragment thereof, guanylin or a fragment thereof, SPP 24 or a fragment thereof, AMBP or a fragment thereof; and serglycin or a fragment thereof.



DIAGNOSTIC METHOD

Field of the Invention

The invention relates to a method for diagnosing or
5 assessing an inflammatory bowel disease in a subject.

Background

Inflammatory bowel diseases (IBD) are chronic immuno-
inflammatory pathologies of the gastrointestinal tract.
10 Crohn's Disease (CD) and Ulcerative colitis (UC) are known as
inflammatory bowel diseases. These diseases are thought to be
affected by many genetic and environmental factors, and have
unknown aetiology. Clinical presentation is non-specific and
diagnosis is based on a clinical, endoscopic, radiological and
15 histological criteria. Disease course is characterised by
relapses and remission. Symptoms are non-specific and include
abdominal pain and diarrhoea.

The clinical presentation of Crohn's Disease and
Ulcerative colitis are similar. An accurate diagnosis of IBD,
20 and the ability to discriminate between Crohn's Disease and
Ulcerative colitis, is important in order to achieve effective
treatment and management of the disease. There is currently no
single diagnostic test for IBD. Invasive diagnostic
investigations, in particular colonoscopy and histopathological
25 evaluation of the inflamed gut wall, remain the standard tool
for diagnosis. Colonoscopy is expensive and invasive, with a
risk of bowel perforation. In particular, colonoscopy is not
suitable for use in acute severe inflammation as the risk of
bowel perforation is too high. Despite investigations using
30 available methods, 15% of patients remain undifferentiated and
are therefore termed as having indeterminate colitis.

Diagnostic antibody markers that have been tried include anti-Saccharomyces cerevisiae antibody (ASCA) and peri-nuclear anti-neutrophil cytoplasmic antibody (P-ANCA). However, diagnosis with these markers is less effective due to their
5 limited sensitivity and specificity.

What is needed is a convenient and reliable method for diagnosing and assessing IBD in subjects.

Summary

A first aspect provides a method of diagnosing or
10 assessing an inflammatory bowel disease (IBD) in a subject, comprising comparing the level of one or more markers in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the marker is selected from the group consisting of:

- 15 i) Secretogranin-1 or a fragment thereof;
- ii) Guanylin or a fragment thereof;
- iii) SPP 24 or a fragment thereof;
- iv) AMBP or a fragment thereof; and
- 20 v) Serglycin or a fragment thereof.

A second aspect provides a method of diagnosing or assessing an inflammatory bowel disease in a subject, comprising comparing the level of SPP 24 or a fragment thereof in a tissue or body fluid of the subject relative to a reference value for
25 the one or more markers, wherein the reference value is the level of SPP 24 or a fragment thereof in a subject not suffering from IBD, and wherein IBD is diagnosed when the level of SPP 24 or a fragment thereof is elevated relative to the reference value.

30 A third aspect provides a method of diagnosing or assessing an inflammatory bowel disease in a subject, comprising comparing the level of serglycin or a fragment thereof in a

tissue or body fluid of the subject relative to a reference value for serglycin , and wherein the reference value is the level of serglycin in a subject not suffering from IBD, and wherein IBD is diagnosed when the level of serglycin is reduced relative to the reference value.

A fourth aspect provides a method of diagnosing or assessing an inflammatory bowel disease in a subject, comprising comparing the level of one or more markers in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the one or more markers is selected from the group consisting of SPP 24 or a fragment thereof, and serglycin or a fragment thereof, and wherein the reference value is the level of the one or more markers in a subject not suffering from IBD, and wherein IBD is diagnosed when the level of SPP 24 or a fragment thereof is elevated relative to the reference value, and/or the level of serglycin or a fragment thereof is reduced relative to the reference value.

A fifth aspect provides a method of assessing whether a subject suffering from IBD is suffering from Crohn's Disease, comprising comparing the level of one or more markers selected from the group consisting of: secretogranin-1 or a fragment thereof; AMBP or a fragment thereof; and guanylin or a fragment thereof, in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from Ulcerative colitis, and wherein Crohn's Disease is diagnosed when the level of the one or more markers is reduced relative to the reference value.

A sixth aspect provides a method of assessing whether a subject suffering from IBD is suffering from Ulcerative colitis, comprising comparing the level of one or more markers selected from the group consisting of secretogranin-1 or a fragment thereof and guanylin or a fragment thereof, in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from Crohn's disease,

and wherein Ulcerative colitis is diagnosed when the level of the one or more markers is increased relative to the reference value.

A seventh aspect provides a method of assessing whether a subject suffering from IBD is suffering from Ulcerative colitis, comprising comparing the level of guanylin or a fragment thereof in a tissue or body fluid of the subject relative to a reference value, wherein the reference value is the level of guanylin or a fragment thereof in a subject suffering from Crohn's disease, and wherein the patient is assessed as suffering from Ulcerative colitis when the level of guanylin or a fragment thereof is elevated relative to the reference value.

An eighth aspect provides a method of assessing the severity of Crohn's disease in a subject, comprising comparing the level of one or more markers selected from the group consisting of SPP 24 or a fragment thereof and secretogranin-1 or a fragment thereof in tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from Crohn's disease of predetermined severity, and wherein the Crohn's disease is diagnosed as more severe than the Crohn's disease of predetermined severity when the level of the one or more markers is elevated relative to the reference value.

A ninth aspect provides a method of assessing the severity of Ulcerative colitis in a subject, comprising comparing the level of one or more markers selected from the group consisting of SPP24 or a fragment thereof and secretogranin-1 or a fragment thereof in tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from Ulcerative colitis in remission, and wherein Ulcerative colitis is diagnosed as active when the level of secretogranin-1 or fragment thereof is elevated relative to the reference value, and/or the level of SPP24 or a fragment thereof is reduced relative to the reference value.

A tenth aspect provides a method of assessing the severity of IBD in a subject suffering from IBD, comprising comparing the level of secretogranin-1 or a fragment thereof in a tissue or body fluid of the subject relative to a reference value for secretogranin-1 or a fragment thereof, wherein the reference value is the level of secretogranin-1 or a fragment thereof in a subject suffering from IBD of predetermined severity, and wherein IBD is diagnosed as more severe than the IBD of predetermined severity when the level of secretogranin-1 or a fragment thereof is elevated relative to the reference value. In one embodiment, the IBD of predetermined severity is IBD in remission.

An eleventh aspect provides a method of assessing whether a subject with no symptoms or few symptoms of IBD is suffering from Ulcerative colitis in remission, comprising comparing the level of one or more markers selected from the group consisting of SPP24 or a fragment thereof and secretogranin-1 or a fragment thereof in a tissue or body fluid of the subject relative to a reference value, wherein the reference value is the level of the one or more markers in a subject suffering from active Ulcerative colitis, and wherein Ulcerative colitis in remission is diagnosed when the level of secretogranin-1 or fragment thereof is reduced relative to the reference value, and/or the level of SPP 24 or fragment thereof is elevated relative to the reference value.

A twelfth aspect provides a method for assessing whether a subject showing no symptoms or few symptoms of IBD is suffering from Crohn's disease or Ulcerative colitis in remission, comprising comparing the level of SPP 24 or a fragment thereof in a tissue or body fluid of the subject relative to a reference value, wherein the reference value is the level of the one or more markers in a subject not suffering from IBD, and wherein the subject is assessed as suffering from Crohn's disease in remission or Ulcerative colitis in remission when the level of SPP 24 or a fragment thereof is elevated relative to the reference value.

A thirteenth aspect provides a method of assessing whether a subject is suffering from active Ulcerative colitis or Ulcerative colitis in remission, comprising comparing the level of one or more markers selected from the group consisting of SPP
5 24 or a fragment thereof and secretogranin-1 or a fragment thereof, in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from active Ulcerative colitis, and wherein
10 the subject is assessed as suffering from ulcerative colitis in remission when the level of secretogranin-1 or fragment thereof is reduced relative to the reference value, and/or the level of SPP 24 or a fragment thereof is elevated relative to the reference value.

15 In one embodiment, a sample of the tissue or body fluid is obtained from the subject and the level of the one or more markers in the sample compared with the reference value.

An alternative aspect provides use of one or more markers selected from the group consisting of:

- 20 i) Secretogranin-1 or a fragment thereof;
ii) Guanylin or a fragment thereof;
iii) SPP 24 or a fragment thereof;
iv) AMBP or a fragment thereof; and
v) Serglycin or a fragment thereof,

25 for diagnosing or assessing an IBD in a subject.

An alternative provides one or more markers selected from the group consisting of:

- i) Secretogranin-1 or a fragment thereof;
ii) Guanylin or a fragment thereof;
30 iii) SPP 24 or a fragment thereof;

iv) AMBP or a fragment thereof; and

v) Serglycin or a fragment thereof,

for use in diagnosing or assessing an IBD in a subject.

A fourteenth aspect provides a diagnostic device or
5 protein array for diagnosing or assessing an IBD in a subject,
comprising one or more markers selected from the group
consisting of:

(i) Secretogranin-1 or a fragment thereof;

(ii) Guanylin or a fragment thereof;

10 (iii) SPP 24 or a fragment thereof;

(iv) AMBP or a fragment thereof; and

(v) Serglycin or a fragment thereof,

or an antibody or fragment thereof which specifically
binds the one or more markers.

15 A fifteenth aspect provides one or more markers selected
from the group consisting of:

(i) Secretogranin-1 or a fragment thereof;

(ii) Guanylin or a fragment thereof;

(iii) SPP 24 or a fragment thereof;

20 (iv) AMBP or a fragment thereof; and

(v) Serglycin or a fragment thereof,

for diagnosing or assessing an IBD in a subject.

A sixteenth aspect provides a kit for diagnosing or
25 assessing an IBD in a subject, comprising one or more markers
selected from the group consisting of:

(i) Secretogranin-1 or a fragment thereof;

- (ii) Guanylin or a fragment thereof;
- (iii) SPP 24 or a fragment thereof;
- (iv) AMBP or a fragment thereof; and
- (v) Serglycin or a fragment thereof,

5 or an antibody or fragment thereof which specifically binds the one or more markers.

A seventeenth aspect provides a composition comprising one or more peptides selected from the group consisting of:

- (i) ADQTVLTEDEK (SEQ ID NO: 2)
- 10 (ii) VTVQDGNFSFSLESVK (SEQ ID NO: 4)
- (iii) VSAQQVQGVHAR (SEQ ID NO: 6)
- (iv) VNSQSLSPYLFR (SEQ ID NO: 7)
- (v) NLPSDSQDLGQHGLEED (SEQ ID NO: 9)
- (vi) HHGPTITAK (SEQ ID NO: 11).

15 An eighteenth aspect provides a method of diagnosing Ulcerative colitis in a subject, comprising comparing the level of one or more markers in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the one or more markers is selected from the group
20 consisting of:

- (i) secretogranin-1 or a fragment thereof;
 - (ii) guanylin or a fragment thereof;
- wherein the reference value is the level of the one or more markers in a subject not suffering from IBD, and wherein
25 Ulcerative colitis is diagnosed when the level of the one or more markers is elevated relative to the reference value.

An nineteenth aspect provides a method of diagnosing Crohn's disease in a subject, comprising comparing the level of a first marker and one or more second markers in a tissue or

body fluid of the subject relative to a reference value for each of the markers, wherein the first marker is SPP24 or a fragment thereof and the second marker is selected from the group consisting of:

- 5 (ii) secretogranin-1 or a fragment thereof; and
 (ii) guanylin or a fragment thereof;

wherein the reference value is the level of the first and second markers in a subject not suffering from IBD, and wherein Crohn's disease is diagnosed when the level of first marker is elevated
10 relative to the reference value, and the level of the one or more second markers is not elevated relative to the reference value.

Brief Description of the Drawings

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Figure 1 shows the full length amino acid sequence of A. human secretogranin-1 (SEQ ID NO: 1); B. human guanylin (SEQ ID NO: 3); C. human SPP 24 (SEQ ID NO: 5); D. human serglycin (SEQ ID NO: 8); and E. human AMBP (SEQ ID NO: 10). Peptides within the
20 full length sequences that have been detected following MRM of enzyme digested samples and and/or ion count MS as described herein are underlined and in bold.

Figure 2A and 2B are graphs showing relative abundance of the
25 peptide VSAQQVQGVHAR (SEQ ID NO: 6) and VNSQSLSPYLFR (SEQ ID NO: 7) in subjects suffering from IBD (IBD), subjects not suffering from IBD (C), and subjects suffering from rheumatoid arthritis (RA), as determined by MRM analysis of enzyme digested serum samples.

30

Figure 2C is a graph of the relative abundance of the protein containing the peptide VSAQQVQGVHAR (SEQ ID NO: 6) and VSNQSLSPYLFR (SEQ ID NO: 7) in pooled serum samples from subjects suffering from IBD (IBD), or not suffering from IBD
35 (control, C), as determined by ion count MS.

Figure 3 is a graph showing the relative abundance of the peptide NLPSPDSQDLGQHGLEED (SEQ ID NO: 9) in subjects suffering from IBD (IBD), subjects not suffering from IBD (C), and
5 subjects suffering from rheumatoid arthritis (RA), as determined by MRM analysis of enzyme digested serum samples.

Figures 4A and 4B and 4C are graphs showing the relative abundance of: A. ADQTVLTEDEK (SEQ ID NO: 2); and B. HHGPTITAK
10 (SEQ ID NO: 11); and C. VTVQDGNFSFSLESVK (SEQ ID NO: 4), in subjects suffering from Ulcerative colitis (UC) or Crohn's disease (CD), as determined by MRM analysis of trypsin digested serum samples.

15 Figure 5 is a graph showing the relative abundance of the peptides ADQTVLTEDEK (SEQ ID NO: 2), HHGPTITAK (SEQ ID NO: 11); and VTVQDGNFSFSLESVK (SEQ ID NO: 4), in pooled samples from subjects suffering from UC, or CD, as determined by ion count MS.

20

Figure 6 is a graph showing the distribution of mean amounts (pg) of the peptides VNSQSLSPYLFR (SEQ ID NO: 7), NLPSPDSQDLGQHGLEED (SEQ ID NO: 9), VSAQQVQGVHAR (SEQ ID NO: 6) and VTVQDGNFSFSLESVK (SEQ ID NO: 4) following MRM of enzyme
25 digested serum samples from: healthy patients (C); patients suffering from Rheumatoid arthritis (RA); patients suffering from Crohn's disease (CD); patients suffering from Ulcerative colitis (UC); and synovial fluid from an otherwise healthy patient suffering from inflamed synovium (Syn).

30

Figure 7 shows the distribution of mean amounts (pg) of the peptides VNSQSLSPYLFR (SEQ ID NO: 7) and VSAQQVQGVHAR (SEQ ID NO: 6) following MRM of enzyme digested serum samples from: healthy patients (H); and patients suffering from IBD (IBD).
35 (p=<0.0002 for both peptides).

Figure 8 shows the distribution of mean amounts (pg) of the peptides VNSQSLSPYLFR (SEQ ID NO: 7) ($p=0.004$) and VSAQQVQGVHAR (SEQ ID NO: 6) ($p=0.002$) and AMBP HHGPTITAK (SEQ ID NO: 11) ($p=0.01$) following MRM of enzyme digested serum samples from healthy patients (H) and patients suffering from Ulcerative colitis in remission (UC Rem); and B. healthy patient (H) and patient suffering from Crohn's disease in remission (CDRem). ($p<0.002$ UC for both peptides, CD $p=$ VNS 0.017, VSAQ $p=0.048$)

Figure 9 shows the distribution of mean amounts (pg) of the peptide VTVQDGNFSFSLESVK (SEQ ID NO: 4) and ADQTVLTEDEK (SEQ ID NO: 2) following MRM of enzyme digested serum samples from patients suffering from Crohn's disease (CD) or Ulcerative colitis (UC).

Figure 10 shows the distribution of mean amounts (pg) of the peptides VNSQSLSPYLFR (SEQ ID NO: 7), VSAQQVQGVHAR (SEQ ID NO: 6) and ADQTVLTEDEK (SEQ ID NO: 2) following LC-MRM of enzyme digested serum samples from patients suffering from Ulcerative colitis in remission (Quiescent) or suffering from active Ulcerative colitis as determined by UC DAI score (ActiveUC).

Figure 11 shows the distribution of mean amounts (pg) of the peptides VNSQSLSPYLFR (SEQ ID NO: 7), VSAQQVQGVHAR (SEQ ID NO: 6) and ADQTVLTEDEK (SEQ ID NO: 2) following LC-MRM of enzyme digested serum samples from patients suffering from Crohn's disease in remission (Quiescent) and patients suffering from increasing severity of Crohn's disease (mild to severe).

Figure 12 shows the distribution of mean amounts of the peptide ADQTVLTEDEK (SEQ ID NO: 2) following MRM of enzyme digested serum samples from subjects suffering from IBD in remission (Quiescent), and severe UC or CD.

Detailed Description

The invention relates in one aspect to a method of diagnosing or assessing an inflammatory bowel disease in a subject. The inflammatory bowel disease is diagnosed or assessed by comparing the level of one or more markers in tissue or body fluid of the subject relative to a reference value for the one or more markers.

As used herein, "Inflammatory bowel disease" refers to Crohn's disease and Ulcerative colitis, collectively. Crohn's disease (CD) is an inflammation anywhere along the digestive tract, including the large intestine, and involves all layers of the intestinal wall. Common symptoms of CD include chronic diarrhea fever, abdominal pain, weight loss and lack of appetite. Ulcerative colitis (UC) is an inflammation of the large intestine and is associated with ulcers in the inner layers of the lining of the large intestine. Common symptoms of UC include bloody diarrhea, fever and abdominal pain.

As used herein, a "marker" is a molecular indicator of a specific biological property or condition. A marker may be, for example, a protein, or a fragment of a protein such as a peptide.

The marker is selected from the group consisting of:

- i) Secretogranin-1 or a fragment thereof;
- ii) Guanylin or a fragment thereof;
- iii) Spp24 or a fragment thereof;
- iv) AMBP or a fragment thereof; and
- v) Serglycin or a fragment thereof.

Secretogranin-1 is also known as Chromogranin-B. The full length amino acid sequence of human secretogranin-1 is shown in Figure 1 (SEQ ID NO: 1). In embodiments in which the marker is secretogranin-1 or a fragment thereof, the secretogranin-1 or fragment thereof may:

- i) comprise an amino acid sequence of SEQ ID NO: 1 or fragment thereof;
- ii) comprise the amino acid sequence ADQTVLTEDEK (SEQ ID NO: 2);
- 5 iii) consist essentially of the amino acid sequence of SEQ ID NO: 2; or
- iv) consist of the amino acid sequence of SEQ ID NO: 2.

Guanylin is also known as Guanylate cyclase activator 24, and Guanylate cyclase activator 2. The full length amino acid
10 sequence of human guanylin is shown in Figure 1 (SEQ ID NO: 3).
In embodiments in which the marker is Guanylin or a fragment thereof, the Guanylin or fragment thereof may:

- i) comprise an amino acid sequence of SEQ ID NO: 3 or fragment thereof;
- 15 ii) comprise an amino acid sequence VTVQDGNFSFSLESVK (SEQ ID NO: 4);
- iii) consist essentially of the amino acid sequence of SEQ ID NO: 4;
- iv) consist of the amino acid sequence of SEQ ID NO: 4.

20 SPP24 is also known as secreted phosphoprotein 24 or secreted phosphoprotein 2. The full length amino acid sequence of human SPP24 is shown in Figure 1 (SEQ ID NO: 5). In embodiments in which the marker is SPP24 or a fragment thereof, the SPP24 or fragment thereof may:

- 25 i) comprise an amino acid sequence of SEQ ID NO: 5 or fragment thereof;
- ii) comprise an amino acid sequence VSAQQVQGVHAR (SEQ ID NO: 6);

- iii) consist essentially of the amino acid sequence of SEQ ID NO: 6;
- iv) consist of the amino acid sequence of SEQ ID NO: 6;
- 5 v) comprise an amino acid sequence VNSQSLSPYLFR (SEQ ID NO: 7);
- vi) consist essentially of the amino acid sequence of SEQ ID NO: 7; or
- vii) consist of the amino acid sequence of SEQ ID NO: 7.

10 Serglycin is also known as proteoglycan 1 core protein, secretory granule core protein, Hematopoietic proteoglycan core protein, Platelet proteoglycan core protein or secretory granule proteoglycan core protein. The full length amino acid sequence of human serglycin (SEQ ID NO: 8) is shown in Figure 1. In
15 embodiments in which the marker is Serglycin or a fragment thereof, the Serglycin or fragment thereof may:

- i) comprise an amino acid sequence of SEQ ID NO: 8 or fragment thereof;
- ii) comprise an amino acid sequence NLPDSQDLGQHGLEED
20 (SEQ ID NO: 9);
- iii) comprise an amino acid sequence GPMFELLPGESNK (SEQ ID NO: 12);
- iv) consist essentially of the amino acid sequence of SEQ ID NO: 9;
- 25 v) consist of the amino acid sequence of SEQ ID NO: 9;
- vi) consist essentially of an amino acid sequence GPMFELLPGESNK (SEQ ID NO: 12);
- vii)** consist of the amino acid sequence of SEQ ID NO: 12.

AMBP or its cleavage products are also known as Alpha-1 microglycoprotein, alpha-1 microglobulin, inter-alpha-trypsin light chain, bikunin, EDC-1, HI-30, uronic acid-rich protein, trypstatin. The full length amino acid sequence of AMBP is shown
5 in Figure 1 (SEQ ID NO: 10). In embodiments in which the marker is AMBP or a fragment thereof, the AMBP or fragment thereof may:

- (i) comprise an amino acid sequence of SEQ ID NO: 10 or fragment thereof;
- (ii) comprise an amino acid sequence HHGPTITAK (SEQ ID NO:
10 11);
- (iii) consist essentially of the amino acid sequence of SEQ ID NO: 11; or
- (iv) consist of the amino acid sequence of SEQ ID NO: 11.

As used herein, a "subject" is a mammal. The mammal can
15 be a human, non-human primate, sheep, mouse, rat, dog, cat, horse, or any other mammals which can suffer from IBD. Typically, the subject is a human.

In one form, the invention relates to a method of diagnosing IBD in a subject. As used herein, "diagnosing IBD in
20 a subject" refers to determining whether a subject is suffering from IBD. A subject suffering from IBD may be a subject suffering from active IBD, or a subject suffering from IBD in remission. A subject suffering from active IBD is a subject which is showing the symptoms of IBD. A subject suffering from
25 IBD in remission is a subject who has suffered from the symptoms of IBD but which is not at the time of testing showing symptoms of IBD. A subject suffering from IBD in remission is suffering from quiescent IBD, and is not cured of the disease. In one embodiment, a subject suffering from IBD is a subject suffering
30 from active IBD. In one embodiment, a subject suffering from IBD is a subject suffering from IBD in remission.

A subject suffering from Crohn's disease may be a subject suffering active Crohn's disease, or from Crohn's disease in remission. A subject suffering from Crohn's disease in remission is a subject who has suffered from the symptoms of Crohn's disease but which is not at the time of testing showing symptoms of Crohn's disease. A subject suffering from Crohn's disease in remission is suffering from quiescent Crohn's disease, and is not cured of the disease. In one embodiment, a subject suffering from Crohn's disease is a subject suffering from active Crohn's disease. In one embodiment, a subject suffering from Crohn's disease is a subject suffering from Crohn's disease in remission.

A subject suffering from Ulcerative colitis may be a subject suffering active Ulcerative colitis, or from Ulcerative colitis in remission. A subject suffering from Ulcerative colitis in remission is a subject who has suffered from the symptoms of Ulcerative colitis but which is not at the time of testing showing symptoms of Ulcerative colitis. A subject suffering from Ulcerative colitis in remission is suffering from quiescent Ulcerative colitis, and is not cured of the disease. In one embodiment, a subject suffering from Ulcerative colitis is a subject suffering from active Ulcerative colitis. In one embodiment, a subject suffering from Ulcerative colitis is a subject suffering from Ulcerative colitis in remission.

The inventors have found that secretogranin-1, Guanylin, SPP24, AMBP and serglycin, or peptides from these proteins, are markers of IBD, CD and/or UC in a subject. The markers are diagnostic of IBD, CD and/or UC. As described herein, the inventors have found that the level of peptides VSAQQVQGVHAR (SEQ ID NO: 6) from SPP24 and VNSQSLSPYLFR (SEQ ID NO: 7) from SPP24, are elevated, and that levels of the peptide NLPDSQDLGQHGLED (SEQ ID NO: 9) from Serglycin, is reduced, following mass spectrometry (MS) analysis of enzyme digested serum samples from subjects suffering from IBD compared to the levels of the same peptides in serum samples of subjects not

suffering from IBD. Thus, the inventors have reasoned that by determining the level of one or more of serglycin or a fragment thereof and/or SPP24 or a fragment thereof relative to levels in, for example, healthy control subjects, it can be determined whether a subject is suffering from IBD. As used herein, a "subject not suffering from IBD" is a subject who does not, and has not, suffered from IBD. Typically, a subject not suffering from IBD is a healthy subject. As used herein, a "healthy subject" is a subject not suffering from IBD or any other detectable inflammation.

In another form, the invention relates to assessing an IBD in a subject. As used herein, "assessing an IBD in a subject" refers to determining the severity of an IBD, or the type of IBD, in a subject. The type of IBD refers to whether the IBD is Crohn's disease or Ulcerative colitis. In one embodiment, the IBD is assessed in a subject known to be suffering from IBD. The inventors have found that the level of a secretogranin-1, and/or guanylin or a fragment thereof, in tissue or body fluid of a subject can be used to distinguish between Crohn's Disease and Ulcerative colitis. As described herein, the inventors have found that subjects suffering from Ulcerative colitis have increased levels of the peptides ADQTVLTEDEK (SEQ ID NO: 2) from secretogranin-1, and VTVQDGNFSFSLESVK (SEQ ID NO: 4) from guanylin, relative to the level of these peptides in subjects suffering from Crohn's disease following MS analysis of enzyme digested serum samples. Accordingly, the inventors reason that increased levels of secretogranin-1 or a fragment thereof and/or guanylin or a fragment thereof, in a subject relative to the level of the peptide in a subject suffering from Crohn's disease is indicative of Ulcerative colitis.

As described herein, the inventors have also found that the levels of VTVQDGNFSFSLESVK (SEQ ID NO: 4) from guanylin are elevated in subjects suffering from Ulcerative colitis relative to the level of these peptides in subjects not suffering from IBD following MS analysis of enzyme digested serum samples.

Accordingly, the inventors reason that increased levels of guanylin or a fragment thereof in a subject relative to the level of the peptide in a subject not suffering from IBD is indicative of Ulcerative colitis.

5 Moreover, as described herein, the inventors have found that increased levels of ADQTVLTEDEK (SEQ ID NO: 2) from secretogranin-1 are indicative of increased severity of Uclerative colitis and Crohn's disease. The present invention therefore allows a determination of the prognosis of IBD in
10 subjects suffering from IBD.

 In one form, the invention relates to assessing whether a subject is not suffering from IBD (e.g. healthy), or whether a subject is suffering from IBD in remission. A subject suffering from IBD in remission is a subject who has previously suffered
15 from symptomatic IBD but is not showing symptoms of IBD at the time of testing. As described herein, the inventors have found that MS analysis of enzyme digested serum samples from subjects suffering from Crohn's Disease or Ulcerative colitis in remission have elevated levels of VSAQQVQGVHAR (SEQ ID NO: 6)
20 from SPP24 and VNSQSLSPYLFR (SEQ ID NO: 7) from SPP24 relative to healthy subjects, and subjects suffering from Ulcerative colitis in remission have elevated levels of VSAQQVQGVHAR (SEQ ID NO: 6) from SPP24, VNSQSLSPYLFR (SEQ ID NO: 7) from SPP24 and HHGPTITAK (SEQ ID NO: 11) from AMBP relative to healthy
25 subjects. Accordingly, elevated levels of SPP24 or a fragment thereof in a subject showing no or few symptoms of IBD compared to levels of SPP24 or a fragment thereof in a healthy subject, are indicative that a subject is suffering from Ulcerative colitis or Crohn's disease in remission, and elevated levels of
30 AMBP or a fragment thereof in a subject showing no or few symptoms of IBD relative to a healthy subject is indicative that the subject is suffering from Ulcerative colitis in remission.

 In one embodiment, the one or more markers is selected from the group consisting of secretogranin-1 or a fragment

thereof, Guanylin or a fragment thereof and SPP24 or a fragment thereof.

In one embodiment, the one or more markers is secretogranin-1 or a fragment thereof. In one embodiment, the one or more markers is Guanylin or a fragment thereof. In one
5 embodiment, the one or more markers is a fragment of SPP24 comprising SEQ ID NO: 6. In one embodiment, the one or more markers is a fragment of SPP24 comprising SEQ ID NO: 7. In one embodiment, one or more markers is AMBP or a fragment thereof.
10 In one embodiment, the one or more markers is serglycin or a fragment thereof.

In various embodiments, the one or more markers used to diagnose IBD is:

- (a) Secretogranin-1 or a fragment thereof;
- 15 (b) Secretogranin-1 or a fragment thereof, Guanylin or a fragment thereof, and SPP24 or a fragment thereof;
- (c) Secretogranin-1 or a fragment thereof, and Guanylin or a fragment thereof;
- (d) Secretogranin-1 or a fragment thereof, and serglycin
20 or a fragment thereof;
- (e) Secretogranin-1 or a fragment thereof, and SPP24 or a fragment thereof;
- (f) Guanylin or a fragment thereof;
- (g) Guanylin or a fragment thereof, and SPP24 or a
25 fragment thereof;
- (h) Guanylin or a fragment thereof, and serglycin or a fragment thereof;
- (i) Guanylin or a fragment thereof, and SPP24 or a
30 fragment thereof, and serglycin or a fragment thereof;

- (j) SPP24 or a fragment thereof, and serglycin or a fragment thereof;
- (k) Secretogranin-1 or a fragment thereof, Guanylin or a fragment thereof, and serglycin or a fragment thereof;
- (l) Secretogranin-1 or a fragment thereof, SPP24 or a fragment thereof, and serglycin or a fragment thereof;
- (m) Secretogranin-1 or a fragment thereof, Guanylin or a fragment thereof, SPP24 or a fragment thereof, and serglycin or a fragment thereof;
- (n) SPP24 or a fragment thereof;
- (o) Secretogranin-1 or a fragment thereof, and SPP24 or a fragment thereof.

In one embodiment, the SPP24 or a fragment thereof is a fragment of SPP24 comprising SEQ ID NO: 6. In one embodiment, the SPP 24 or a fragment thereof is a fragment of SPP 24 comprising SEQ ID NO: 7.

In one embodiment, the Serglycin or a fragment thereof is a fragment of Serglycin comprising SEQ ID NO: 9. In another embodiment, the Serglycin or a fragment thereof is a fragment of serglycin comprising SEQ ID NO: 12.

In one embodiment, comparing the level of the one or more markers comprises determining the level of the one or more markers.

The level of the one or more markers of a subject may be determined by any known methods for determining the level of a protein in a tissue or body fluid. The method may be a direct method, in which the level of protein or fragment thereof is determined directly, or may be determined indirectly. Examples of direct methods include immunoassay and mass spectrometry.

Examples of indirect methods include determining the level of expression of mRNA for a protein or peptide.

In one embodiment, the level of the one or more markers may be determined by obtaining a sample of the tissue or body fluid from the subject. The sample may be, for example, blood, serum, plasma, faeces, tissue, urine, tears, saliva, cells, organs, bone marrow, cerebrospinal fluid, sweat, bile, pancreatic juice, etc.

In one embodiment, the sample is a body fluid. The body fluid may be blood, serum, plasma, urine, feces, saliva, gastric juice, tears, sweat, bile, pancreatic juice. Typically, the body fluid is serum. As described herein, the inventors have found that the level of a peptide or protein comprising the amino acid sequence set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and/or 12 in a serum sample from a subject can be used to diagnose whether a subject is suffering from IBD or to assess the severity or type of IBD. Thus, peptides or proteins comprising the amino acid sequence set forth in SEQ ID Nos: 1 to 12 are serum markers of IBD or the severity or type of IBD. The ability to use a serum sample provides a relatively convenient and rapid means by which to assess or diagnose IBD in a subject. As mentioned above, prior to the present invention, no markers were reliable for assessing or diagnosing IBD. The markers may be used individually, or a combination of the markers may be used, to diagnose or assess IBD.

In another embodiment, the sample is a tissue. The tissue may be any sample from the gastrointestinal tract, including rectum, colon, small intestinal tract. The sample may be all layers from the gastrointestinal tract, or may be the mucosal layer, or the epithelial layer.

Methods for obtaining tissue and body fluid samples from subjects are known in the art.

The sample may be processed to enhance detectability of the markers. For example, the sample may be fractionated to enrich for markers of a particular size range. In this regard, a sample may be fractionated to enrich for peptides or proteins of a particular size range. Methods for fractionation of peptides and proteins in a sample are known in the art and are described in, for example, Ly and Wasinger (2008) *Proteomics* 8(20): pp 4197-4208; Echan et al. (2005) *Proteomics* 5: pp 3292-3303; Omenn (2005) *Proteomics* 5: pp 3226-3245. Examples of methods for size fractionation of peptides or proteins include size exclusion chromatography, ion exchange chromatography, affinity chromatography, gel electrophoresis. The sample may be processed to enrich for nucleic acid such RNA, more typically mRNA. Methods for enrichment of RNA, including mRNA, are known in the art and are described in Simpson R. J., ed. *Proteins and Proteomics: a Lab Manual*. 2003 Cold Spring Harbor Laboratory Press 926; Sambrook, J., Russet D.W., ed. *Molecular Cloning: A Laboratory Manual Volume 1, 2, 3*. 2001. Cold Spring Harbor Laboratory Press.

Once a sample has been obtained from the subject, the level of the one or more markers in the sample is compared with the reference value. The term "level" refers to an indication of abundance. Thus, the "level of one or more markers" refers to an indication of the abundance of one or more markers. The level of one or more markers may be a measure of the one or more markers, such as a measure of the amount of the one or more markers per unit weight or volume. The level of one or more markers may be a ratio, such as a ratio of the amount of one or more markers in a sample relative to the amount of the one or more markers of a reference value or in a control subject.

In one embodiment, the level of the one or more markers in a sample is the concentration of the one or more markers in the tissue or body fluid. The concentration of the one or more markers may be measured in any manner that is suitable for measuring concentrations of the marker in body fluids or tissue.

For example, the level of the one or more markers may be determined using mass spectrometry or immunoassay.

In one embodiment, the level of the one or more markers in a sample may be determined using mass spectrometry. Examples of suitable mass spectrometry include: ionisation sources such as EI, CI, MALDI, ESI, and analysis such as Quad, ion trap, TOF, FT or combinations thereof, spectrometry, isotope ratio mass spectrometry (IRMS), thermal ionisation mass spectrometry (TIMS), spark source mass spectrometry, Multiple Reaction Monitoring (MRM) or SRM. The mass spectrometry may be conducted in combination with 2D gel electrophoresis, high performance liquid chromatography (HPLC) or other prefractionation or enrichment techniques. Methods for quantitation of molecules by two-dimensional gel electrophoresis, HPLC and mass spectrometry such as MALDI and SELDI are known in the art and are described in, for example, Simpson R. J., ed. Proteins and Proteomics: a Lab Manual. 2003 Cold Spring Harbor Laboratory Press; Sanchez, J. C. et al. Biomedical Applications of Proteomics 2004, Wiley-Blackwell. 425. Methods such as prefractionation are known and described in Ly and Wasinger (2008) Proteomics, 8(20): pp 4197-4208. Methods such as MRM are known in the art and described in, for example, Anderson and Hunter (2006) MCP, 5(4): pp.573-589.

In one embodiment, the level of the one or more markers in a sample may be determined using MRM with a reverse-polynomial dilution (RPD) calibration or a stable-isotope dilution (SID) calibration. In one embodiment, the level of the one or more biomarkers in a sample is determined using RPD when MRM is used.

The level of the one or more markers may be determined using immunoassays. An immunoassay is an assay that uses an antibody to specifically bind to an antigen (e.g. the marker). The antibody may be a polyclonal, monoclonal, Fab, F(ab)₂, scFv, diabody, scFab etc. Immunoassays using antibodies include immunoblots, western blots, Enzyme linked Immunosorbant Assay

(ELISA), Enzyme immunoassay (EIA), radioimmune assay.

Immunoassay methods for detection and determination of levels of an antigen are known in the art and are described in, for example, Antibodies: A Laboratory Manual (1988); Monoclonal

5 Antibodies: Principles and Practice (2nd Edition, 1986); Methods in Cell Biology: Antibodies in Cell Biology, volume 37 (Asai, ed. 1993); Basic Clinical Immunology (Stits & Terr, eds., 7th ed. 1991). Generally, a sample obtained from a subject can be contacted with the antibody that specifically binds the marker.

10 The antibody may be immobilised on a solid support such as a stick, plate, bead, microbead or array. A sample such as serum, blood, plasma, urine, or saliva is incubated with the antibodies for a period of time sufficient for the antibodies to bind the markers if present, and the mixture washed to remove unbound

15 material. Sample bound to the antibody can then be determined by incubating the mixture with a detection agent such as, for example, a second antibody labelled with a detectable agent such as a fluorescent dye, radiolabels, enzymes (e.g. horseradish peroxidase, alkaline phosphatase, etc.), colloidal gold, etc.

20 Alternatively, the marker in the sample can be detected using an indirect assay in which, for example, a second, labelled antibody is used to detect bound specific antibody, or in a competition or inhibition assay in which, for example, binding of the marker to a labelled specific antibody inhibits binding

25 of the specific antibody to a detection site.

The level of the one or more markers in a sample may be determined indirectly by determining the expression of mRNA for the marker in a tissue sample. In this regard, there is typically a correlation between mRNA levels and protein

30 expression. Accordingly, elevated or reduced levels of mRNA relative to a control is likely to reflect elevated or reduced levels of the protein encoded by that mRNA. The inventors therefore envisage that in some embodiments, the level of mRNA of one or more markers in tissue of a subject relative a

35 reference value can be used to diagnose or assess IBD in a

subject. Methods for assessing the levels of mRNA in a tissue sample include northern blot analysis, RT-PCR, real-time RT-PCR, array analysis. Such methods are known in the art and described in, for example, Sambrook, J., Russet D.W., ed. Molecular Cloning: A Laboratory Manual Volume 1, 2, 3. 2001. Cold Spring Harbor Laboratory Press; Rio, D. C. et al. RNA: A Lab Manual 2011, Cold Spring Harbor Laboratory Press.

The level of the one or more markers in the sample from the subject is compared with a reference value. The "reference value for the one or more markers" is a value which is indicative of the level of the one or more markers in a subject of predetermined disease status. The predetermined disease status may be, for example, not suffering from IBD (e.g. healthy), suffering from active IBD, suffering from IBD of predetermined severity (e.g. mild, moderate, severe, in remission), or suffering from IBD of a predetermined type, such as active Crohn's disease, Crohn's disease in remission, active Ulcerative colitis, Ulcerative colitis in remission.

The reference values may be a predetermined standard value. For example, the reference value may be a predetermined standard value, or a range of predetermined standard values, which represent no illness, or a predetermined type or severity of illness.

The reference value may be the level of the one or more markers in a reference sample from a subject, or a pool of subjects, not suffering from IBD or suffering from IBD or of a predetermined severity or type. As used herein, the "subject suffering from IBD of predetermined severity" is a subject suffering from IBD, the severity of which is known. For example, a subject may be suffering from IBD which is known to be mild, moderate, severe, or in remission. In some forms, the predetermined severity may be active. As used herein, "active" refers to disease which is not in remission, and may be mild, moderate or severe in severity. In some embodiments, the

disease severity is as determined by a disease activity index (DAI) for CD or UC such as, for example, the Harvey-Bradshaw Index for CD, or the Ulcerative Colitis Disease Activity Index for UC. As used herein, a "subject suffering from IBD of a
5 predetermined type" is a subject suffering from IBD in which the IBD is known to be either Crohn's Disease or Ulcerative colitis.

In one embodiment, the reference value is the level of the one or more markers in the tissue or body fluid of a subject, or subjects, having a predetermined disease status.

10 In one embodiment, the level of the one or more markers in a reference sample is the concentration of the one or more markers in the reference sample.

A reference sample may be from a subject not suffering from IBD. By comparing the level of the one or more markers in
15 a tissue or body fluid of a subject with a level of the one or more markers from a reference sample obtained from a single subject, or a plurality of subjects, not suffering from IBD, it is possible to diagnose whether the subject is suffering from IBD, or the severity or type of IBD.

20 The reference sample may be from a subject suffering from IBD of a predetermined severity. In this regard, by comparing, for example, the level of SPP24 or a fragment thereof in a sample obtained from a subject suffering from IBD with a level of the one or more markers from a reference sample obtained from
25 a subject suffering from a mild or less severe form of IBD, it is possible to determine the severity of the disease or whether the disease has progressed to a more severe form.

In various embodiments:

(a) The one or more markers is guanylin or a fragment thereof,
30 wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Ulcerative

colitis is diagnosed when the level of the one or more markers is elevated relative to the reference value.

- (b) The one or more markers is SPP24 or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein IBD is diagnosed when the level of the one or more markers is elevated relative to the reference value.
- (c) The one or more markers is serglycin or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein IBD is diagnosed when the level of the one or more markers is reduced relative to the reference value.
- (d) The one or more markers is guanylin or a fragment thereof and SPP24 or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Ulcerative colitis is diagnosed when the level of the one or more markers is elevated relative to the reference value.
- (e) The one or more markers is SPP24 or a fragment thereof wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Ulcerative colitis is diagnosed when the level of the one or more markers is elevated relative to the reference value.
- (f) The one or more markers is guanylin or a fragment thereof, and SPP24 or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Crohn's disease is diagnosed when the level of SPP 24 or a fragment thereof

is elevated relative to the reference value, and the level of Guanylin or a fragment thereof is not elevated relative to the reference value.

- 5 (g) The one or more markers is guanylin or a fragment thereof, and serglycin or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Crohn's disease is diagnosed when the level of guanylin or a fragment thereof is not elevated relative to the reference value, and the level of serglycin or a fragment thereof is reduced relative to the reference value.
- 10
- (h) The one or more markers is SPP24 or a fragment thereof, and serglycin or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein IBD is diagnosed when the level of SPP24 or a fragment thereof is elevated relative to the reference value, and the level of serglycin or a fragment thereof is reduced relative to the reference value.
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- 20
- (i) The one or more markers is guanylin or a fragment thereof, SPP 24 or a fragment thereof, and serglycin or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Crohn's disease is diagnosed when the level of guanylin or a fragment thereof is not elevated relative to the reference value, the level of serglycin or a fragment thereof is reduced relative to the reference value, and the level of SPP24 or a fragment thereof is elevated relative to the reference value.
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- 30
- (j) The one or more markers is guanylin or a fragment thereof and SPP 24 or a fragment thereof, wherein the reference

value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Crohn's disease is diagnosed when the level of guanylin or a fragment thereof is not elevated relative to the reference value, and the level of SPP24 or a fragment thereof is elevated relative to the reference value.

(k) The one or more markers is AMBP or a fragment thereof, wherein the reference value is the level of the one or more markers of a subject or subjects not suffering from IBD, and wherein Ulcerative colitis is diagnosed when the level of the one or more markers is elevated relative to the reference value.

(l) The one or more markers is SPP 24 or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease of predetermined severity, and wherein Crohn's disease is diagnosed as more severe than the Crohn's disease of predetermined severity when the level of the one or more markers is elevated relative to the reference value.

(m) The one or more markers is AMBP or a fragment thereof, wherein the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from IBD of predetermined severity, and wherein IBD is diagnosed as more severe than the IBD of predetermined severity when the level of the one or more markers is elevated relative to the reference value.

(n) The one or more markers is secretogranin-1 or a fragment thereof, and the reference value is the level of secretogranin-1 or a fragment thereof in the tissue or body fluid of a subject or subjects suffering from ulcerative colitis, and wherein Crohn's Disease is diagnosed in a subject suffering from IBD when the level

of secretogranin-1 or a fragment thereof is reduced relative to the reference value.

- (o) The one or more markers is secretogranin-1 or a fragment thereof, and the reference value is the level of secretogranin-1 or a fragment thereof in the tissue or body fluid of a subject or subjects suffering from Crohn's Disease, and wherein ulcerative colitis is diagnosed when the level of secretogranin-1 or a fragment thereof is elevated relative to the reference value.
- (p) The one or more markers is SPP 24 or a fragment thereof, and the reference value is the level of SPP 24 or a fragment thereof in the tissue or body fluid of a subject or subjects suffering from Ulcerative colitis of predetermined severity, and wherein Ulcerative colitis is diagnosed as more severe than the Ulcerative colitis of predetermined severity when the level of SPP 24 or fragment thereof is reduced relative to the reference value.
- (q) The one or more markers is guanylin or a fragment thereof, and the reference value is the level of guanylin or a fragment thereof in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed when the level of guanylin is elevated relative to the reference value.
- (r) The one or more markers is serglycin or a fragment thereof, and the reference value is the level of serglycin or a fragment thereof in the tissue or body fluid of a subject or subjects suffering from Crohn's disease of predetermined severity, and wherein Crohn's disease of the subject is diagnosed as more severe than the Crohn's disease of predetermined severity when the level of serglycin is reduced relative to the reference value.

- (s) The one or more markers is secretogranin-1 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein
5 Ulcerative colitis is diagnosed when the level of secretogranin-1 or a fragment thereof is elevated relative to the reference value.
- (t) The one or more markers is AMBP or a fragment thereof, and the reference value is the level of the one or more
10 markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed when the level of AMBP or a fragment thereof is elevated relative to the reference value.
- (u) The one or more markers is SPP 24 or a fragment thereof, and the reference value is the level of the one or more
15 markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease of predetermined severity, and wherein Crohn's disease is diagnosed as
20 more severe when the level of SPP 24 of a fragment thereof is elevated relative to the reference value.
- (v) The one or more markers is SPP 24 or a fragment thereof, and the reference value is the level of the one or more
25 markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Crohn's disease or Ulcerative colitis is diagnosed when the level of SPP 24 or a fragment thereof is elevated relative to the reference value.
- (w) The one or more markers is Serglycin or a fragment
30 thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease in remission, and wherein Crohn's disease is diagnosed when the level of

Serglycin or a fragment thereof is reduced relative to the reference value.

- 5 (x) The one or more markers is AMBP or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects in remission from suffering from Crohn's disease or having mild Crohn's disease, and wherein severe Crohn's disease is diagnosed when the level of AMBP or a fragment thereof is elevated relative to the reference value.
- 10 (y) The one or more markers is guanylin or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed when the level of Guanylin or a fragment thereof is elevated relative to the reference value.
- 15 (z) The one or more markers is SPP 24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Ulcerative colitis or Crohn's disease in remission is diagnosed when the subject is showing no or few symptoms of UC or CD and the level of SPP 24 or a fragment thereof is elevated relative to the reference value.
- 20 (aa) The one or more markers is Secretogranin-1 or a fragment thereof, AMBP or a fragment thereof, and SPP 24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed in a subject suffering from IBD when the level of the one or more markers is elevated relative to the reference value.
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- (bb) The one or more markers is AMBP or a fragment thereof, and SPP 24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed in a subject suffering from IBD when the level of the one or more markers is elevated relative to the reference value.
- (cc) The one or more markers is Secretogranin-1 or a fragment thereof, and AMBP or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed in a subject suffering from IBD when the level of the one or more markers is elevated relative to the reference value.
- (dd) The one or more markers is Secretogranin-1 or a fragment thereof, and SPP 24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed in a subject suffering from IBD when the level of secretogranin-1 or a fragment thereof is elevated relative to the reference value.
- (ee) The one or more markers is Secretogranin-1 or a fragment thereof, AMBP or a fragment thereof, and SPP 24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Ulcerative colitis, and wherein Crohn's disease is diagnosed in a subject suffering from IBD when the level of secretogranin-1 or a fragment thereof and AMBP or a fragment thereof is reduced relative to the reference value.
- (ff) The one or more markers is AMBP or a fragment thereof, and SPP 24 or a fragment thereof, and the reference value is

the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Ulcerative colitis, and wherein Crohn's disease is diagnosed in a subject suffering from IBD when the level of AMBP or a fragment thereof is reduced relative to the reference value.

(gg) The one or more markers is Secretogranin-1 or a fragment thereof, and AMBP or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Ulcerative colitis, and wherein Crohn's disease is diagnosed in a subject suffering from IBD when the level of the one or more markers is reduced relative to the reference value.

(hh) The one or more markers is Secretogranin-1 or a fragment thereof, and SPP 24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Ulcerative colitis, and wherein Crohn's disease is diagnosed in a subject suffering from IBD when the level of secretogranin-1 or a fragment thereof is reduced relative to the reference value.

(ii) The one or more markers is SPP 24 or a fragment thereof and AMBP or a fragment thereof and guanylin or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, and wherein ulcerative colitis is diagnosed in a subject suffering from IBD when the level of the one or more markers is elevated relative to the reference value.

(jj) The one or more markers is a fragment of AMBP or a fragment thereof and guanylin or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or

5 subjects suffering from Crohn's disease , or suffering from mild Crohn's disease, and wherein Crohn's disease is diagnosed in a subject suffering from IBD as severe when the level of the one or more markers is elevated relative to the reference value.

10 (kk) The one or more markers is serglycin or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's disease in remission, and wherein Crohn's disease is diagnosed in a subject suffering from IBD as active when the level of the one or more markers is reduced relative to the reference value.

15 (ll) The one or more markers is guanylin or a fragment thereof, and SPP24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Ucerative colitis is diagnosed when the level of the one or more markers is elevated relative to the reference value.

20 (mm) The one or more markers is SPP 24 or a fragment thereof, and the reference value is the level of SPP24 or a fragment thereof in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Ulcerative colitis or Crohn's disease in remission is diagnosed in a subject showing no or few symptoms of IBD when the level of SPP 24 or a fragment thereof is elevated relative to the reference value. The one or more markers is AMBP or a fragment thereof, and the reference value is the level of AMBP in the tissue or body fluid of a subject or subjects not suffering from IBD, and wherein Ulcerative Colitis in remission is diagnosed in a subject showing no or few symptoms of IBD when the level of AMBP is elevated relative to the reference value.

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- (nn) The one or more markers is guanylin or a fragment thereof, and the reference value is the level of guanylin or a fragment thereof in the tissue or body fluid of a subject or subjects suffering from Crohn's disease, wherein
5 Ulcerative colitis is diagnosed in a subject suffering from IBD when the level of guanylin or a fragment thereof is elevated relative to the reference value.
- (oo) The one or more markers is guanylin or a fragment thereof, and the reference value is the level of guanylin or a
10 fragment thereof in the tissue or body fluid of a subject or subjects not suffering from IBD, wherein Ulcerative colitis is diagnosed when the level of guanylin or a fragment thereof is elevated relative to the reference value.
- 15 (pp) The one or more markers is SPP24 or a fragment thereof and secretogranin-1 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject or subjects suffering from Crohn's Disease of a predetermined severity, wherein
20 Crohn's Disease of greater severity than the predetermined severity is diagnosed in a subject suffering from Crohn's Disease when the level of the one or more markers is elevated relative to the reference value.
- 25 (qq) The one or more markers is SPP24 or a fragment thereof and/or secretogranin-1 or a fragment thereof, and the reference value is the level of one or more markers in the tissue or body fluid of a subject or subjects suffering from active Ulcerative Colitis, wherein Ulcerative Colitis in remission is diagnosed in a subject showing no or few
30 symptoms of Ulcerative Colitis when the level of SPP24 or a fragment thereof is elevated relative to the reference value, and/or the level of secretogranin-1 or a fragment thereof is reduced relative to the reference value.

(rr) The one or more markers is SPP24 or a fragment thereof, and the reference value is the level of SPP24 or a fragment thereof in the tissue or body fluid of a subject or subjects not suffering from IBD, wherein IBD in remission is diagnosed in a subject showing no or few symptoms of IBD when the level of SPP24 or a fragment thereof is elevated relative to the reference value.

(ss) The one or more markers is secretogranin-1 or a fragment thereof, and the reference value is the level of secretogranin-1 or a fragment thereof in the tissue or body fluid of a subject or subjects suffering from IBD of predetermined severity, wherein IBD more severe than the predetermined severity is diagnosed in a subject when the level of secretogranin-1 or a fragment thereof is increased relative to the reference value.

(tt) The one or more markers is SPP 24 or a fragment thereof and Guanylin or a fragment thereof, a first reference value is the level of SPP24 or a fragment thereof in the tissue or body fluid of a subject not suffering from IBD, and a second reference value is the level of Guanylin of a fragment thereof in a subject or subjects suffering from CD, wherein UC in remission is diagnosed in a subject showing no or few symptoms of IBD when the level of SPP24 or a fragment thereof is elevated relative to the first reference value, and the level of guanylin or a fragment thereof is elevated relative to the second reference value.

In one form, Guanylin or a fragment thereof is the peptide VTVQDGNFSFSLESVK (SEQ ID NO: 4).

In one form, Secretogranin-1 or a fragment thereof is the peptide ADQTVLTEDK (SEQ ID NO: 2).

In one form, a fragment of SPP 24 comprising SEQ ID NO: 6 is the peptide VSAQQVQGVHAR (SEQ ID NO: 6).

In one form, Serglycine of a fragment thereof is the peptide NLPSDSQDLGQHGLEED (SEQ ID NO: 9) or the peptide GPMFELLPGESNK (SEQ ID NO: 12).

In one form, AMBP or a fragment thereof is the peptide
5 HHGPTITAK (SEQ ID NO: 11).

In one form, a fragment of SPP 24 comprising SEQ ID NO: 7 is the peptide VNSQSLSPYLFR (SEQ ID NO: 7).

The methods described herein may be used independently of other diagnostic tests, or may be used in combination with other
10 diagnostic tests.

As used herein, the term "elevated" means more than or greater than. Typically, a level "elevated relative to the reference value" is a level that is statistically significantly more than or greater than the reference level. A level may be
15 elevated relative to a reference value by any amount that is statistically significant more than the reference value. Typically, levels greater than 1.2 fold are significant. In various embodiments, the level may be elevated by about 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0 fold or greater.

20 As used herein, the term "reduced" means less than or lower than. Typically, a level "reduced relative to the reference value" is a level that is statistically significantly less than or lower than the reference level. A level may be reduced relative to a reference value by any amount that is
25 statistically significant less than the reference value. Typically, levels that are reduced by than 1.2 fold or more are significant. In various embodiments, the level may be reduced by about 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0 fold or greater.

30 As used herein, the term "not elevated" means not statistically significantly more than or greater than. A level that is "not elevated relative to the reference value" may be a level that is not statistically significantly more than the

reference value, or is less than the reference value.

Statistical significance may be determined by any methods known in the art, such as, for example, a student t test.

In one embodiment, the method of diagnosing or assessing
5 comprises the further step of treating the subject for IBD, UC
or CD if the subject is diagnosed or assessed as suffering from
IBD, UC or CD. Methods for the treatment of IBD, CD and UC are
known in the art and are described in, for example, D.C.
Baumgart, Sandborn, W.J., Inflammatory bowel disease: clinical
10 aspects and established and evolving therapies, The Lancet 369
(2007) 1641-1657. Treatments for UC and CD include
administration of anti-inflammatory agents such as 5-
aminosalicylic acid, mesalazine, sulfasalazine, mesalamine,
olsalazine, balsalazide; corticosteroids; immune modifiers such
15 as thiopurines such as 6-mercaptopurine, azathioprine,
calineurin inhibitors such as cyclosporine A, tacrolimus;
methotrexate, anti-TNF agents such as infliximab, adalimumab,
certolizumab, as well as dietary management.

The invention further relates to a diagnostic peptide
20 selected from the group consisting of: ADQTVLTEDK (SEQ ID NO:
2); VTVQDGNFSFSLESVK (SEQ ID NO: 4); VSAQQVQGVHAR (SEQ ID NO:
6); VNSQSLSPYLFR (SEQ ID NO: 7); NLPSDSQDLGQHGLEED (SEQ ID NO:
9); HHGPTITAK (SEQ ID NO: 11). In one embodiment, the diagnostic
peptides are isotopically labelled. The peptides may be
25 labelled with any isotope suitable for use in mass spectrometry.
The isotope may be, for example, C¹³ or C¹³ and N¹⁵.

The invention further relates to a composition comprising
one or more peptides selected from the group consisting of:
secretogranin-1 or a fragment thereof, such as ADQTVLTEDK (SEQ
30 ID NO: 2); guanylin or a fragment thereof, such as
VTVQDGNFSFSLESVK (SEQ ID NO: 4); SPP 24 or a fragment thereof,
such as VSAQQVQGVHAR (SEQ ID NO: 6) or VNSQSLSPYLFR (SEQ ID NO:
7); Serglycin or a fragment thereof, such as NLPSDSQDLGQHGLEED
(SEQ ID NO: 9); AMBP or a fragment thereof, such as HHGPTITAK

(SEQ ID NO: 11);. In one embodiment, the diagnostic peptides are isotopically labelled. The peptides may be labelled with any isotope suitable for use in mass spectrometry. The isotope may be, for example, C^{13} or C^{13} and N^{15} .

5 The invention further relates to kits for diagnosing or assessing IBD in a subject. The kits can be used to carry out the method of the invention. In one form, the kit comprise antibodies which specifically bind to the one or more markers. In another form, the kit comprises nucleic acid such as primers
10 for amplifying the one or more markers using, for example, RT-PCR, or probes, for detection of mRNA. In another form, the kits comprise peptides from the one or more markers for use in mass spectrometry.

 The kits may comprise a control sample such as a sample
15 from a subject or subjects not suffering from IBD, or suffering from IBD of a predetermined severity or type. Kits comprising antibodies may comprise a solid support on which the antibody is immobilised, and a second antibody conjugated to a detectable group. Kits comprising nucleic acids may comprise fluorescent
20 labels for detection of hybridisation. The kits may comprise solid supports such as arrays or beads.

 Typically, the kits comprise instructions for use.

 In the claims which follow and in the preceding description of the invention, except where the context requires
25 otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, ie. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the
30 invention. It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a peptide" includes a plurality of such peptides, and so forth. Unless defined

otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

5 The invention will now be further described by way of reference only to the following non-limiting examples. It should be understood, however, that the examples following are illustrative only, and should not be taken in any way as a restriction on the generality of the invention described above.

10

Examples

Example 1: Discovery phase, Identification of diagnostic markers for differentiation between Healthy and IBD and UC and CD as well as severity.

15

Sample pooling and preparation prior to MF10 fractionation

Five plasma samples, 30µL each, were pooled into each group defined by their clinical activity with the addition of 15µL of 10% protease inhibitor (*Roche, Basel, Switzerland*) resulting in 11 pools of grouped samples as listed below in Table-1. Prior to the MF10 fractionation, the pooled samples were diluted with 150µL of 180mM Tris/20mM EACA/4M urea buffer, pH 10.2 and centrifuged for 10 minutes to isolate the supernatant from the plasma debris.

25

Table-1. Disease characteristics of pooled group samples. Each pool was an equal mix of 5 patients based on the concentration of total protein.

Table 1

Healthy control group	Clinical Remission group	Mild clinical activity group	Moderate-severe clinical activity group
Control Pool A Control Pool C	UC pool A CD pool A CD pool C	UC pool B UC pool C CD pool B CD pool D	UC pool D CD pool E

30

MF10 partitioning Methods

MicroFlow MF10 (*NuSep, Frenchs Forest, Sydney, Australia*) presents a unique in-solution electrophoresis method to enrich for low mass proteins. Proteins less than 25kDa are an area of the proteome that are not well explored and challenging to detect using 2D-SDS-PAGE or LCMS methods. MF10 allows for the enrichment of this often omitted section of the proteome. MicroFlow MF10 (*NuSep, Frenchs Forest, Sydney, Australia*) partitioning methods were done as per the methods described in Ly and Wasinger (2008) *Proteomics*, 8(20): pp. 4197-4208. Any modifications made to the description are mentioned in brief. For protein separation, we used a 5 chamber cartridge assembly method using 5, 25, 45, 65 and 125kDa polyacrylamide membranes (*NuSep, French Forest, Sydney, Australia*) and 1 kDa regenerated cellulose membrane (*Millipore, Massachusetts, USA*), resulting in the following fractions; F1, F2, F3, F4 and F5. This essentially created 1kDa to 25kDa fractions of enriched plasma proteins. These cartridge assemblies have 2 separate lanes of chambers which allow two different samples to be fractionated simultaneously.

MF10 pooled plasma fractionation

The cartridge assemblies were loaded to the separation unit of MF10 with 100mL of 90mM Tris/10mM EACA/2M urea buffer, pH 10.2 circulating around the electrodes for 10 minutes prior to sample loading. One hundred and forty micro liters of the pooled plasma sample was loaded onto both lanes of the F1 chamber and for the 2x4 remaining chambers, 140µL of 90mM Tris/10mM EACA/2M urea buffer, pH 10.2 was loaded. Fractionation was performed first at 50V for 30mins followed by 250V for 2h30mins at 15°C. After the separation, each fraction from both lanes was collected using gel-loading tips

and pooled together to give 5 distinct mass defined fractions.

Trypsin digestion and sample preparation for LC-MS/MS

5 Fraction 4 and 5 samples were cleaned with C18 stage tips (*Proxeon, Odense, Denmark*), speedvac dried and rehydrated with 50 μ L of 50mM ammonium bicarbonate, pH 8.0. Fraction 1, 2 and 3 samples were acetone precipitated, cold centrifuged and the dry pellets were rehydrated in 200 μ L of 50mM ammonium
10 bicarbonate, pH 8.0 for fraction 1 and 2, and 100 μ L for fraction 3. Subsequently, trypsin was added to all fractions at a ratio of approximately 1:100, trypsin/total protein and incubated overnight at 37°C. Following digestion, 4 μ L of formic acid was added to stop the reaction and the samples
15 were speedvac dried. All fractions were re-suspended in 10 μ L of buffer A (2% acetic acid, 0.1% formic acid) prior to LC-MS/MS. One micro liter of F5, 0.5 μ L of F4, 0.2 μ L of F3 and 0.1 μ L of F1 and F2 each were loaded onto the nano-LC.

20 LC-MS/MS configuration

An LTQ-FT Ultra mass spectrometer (*Thermo Electron, Bremen, Germany*) was used to analyze the plasma fractions. As per Ly and Wasinger (2008), peptides were separated by nano-LC using an Ultimate 3000 HPLC and autosampler system (*Dionex, Amsterdam, Netherlands*).¹⁴ 1800V was applied to low volume tee
25 (*Upchurch Scientific, WA, USA*) and the column tip was positioned ~0.5cm from the heated capillary (T=200°C) of the LTQ-FT. The instrument operated in DDA mode, with positive ions generated by electrospray. A survey scan of m/z 350-1750
30 was acquired in the FT ICR cell. Collision induced dissociation was used by the linear ion trap in which up to seven of the most abundant ions (>2000 counts) with charge states of +2 or +3 were successively isolated and fragmented. Mass to charge ratios selected for MS/MS were dynamically

excluded for 60 seconds. 'Mascot Daemon/extract_msn' (*Matrix Science, London, England*) was used with default parameters to generate peak lists, which were submitted to the database search program Mascot. MS was then repeated depending on the technical variations observed. Most fractions required under 3 repeats.

Protein database search, identification and assessment

The Swiss prot release 15 protein database was searched using the Mascot search algorithm with 6ppm peptide tolerance, 0.6Da fragment tolerance, and no enzyme selected. Progenesis LC-MS version 2.4 (*Nonlinear Dynamics, Newcastle upon Tyne, UK*) was used to simultaneously compare and assess for differential abundance based on peptide ion counting. A statistical model, analysis of variance (ANOVA), was used to calculate the p-values of differential abundances. Briefly, ion intensity maps from each run were aligned to a reference sample and ion feature matching was achieved by aligning consistent ion m/z and retention times. The peptide intensities were normalized against total intensity (sample specific log-scale abundance ratio scaling factor) and compared between groups by one-way analysis of variance (ANOVA, $P \leq 0.05$ for statistical significance) and *post hoc* multiple comparison procedures. Type I errors were controlled for by False Discovery Rate (FDR) with q value significance set at 0.01. Peptides exhibiting a statistically significant 1.5-fold or greater abundance difference between groups were identified using the database search program Mascot (*Matrix Science, London, UK, www.matrixscience.com*). MS/MS spectra of differentiating peptides were searched against the Swiss-Prot database (version 15) using Mascot. Parent and fragment ions were searched with tolerances of ± 4 ppm and

±0.5 Da, respectively. Peptide charge states were set at +2 and +3. 'No enzyme' was specified. Proteins and peptides were considered confidently identified when matches had a high ion score >20 and were statistically significant and at least semi-tryptic. Following identification a filter was applied to select proteins of human origin.

These hypothesis-free proteomic analysis of differences between control, Crohns disease and Ulcerative colitis patients resulted in the analysis of over 11,000 ions for differential assessment; the majority of which as expected remained at the same abundance levels between the groups. 58 proteins were identified as significantly ($p < 0.05$) different in abundance levels and 6 peptides were chosen from this hypothesis -free experiment for further validation using MRM. These peptides were ADQTVLTEDK (SEQ ID NO: 2) from Secretogranin-1, VTVQDGNFSFSLESVK (SEQ ID NO: 4) from Guanylin, VSAQQVQGVHAR (SEQ ID NO: 6) from SPP 24, VNSQSLSPYLFR (SEQ ID NO: 7) from SPP 24, NLPSDSQDLGQHGLEED (SEQ ID NO: 9) from Serglycin; and HHGPTITAK (SEQ ID NO: 11) from AMBP.

Example 2: Absolute quantitation of chosen diagnostic markers for differentiation between Healthy, IBD, CD and UC as well as severity

2.1 Plasma and serum samples

A total of 104 participants, including 56 CD patients, 27 UC patients, 9 healthy controls and 12 rheumatoid arthritis (RA) patients, were recruited. All IBD and RA patients were recruited from IBD and RA outpatient clinics at Bankstown-Lidcombe and Concord Repatriation General Hospitals, New South Wales, Australia. All participants provided written informed consent before partaking in this

study. All IBD patients had been previously diagnosed with CD or UC according to standard clinical, endoscopic and histological criteria. Disease activity at the time of recruitment was assessed using the Crohn's Disease Activity Index for patients with CD and the partial Mayo score for patients with UC. All RA patients had been previously diagnosed by a rheumatologist according to standard criteria. Subject details and disease characteristics were obtained at time of recruitment, and are shown in Table 2.

Table 2. Participant characteristics

Group (participants)	Crohn's Disease (n=56)	Ulcerative Colitis (n=27)	Healthy Controls (n=9)	Rheumatoid arthritis (n=12)
Plasma samples obtained	59	32	9	12
Mean Age ($\pm$ SD)	37 ($\pm$ 15)	39 ($\pm$ 15)	31 ($\pm$ 11)	56 ($\pm$ 14)
Males (%)	30 (51%)	10 (31%)	3 (25%)	3 (33%)
Females (%)	29 (49%)	22 (69%)	9 (75%)	6 (67%)
Disease activity at collection				
Remission	27 (47%)	11 (34%)		
Mild activity	18 (30%)	9 (28%)		
Moderate activity	8 (13%)	5 (16%)		
Severe activity	6 (10%)	7 (22%)		
Disease phenotype				
Inflammatory	19 (32%)			
Stricturing	15 (25%)			
Penetrating	25 (42%)[18 ^a]			

^aThis includes 18 samples collected from patients with penetrating phenotype and perianal disease.

Blood samples were collected by standard venepuncture technique with EDTA-vacutainers. Samples were centrifuged immediately for 10 minutes at 4000 revolutions per minute at room temperature to isolate the plasma from the cellular components of the blood. The plasma was immediately extracted and stored at -80°C in 100 μL aliquots. A second plasma sample was collected from 8

patients when they developed changes in their disease profile at subsequent consultations.

Development of MRM method

Candidate peptides were selected based on LC-MS/MS experiments described in Example 1. Skyline software v1.3 (MacCoss Lab, Washington, USA) and MS/MS spectra from previous LC-MS/MS experiments were used to generate a scheduled MRM method consisting of 4-7 transitions per peptide.

10 Stable isotope-labelled peptides

Synthetic light and ^{13}C -labelled heavy peptides for each candidate peptide were obtained in powder form from Sigma-Aldrich (Missouri, USA) at greater than 95% purity and dissolved in 0.1% TFA and 5% formic acid to a 1mg/ml concentration.

Standard curve generation

Serial dilutions of each light synthetic peptide were prepared and made up to a final volume of 10 μL . Standard curves were prepared within the previously established linear working range of each peptide. Heavy peptides were spiked into each dilution (and patient sample) at the same concentration for normalisation of technical variability.

Sample preparation for MRM

Individual patient samples were analysed by MRM. A plasma volume of 2 μL from each sample was added to 48 μL of ammonium bicarbonate 50mM, pH8 and digested overnight at 37°C with 1 μg Trypsin. Five μL of formic acid was added to end digestion and samples were dried down in a SpeedVac and resuspended in 0.1% formic acid. Peptide samples were purified using 2 passes through C18 Stage tips

(*ThermoScientific, Illinois, USA*) and dried down. Cleaned peptide samples were then resuspended in 10 μ L of 0.1% formic acid and heavy labelled peptides added to a final concentration of 100 fmol/ μ L.

5 **MRM analysis**

Two μ L injections of each sample was analysed in a 4000 Q-Trap mass spectrometer (*AB SCIEX, Massachusetts, USA*) coupled to an Ultimate 3000 HPLC and autosampler system (*Dionex, Amsterdam, Netherlands*) for the selected
10 transitions.

MRM data analysis

MRM data were processed using Skyline software v1.3 (*MacCoss Lab, Washington, USA*). For each target peptide, relative quantitation was performed by ratio comparison of
15 total transition peak areas between samples. Absolute quantitation was performed by normalization of peak areas to the heavy isotope internal standard, and concentration calculated according to the constructed standard curves for each peptide.

20 SPSS Statistics 20 program (*IBM, New York, USA*) was used for statistical analysis to perform Kruskal-Wallis tests, Mann-Whitney U tests and Spearman's rank correlation, and generate receiver operating characteristic (ROC) curves.

25 **Experiment 3: Confirmation of Proteins/ peptides**

LC-MS/MS using LTQ-FT instruments

An LTQ-FT Ultra mass spectrometer (*Thermo Electron, Bremen, Germany*) was used to analyse protein band fractions. As per Ly and Wasinger[9], peptides were
30 separated by nano-LC using an Ultimate 3000 HPLC and autosampler system (*Dionex, Amsterdam, Netherlands*). 1800V

was applied to low volume tee (*Upchurch Scientific, WA, USA*) and the column tip positioned ~0.5cm from the heated capillary (T=200°C) of a LTQ-FT. The instrument operated in DDA mode, with positive ions generated by electrospray.
5 A survey scan of m/z 350-1750 was acquired in the FT ICR cell.

Collision induced dissociation was used by the linear ion trap in which up to seven of the most abundant ions (>2000
10 counts) with charge states of +2 or +3 were successively isolated and fragmented. Mass to charge ratios selected for MS/MS were dynamically excluded for 60 seconds.

Identification and assessment of proteins

'Mascot Daemon/extract_msn' (*Matrix Science, London, England*) was used with default parameters to generate peak
15 lists, which were submitted to the database search program Mascot. The Swiss-Prot release 15 protein database was searched using the Mascot search algorithm with 6ppm peptide tolerance, 0.6Da fragment tolerance, and no enzyme
20 selected.

4. Results

a. Peptides which diagnose IBD

25 The absolute abundance of peptides in samples from healthy patients (C) or patients suffering from IBD (IBD) or rheumatoid arthritis (RA) as determined by MRM is shown in Figure 2A and 2B, or by relative abundance ion count is shown in Figure 2C and 3.

30

As can be seen from Figures 2A, 2B, and 2C, levels of the peptides VSAQQVQGVHAR (SEQ ID NO: 6) and VNSQSLSPYLFR (SEQ ID

NO: 7) from SPP 24, are elevated in serum from patients suffering from IBD as compared to levels of the same peptides in control patients (i.e. patients not suffering from IBD).

- 5 Referring to Figure 3, it can be seen that absolute abundance levels of the peptide NLPSPDSQDLGQHGLED (SEQ ID NO: 9) from Serglycin is reduced in serum from patients suffering from IBD as compared to levels of the same peptides in control patients (i.e. patients not suffering from IBD).

10

b. Peptides which distinguish between Ulcerative colitis and Crohn's disease

The relative abundance of peptides in samples from
15 patients suffering from Ulcerative colitis (UC) and Crohn's disease (CD) as determined by absolute abundance using MRM is shown in Figures 4A, 4B and 4C, and as determined by relative abundance ion count MS is shown in Figure 5.

20

As can be seen from Figures 4A to 4C and 5, the level of the peptides ADQTVLTEDK (SEQ ID NO: 2) from Secretogranin-1, HHGPTITAK (SEQ ID NO: 11) from AMBP, VTVQDGNFSFSLESV (SEQ ID NO: 4) from guanylin, are elevated in serum from patients suffering
25 from Ulcerative colitis as compared to the level of the same peptides in patients suffering from Crohn's disease.

c. Peptides which assess the severity of IBD

30 The relative abundance of peptides in samples from patients: in remission from suffering from Crohn's disease (Qiescent); 1, suffering from mild Crohn's disease (mild); 2, Suffering from moderate Crohn's disease (moderate); or 3, suffering from severe Crohn's disease (severe), is shown in Figures 11.

35

As can be seen from Figure 11, the level of the peptides VSAQQVQGVHAR (SEQ ID NO: 6) from SPP 24, VNSQSLSPYLFR (SEQ ID NO: 7) from SPP 24 are elevated in serum from patients suffering from severe or active Crohn's disease as compared to the level of the same peptides in serum from patients in remission from suffering from Crohn's disease.

The relative abundance of the peptide in samples from patients: in remission from suffering from IBD (Qiescent); or suffering from severe UC or CD, is shown in Figures 12. As can be seen from Figure 12, ADQTVLTEDK (SEQ ID NO: 2) from Secretogranin-1 is elevated in subjects suffering from severe UC or CD.

15 **Example 3: Assessment of Serum Using Reverse-Polynomial Dilution (RPD) Calibration in Multiple Reaction Monitoring (MRM) Plasma Assays**

RPD was used in conjunction with MRM to assess the levels of peptides in cohorts of UC and CD patients.

Light (crystalline powder) and stable isotope-labelled synthetic AQUA (heavy) peptides for SEQ ID NOS: 2, 4, 6, 7, 9 and 11 were obtained from Sigma-Aldrich (Missouri, USA) at greater than 95% purity. Isotopically labelled forms contained either a C-terminal N¹⁵, C¹³ on Arg, Lys or internal Leu. Peptides are shown in Table 1.

Peptide	Name	SEQ ID NO
HHGPTITAK	AMBP	11
NLPSDSQDLGQHGLEED	Serglycine	9
VNSQSLSPYLFR	SPP24	7
VSAQQVQGVHAR	SPP24	6
ADQTVLTEDK	Secretogranin-1	2

VTVQDGNFSFSLES VK	Guanylin	4
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Amino acid residues in bold are those labelled with C¹³, N¹⁵.

All peptides were aliquoted into 1 nM amounts following amino acid analysis. Amino acid analysis was carried out at the Australian Proteome Analysis Facility with all peptides made up in stock amounts to 1 mg/ml with 50% acetonitrile, 5% acetic acid, 0.1% trifluoroacetic acid (TFA). Briefly, 20 µg amounts of synthetic peptides were reconstituted in 200 µl of 20% acetonitrile, 0.1% TFA, put into 10 µl aliquots and dried down. These samples were put through 24 hr gas phase hydrolysis with 6M HCl at 110°C and analysed in duplicate using the Waters AccQTag Ultra chemistry on a Waters Acquity UPLC. The quantitative values were averaged and used for subsequent analysis.

Human plasma samples were obtained from Concord repatriation General Hospital, Sydney Australia. The collection of samples was approved by the Sydney Local Health District Human Research Ethics Committee (Approval Code: CH62/6/2011-154). Plasma samples were trypsin digested at a 100:1 protein to enzyme ratio, made up to 100 ml with 50mM NH₄HCO₃ (AMBIC) and incubated at 37 °C overnight. 5µl of neat formic acid was used to terminate the digestion reaction, the samples dried, resuspended in 0.1% formic acid and desalted by 3 successive passes through C18 stage-tips (Thermo Fisher Scientific, MA, USA). Protein content was determined using the GE Healthcare 2-D Quant Kit (Uppsala, Sweden). The starting concentration of plasma used was 58 µg/µl and peptide peptide digested samples were reconstituted in 0.1% formic acid and aliquoted so that all experimental samples contained the same approximate final analysis of 0.1 µg/µl.

RPD calibration was carried out with serial dilutions of 0.5, 1, 5, 25, 50 and 100fmol of C13N15-labeled peptide that was normalised against the constant endogenous light target peptide signal from the plasma-digest background. RPD

5 datapoints were fitted with a 2nd order polynomial regression equation. All transitions were used for quantitation of each target peptide and the calibration standard was run in triplicate.

10 MRM transition lists consisting of 4-7 transitions per peptide precursor were developed in Skyline SRM Environment v1.4 (MacCoss lab, UW), and are show in Table 4 and refined by iterative experimentation and optimization using a 4000Qtrap mas spectrometer (AB SCIEX, MA, USA) coupled to an

15 ultimate 3000 HPLC and autosampler system (Dionex, Amsterdam, Netherlands). Samples were concentrated and desalted onto a micro C18 precolumn (500 µm x 2mm, Michrom Bioresources, USA) with H₂O:CH₃CN (98:2, 0.05% v/v TFA) at 15 ml/minute. After 4 min washing the pre-column was automatically switched

20 (Valco10 port valve, Houston, USA) into line with a fritless-nano column manufactured according to Gatlin et al. (1998)Anakl. Biochem. 263(1): 93-101. Peptides were eluted using a linear gradient of H₂O:CH₃CN (36:64, 0.1% (v/v)FA) at ~300 nL/min over 40 min. The pre-column was connected via a

25 fused silica capillary (25cm, 25µm) to a low volume tee (Upchurch Scientific, USA) and introduced into the 4000QTRAP mass spectrometer. Samples were analysed in positive ion mode with an ion spray voltage of 2.4 kV, curtain gas flow of 20 and nebulizing gas flow of 5. For MRM analysis,

30 quadropoles were operated in unit resolution, and the dwell time was 66.2 ms. All samples were made to 10 µl and analysed in 1 µl injections. Raw.WIFF files were imported into Skyline SRM Environment for manual inspection and peak area-ratio calculation.

Table 4: LC-ESI-MS/MS MRM method for peptides.

Peptide (SEQ ID NO:)	De- clustering Potential	Collis- ion Energy	Rt(Min ± Window)	MRM Transitions (m/z)						
				Q1	Q3					
11	66.2	23.2	19.0 ± 1.0	481.26	824.46	687.40	630.38	533.33		
				485.27	832.48	695.42	638.40	541.34		
9	98.7	48.6	29.2 ± 1.3	927.41	1240.54	1112.49	997.46	884.37		
				930.92	1247.56	1119.50	1004.48	891.39		
7	82.6	36	35.4 ± 0.7	705.87	1197.63	1110.59	982.54	895.50	782.42	69
				710.88	1207.63	1120.6	992.54	905.51	792.43	70
6	77.8	37.2	21.2 ± 0.6	640.35	1093.59	1022.55	894.49	766.43	667.36	
				645.35	1103.59	1032.56	904.50	776.44	677.37	
2	76.7	31.3	23.5 ± 0.5	624.80	934.47	833.43	734.36	621.27		
				628.81	942.49	841.44	742.37	629.29		
4	95.2	45.8	36.3 ± 2.5	878.94	1329.63	1214.60	1157.58	1043.54	896.47	
				882.94	1337.64	1222.61	1165.59	1051.55	904.48	

The results of the analysis of the levels of the 6 peptides
 5 in groups of subjects suffering from IBD, UC, CD, in
 remission, or not suffering from any disease, are shown in
 Figures 6 to 11.

Figure 6 shows the results of analysis of serum from subjects
 10 from the following groups: control (no disease) (0); subjects
 suffering from rheumatoid arthritis (1); subjects suffering
 from Crohn's disease (2); subjects suffering from Ulcerative
 colitis (3); and inflamed synovial fluid (4). As can be seen
 from Figure 6, elevated levels of SEQ ID NO: 6, 7 distinguish
 15 between control and CD and UC, while elevated levels of SEQ
 ID NO: 4 distinguishes between the control and UC.

Figure 7 shows the levels of SEQ ID NO: 6 and 7 in subjects which either do not suffer from IBD (0) and subjects which do suffer from IBD (1). Both SEQ ID NO: 6 and 7 are elevated in serum of subjects suffering from IBD when compared to levels
5 in subjects not suffering from IBD.

Figure 8 shows the distribution of mean amounts of peptides having SEQ ID NO: 6 and 7 from serum of subjects which are either: A: healthy (0) or suffering from UC in remission; or
10 B. healthy (0) or suffering from CD in remission (1). Both SEQ ID NO: 6 and 7 are elevated in subjects suffering from UC in remission compared to healthy subjects. Similarly, SEQ ID NO: 6 and 7 are elevated in subjects suffering from CD in remission compared to healthy subjects. Accordingly, SEQ ID
15 NOs 6 and 7 are capable of distinguishing between healthy subjects and those suffering from UC or CD in remission.

Figure 9 shows the levels of SEQ ID NO: 4 in subjects suffering from UC (0) and CD (1). Levels of SEQ ID NO: 4 are elevated in subjects suffering from UC relative to this
20 subjects suffering from CD.

Figure 10 shows the level of peptides having SEQ ID NOs: 2, 6 and 7 in subjects suffering from UC in remission (0) and active UC (1). SEQ ID NO: 2 is reduced in serum of subjects
25 suffering from UC in remission compared with subjects suffering from active UC. SEQ ID NO: 6 and 7 is elevated in serum of subjects suffering from UC in remission compared to the levels in subjects suffering from active UC.

30 Figure 11 shows a comparison of the level of peptides having SEQ ID NOS: 2, 6 and 7 in subjects suffering from increasingly severe forms of CD (1 to 3). Levels of peptides having SEQ ID NOS: 2, 6 and 7 were elevated in subjects suffering from severe CD.

The correlation of peptides having SEQ ID NOs: 2, 4, 6 and 7 were assessed for with IBD, CD and UC were assessed for significance using a relative operating characteristic (ROC) analysis. The results of the ROC analysis are shown in Table 3.

Table 3

	Condition	Peptides	AUC	probat
1	Peptides which distinguish between severe CD vs CD in remission	VSAQQVQGVHAR_t VNSQSLSPYLFR ADQTVLTEDEK_t	0.75 0.8 0.833	0.068 0.028 0.015
2	Peptides which distinguish between active IBD vs inactive IBD	ADQTVLTEDEK_t	0.867	0.045
3	Peptides which distinguish between active IBD vs inactive IBD	VSAQQVQGVHAR_t VNSQSLSPYLFR	0.833 0.900	0.068 0.028
4	Peptide which distinguish between CD in remission vs Healthy subject	VSAQQVQGVHAR_t VNSQSLSPYLFR	0.818 0.763	0.017 0.048
5	Peptides which distinguish between UC in remission vs Healthy subject	VSAQQVQGVHAR_t VNSQSLSPYLFR	0.964 1.000	0.004 0.002
6	Peptides which distinguish between UC vs CD	VTVDGNFSFSLESVK_t	0.778	0.006
7	Peptides which distinguish between IBD vs Healthy subjects	VSAQQVQGVHAR_t VNSQSLSPYLFR	0.852 0.865	0.0004 0.0002

10 A correlation analysis of the peptides was carried out using Spearman's Rho. The results are shown in Table 4.

Table 4: Results of Spearman's Rho analysis

		SPP24 SEQ ID NO: 7	SPP24 SEQ ID NO: 6	AMBP SEQ ID NO: 11	Serglycin SEQ ID NO: 9	Secretogranin-1 SEQ ID NO: 2	Guanylin SEQ ID NO: 4
SPP24 SEQ ID NO: 7	Correlation Coefficient	1.000	.574**	.405**	.036	.217	.431**
	Sig. (2-tailed)	.000	.000	.002	.790	.108	.001
	N	56	56	56	56	56	56
SPP24 SEQ ID NO: 6	Correlation Coefficient	.574**	1.000	.688**	-.260	-.131	.181
	Sig. (2-tailed)	.000	.	.000	.053	.335	.182

	N	56	56	56	56	56	56
AMBP SEQ ID NO: 11	Correlation Coefficient	.405**	.688**	1.000	-.327*	-.089	.294*
	Sig. (2-tailed)	.002	.000	.	.014	.512	.028
	N	56	56	56	56	56	56
Serglycin SEQ ID NO: 9	Correlation Coefficient	.036	-.260	-.327*	1.000	.140	.049
	Sig. (2-tailed)	.790	.053	.014	.	.303	.720
	N	56	56	56	56	56	56
Secretogranin-1 SEQ ID NO: 2	Correlation Coefficient	.217	-.131	-.089	.140	1.000	.651**
	Sig. (2-tailed)	.108	.335	.512	.303	.000	.000
	N	56	56	56	56	56	56
Guanylin SEQ ID NO: 4	Correlation Coefficient	.431**	.181	.294*	.049	.651**	1.000
	Sig. (2-tailed)	.001	.182	.028	.720	.000	.000
	N	56	56	56	56	56	56

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Summary

5 Using RPD in conjunction with MRM on enzyme digests of serum samples:

- (a) peptides of segretogranin-1 and SPP24 are elevated in subjects suffering from severe CD relative to subjects in remission from CD;
- 10 (b) peptides from SPP24 are elevated in subjects suffering from UC in remission compared to subjects suffering from active UC, and peptides from secretogranin-1 are reduced

in subjects suffering from UC in remission compared to subjects suffering from active UC;

5 (c) peptides from SPP24 are elevated in subjects suffering from UC and CD in remission when compared to healthy subjects (i.e. subjects not suffering from IBD);

(d) peptides from Guanylin are elevated in subjects suffering from UC relative to subjects suffering from CD;

10 (e) peptides from SPP24 are elevated in subjects suffering from IBD relative to healthy subjects (i.e. subjects not suffering from IBD).

15

CLAIMS:

1. A method of diagnosing or assessing an inflammatory bowel disease in a subject, comprising comparing the level of one or more markers in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the marker is selected from the group consisting of:
 - (i) Secretogranin-1 or a fragment thereof;
 - (ii) guanylin or a fragment thereof;
 - (iii) SPP 24 or a fragment thereof;
 - (iv) AMBP or a fragment thereof; and
 - (v) serglycin or a fragment thereof.
2. The method of claim 1, wherein the Secretogranin-1 or a fragment thereof is a peptide comprising SEQ ID NO: 2.
3. The method of claim 1, wherein the guanylin or a fragment thereof is a peptide comprising SEQ ID NO: 4.
4. The method of claim 1, wherein the SPP 24 (S) or a fragment thereof is a peptide comprising SEQ ID NO: 6.
5. The method of claim 1, wherein the SPP 24 (N) or a fragment thereof is a peptide comprising SEQ ID NO: 7.
6. The method of claim 1, wherein the AMBP or a fragment thereof is a peptide comprising SEQ ID NO: 11.
7. The method of claim 1, wherein the serglycin or a fragment thereof is a peptide comprising SEQ ID NO: 9.
8. The method of any one of claims 1 to 7, wherein the one or more markers is SPP 24 or a fragment thereof, and the reference value is the level of SPP 24 or a fragment thereof in the tissue or body fluid of a subject not suffering from IBD, wherein IBD is diagnosed when the level of SPP24 or a fragment thereof is elevated relative to the reference value.
9. The method of claim 1, wherein the one or more markers is guanylin or a fragment thereof, and the reference value is the level of the one or more markers in the

tissue or body fluid of a subject not suffering from IBD, and wherein Ulcerative colitis is diagnosed when the level of guanylin or a fragment thereof is elevated relative to the reference value.

- 5 10. The method of claim 1, wherein the subject shows no or few symptoms of IBD, the one or more markers is SPP24 or a fragment thereof, and the reference value is the level of the one or more markers in the tissue or body fluid of a subject not suffering from IBD, wherein the subject is diagnosed with CD or UC in remission when the level of the one or more markers is elevated relative to the reference value.
- 10
11. The method of claim 1, wherein the one or more markers is selected from the group consisting of SPP24 or a fragment thereof and secretogranin-1 or a fragment thereof, and the reference value for the one or more markers is the level of the one or more markers in a tissue or body fluid of a subject suffering from active Ulcerative colitis, wherein the subject is diagnosed as suffering from Ulcerative colitis in remission when the level of SPP24 or a fragment thereof is elevated relative to the reference value, and/or the level of secretogranin-1 is reduced relative to the reference value.
- 15
- 20
- 25 12. The method of claim 1, wherein the one or more markers is selected from the group consisting of SPP24 or a fragment thereof and secretogranin-1 or a fragment thereof, and the reference value is the level of the one or more markers in a subject suffering from Crohn's Disease of a predetermined severity, wherein the subject is assessed as suffering from CD of greater severity than the predetermined severity when the level of the one or more markers is elevated relative to the reference value.
- 30

13. The method of any one of claims 1 to 12, wherein the sample is a serum sample.
14. A method of diagnosing or assessing an inflammatory bowel disease in a subject, comprising comparing the level of one or more markers in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the marker is selected from the group consisting of SPP 24 or a fragment thereof, and serglycin or a fragment thereof, and wherein the reference value is the level of the one or more markers of a subject not suffering from IBD, and wherein IBD is diagnosed when the level of SPP 24 or a fragment thereof is elevated relative to the reference value, and/or the level of serglycin or a fragment thereof is reduced relative to the reference value.
15. A method of assessing whether a subject suffering from IBD is suffering from Crohn's Disease, comprising comparing the level of one or more markers selected from the group consisting of secretogranin-1 or a fragment thereof, SPP24 or a fragment thereof, guanylin or a fragment thereof, and AMBP or a fragment thereof, in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from Ulcerative colitis, and wherein Crohn's Disease is diagnosed when the level of the one or more markers is elevated relative to the reference value.
16. A method of assessing whether a subject suffering from IBD is suffering from Crohn's Disease, comprising comparing the level of one or more markers selected from the group consisting of: secretogranin-1 or a fragment thereof; AMBP or a fragment thereof; and guanylin or a

fragment thereof, in a tissue or body fluid of the subject relative to a reference value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from
5 Ulcerative colitis, and wherein Crohn's Disease is diagnosed when the level of the one or more markers is reduced relative to the reference value.

17. A method of assessing whether a subject suffering from
10 IBD is suffering from Ulcerative colitis, comprising comparing the level of one or more markers selected from the group consisting of secretogranin-1 or a fragment thereof; and guanylin or a fragment thereof, in a tissue or body fluid of the subject relative to a reference
15 value for the one or more markers, wherein the reference value is the level of the one or more markers in a subject suffering from Crohn's disease, and wherein Ulcerative colitis is diagnosed when the level of the one or more markers is increased relative to the
20 reference value.

18. A method of assessing whether a subject suffering from
IBD is suffering from Ulcerative colitis, comprising
25 comparing the level of guanylin or a fragment thereof in a tissue or body fluid of the subject relative to a reference value, wherein the reference value is the level of guanylin or a fragment thereof in a subject suffering from Crohn's disease, and wherein the patient is assessed as suffering from Ulcerative colitis when
30 the level of guanylin or a fragment thereof is elevated relative to the reference value.

19. A method of assessing the severity of Crohn's disease
in a subject, comprising comparing the level of one or
35 more markers selected from the group consisting of SPP

24 or a fragment thereof and secretogranin-1 or a
fragment thereof in tissue or body fluid of the subject
relative to a reference value for the one or more
markers, wherein the reference value is the level of the
one or more markers in a subject suffering from Crohn's
disease of predetermined severity, and wherein the
Crohn's disease is diagnosed as more severe than the
Crohn's disease of predetermined severity when the level
of the one or more markers is elevated relative to the
reference value.

20. A method of assessing the severity of Ulcerative
colitis in a subject, comprising comparing the level of
one or more markers selected from the group consisting
of SPP24 or a fragment thereof and secretogranin-1 or a
fragment thereof in tissue or body fluid of the subject
relative to a reference value for the one or more
markers, wherein the reference value is the level of the
one or more markers in a subject suffering from
Ulcerative colitis in remission , and wherein Ulcerative
colitis is diagnosed as active when the level of
secretogranin-1 or fragment thereof is elevated relative
to the reference value, and/or the level of SPP24 or a
fragment thereof is reduced relative to the reference
value.

21. A method of assessing the severity of IBD in a subject
suffering from IBD, comprising comparing the level of
secretogranin-1 or a fragment thereof, in a tissue or
body fluid of the subject relative to a reference value
for the one or more markers, wherein the reference value
is the level of the one or more markers of a subject
suffering from IBD of predetermined severity, and
wherein IBD is diagnosed as more severe than the IBD of
predetermined severity when the level of secretogranin-1

or a fragment thereof is elevated relative to the reference value. In one embodiment, the IBD of predetermined severity is IBD in remission.

5

22. A method of assessing whether a subject no symptoms or few symptoms of IBD is suffering from Ulcerative colitis in remission, comprising comparing the level of one or more markers selected from the group consisting of SPP24 or a fragment thereof and secretogranin-1 or a fragment thereof with a reference value, wherein the reference value is the level of the one or more markers of a subject suffering from active Ulcerative colitis, and wherein Ulcerative colitis in remission is diagnosed when the level of secretogranin-1 or fragment thereof is reduced relative to the reference value, and/or the level of SPP24 or fragment thereof is elevated relative to the reference value.

20

23. A method for assessing whether a subject showing no symptoms or few symptoms of IBD is suffering from Crohn's disease or Ulcerative colitis in remission, comprising comparing the level of SPP 24 or a fragment thereof in a tissue or body fluid of the subject relative to a reference value, wherein the reference value is the level of the one or more markers of a subject not suffering from IBD, and wherein the subject is assessed as suffering from Crohn's disease in remission or ulcerative colitis in remission when the level of SPP 24 or a fragment thereof is elevated relative to the reference value.

35

24. A diagnostic device or protein array for diagnosing or assessing an IBD in a subject, comprising one or more markers selected from the group consisting of:

(vi) Secretogranin-1 or a fragment thereof;

(vii) Guanylin or a fragment thereof;

(viii) SPP 24 or a fragment thereof;

(ix) AMBP or a fragment thereof; and

5 (x) Serglycin or a fragment thereof.

25. A method of diagnosing Ulcerative colitis in a subject,
comprising comparing the level of one or more markers in
a tissue or body fluid of the subject relative to a
reference value for the one or more markers, wherein the
10 marker is selected from the group consisting of:

(i) secretogranin-1 or a fragment thereof;

(ii) guanylin or a fragment thereof;

wherein the reference value is the level of the one or
more markers of a subject not suffering from IBD, and
15 wherein Ulcerative colitis is diagnosed when the level of
the one or more markers is elevated relative to the
reference value.

26. A method of diagnosing Crohn's disease in a subject,
20 comprising comparing the level of a first marker and one
or more second markers in a tissue or body fluid of the
subject relative to a reference value for each of the
markers, wherein the first marker is SPP24 or a fragment
thereof and the second marker is selected from the group
25 consisting of:

(i) secretogranin-1 or a fragment thereof; and

(ii) guanylin or a fragment thereof;

wherein the reference value is the level of the first and
second markers of a subject not suffering from IBD, and
30 wherein Crohn's disease is diagnosed when the level of
first marker is elevated relative to the reference value,
and the level of the one or more second markers is not
elevated relative to the reference value.

Figure 1

A. HUMAN Secretogranin-1

MQPTLLLSLLGAVGLAAVNMPVDNRNHNENGMVTRCIIEVLSNALS KSSAPPITPECRQV
 LKTSRKDV KDKETTENENTKFEVRLLRDPADASEAHSSSRGEAGAPGEEDIQGPTKADT
 EKWAEGGGHSRERADEPQWSLYPSDSQVSEEVKTRHSEKSQREDEEEEGENYQKGERGE
 DSSEKHLLEEPGETQNAFLNERKQASAIKKEELVARSETHAAGHSQEKTHSREKSSQESG
 EETGSQENHPQESKQPRSQEESSEEGEEDATSEVDKRRTRPRHHHGRSRPDRSSQGGSLP
 SEEKGHPQEESSESNVSMASLGEKRDHHS THYRASEEEPEYGEI KGYPGVQAPEDLEWE
 RYRGRGSEEYRAPRPQSEESWDEEDKRNYPSELDKMAHG YGESEEEERGLEPGKGRHHR
 GRGGEPRAYFMSDTREEKRFLGEGHHRVQENQMDKARRHPQGA WKELDRNYLNYGEEGAP
 GKWQQQGD LQDTKENREEARFQDKQYSSHHTAEKRKRLGELFNPYDPLQWKSSH FERRD
 NMNDNFLEGEENELTLNEKNFFPEYN YDWEKKPFSE DVNWGYEKRNLARVPKLDLKRQ
 YDRVAQLDQLLHYRKKS AEFDPDFYDSEEPVSTHQEAENEKDR ADQTVLTEDEK KELENLA
 AMDLELQKIAEKFSQRG (SEQ ID NO: 1)

B. HUMAN Guanylin

MNAFLLSALCLLGAWAALAGG VTVDGNFSFSLESVK KLKDLQEPQEPRVGKLRNFAPIP
 GEPVVPILCSNPNFPEELKPLCKEPNAQEILQRLEEIAEDPGTCEICAYAACTGC (SEQ ID NO: 3)

C. HUMAN Secreted phosphoprotein 24 (SPP24)

MISRMEKMTMMMKILIMFALGMNYWSCSGFPVYDYPSSSLRDALSASVVK VNSQSLSPYL
FRAFRSSLKRVEVL DENNLVMNLEFSIRETTCKRDSGEDPATCAFQRDY YVSTAVCRSTV
KVSAQQVQGVHAR CSWSSSTSESYSSEEMIFGDMLGSHKWRNNYLFGLISDESISEQFYD
 RSLGIMRRVLPPGNRRYPNHRHRARINTDFE (SEQ ID NO: 5)

D. HUMAN Serglycin

MMQKLLKCSRLVLALALILVLESSVQGYPTRRARYQWVRCNPDSNSANCLEEKGPMFELL
 PGESNKIPRLRTDLFPKTRIQLNRIFPLSE DYSGSGFGSGSGSGSGSGSGFLTEMEQDY
 QLVDSDAFHDNLRSLDR NLPSDSQDLGQHGLEED FML (SEQ ID NO: 8)

E. HUMAN Protein AMBP

MRSLGALLLLLSACLAVSAGPVPTPPDNIQVQENFNISRIYGKWNLAIGSTCPWLKKIM
 DRMTVSTLVLGEGATEAEISMTSTRWRKGVCEETSGAYEKTD TDGKFLYHKSKWNITMES
 YVHTNYDEYAI FLTKKFSR HHGPTITAK LYGRAPQLRETLLQDFRVVAQGVGIPEDSIF
 TMADRGE CVPEQEPEPILIPRVRAVL PQEEEGSGGQLVTEVTKKEDSCQLGYSAGPC
 MGMTSRYFYNGTSMACETFYGGCMGNGN NFVTEKECLQTCRTVAACNLPIVRGPCRAFTI
 QLWAFDAVKGKCVLFPYGGCQGN GNKFYSEKECREYCGVPGDGDEELLRFSN (SEQ ID NO: 10)

FIGURE 2A

SPP 24: VNSQSLSPYLFR

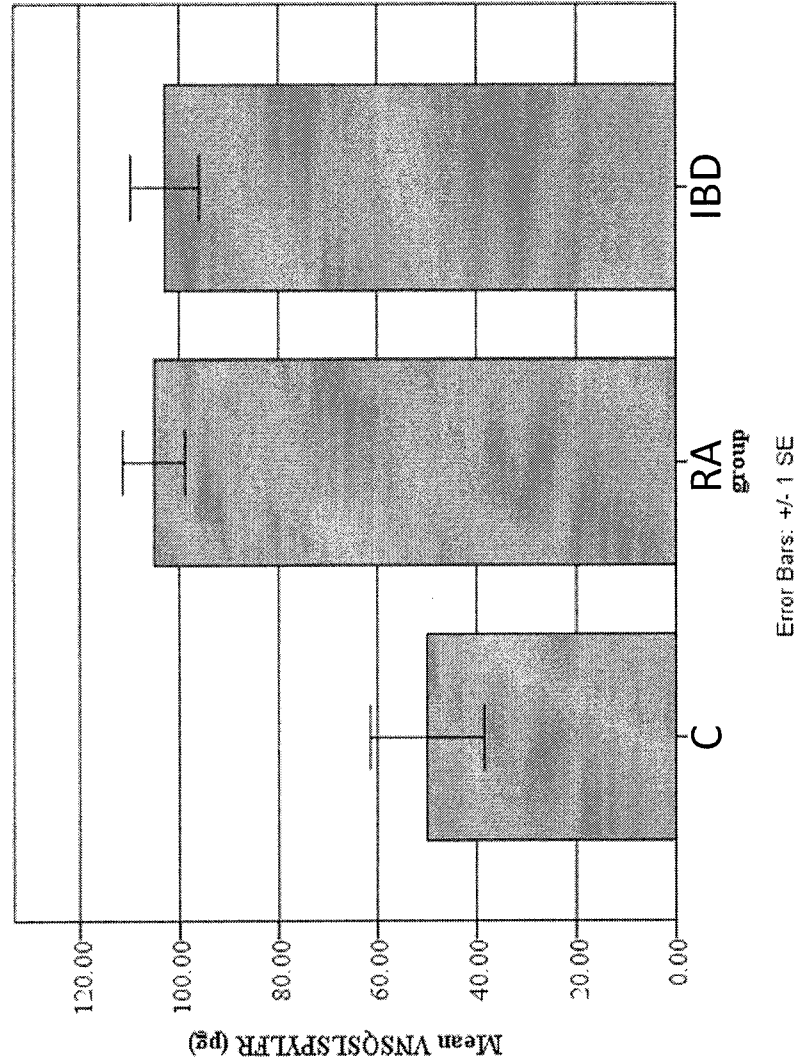


FIGURE 2B

SPP 24: VSASQQVQGVHAR

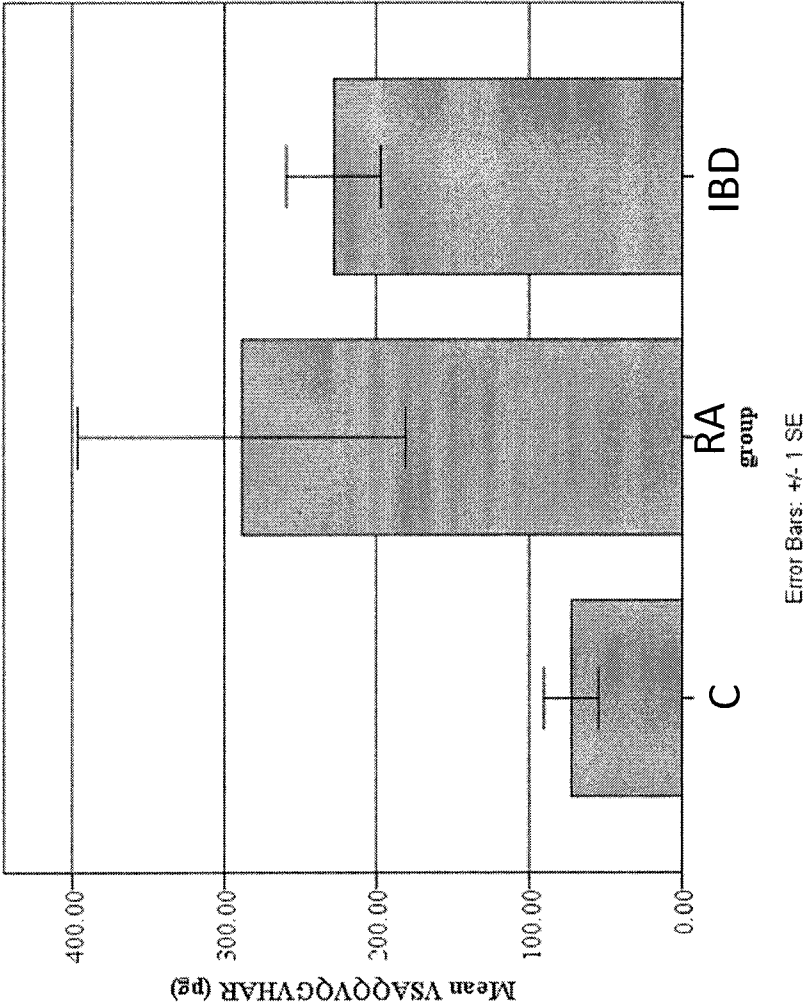


FIGURE 2C

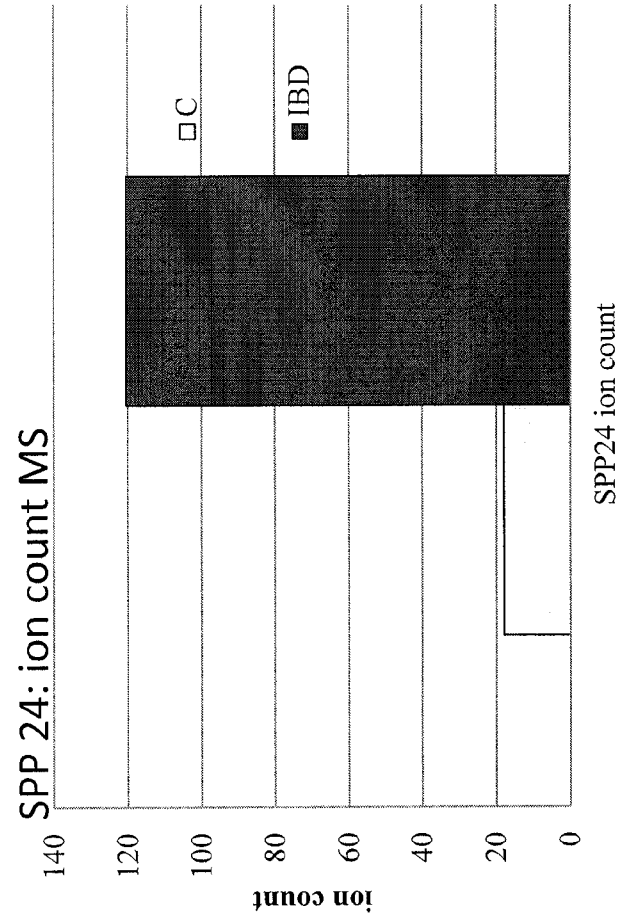


FIGURE 3

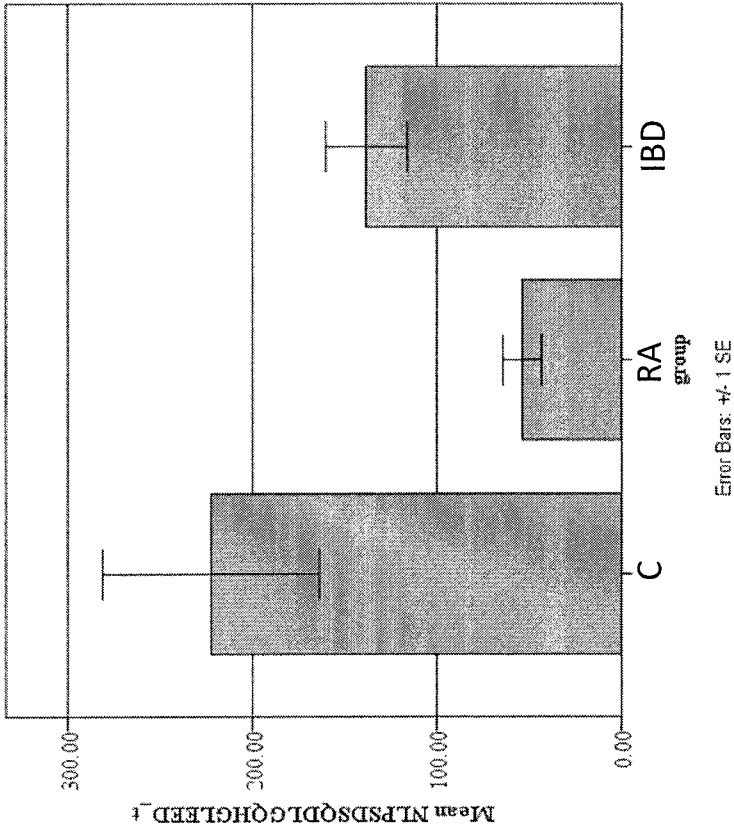


FIGURE 4A

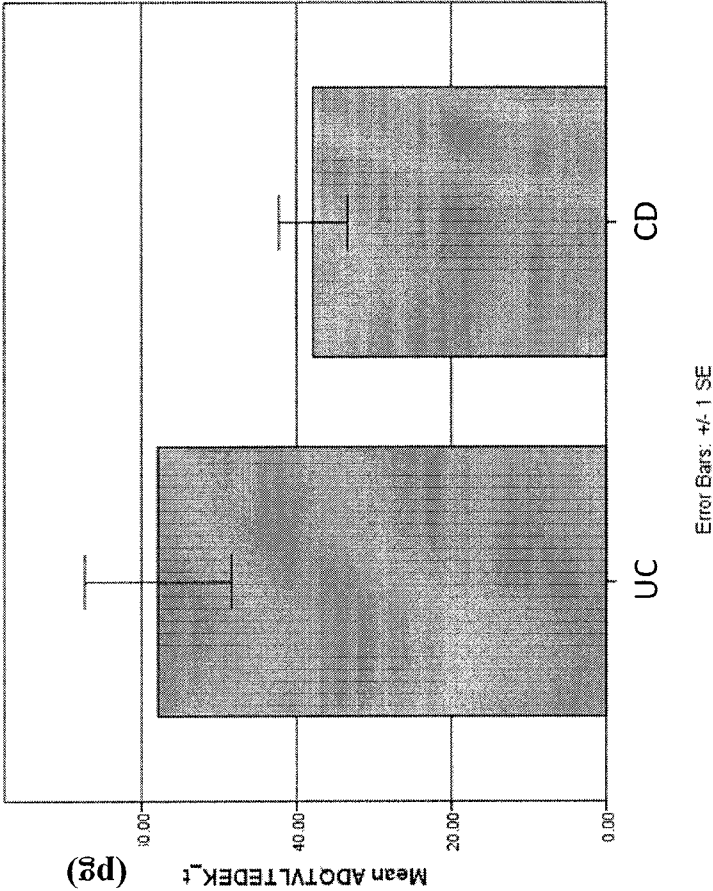


FIGURE 4B

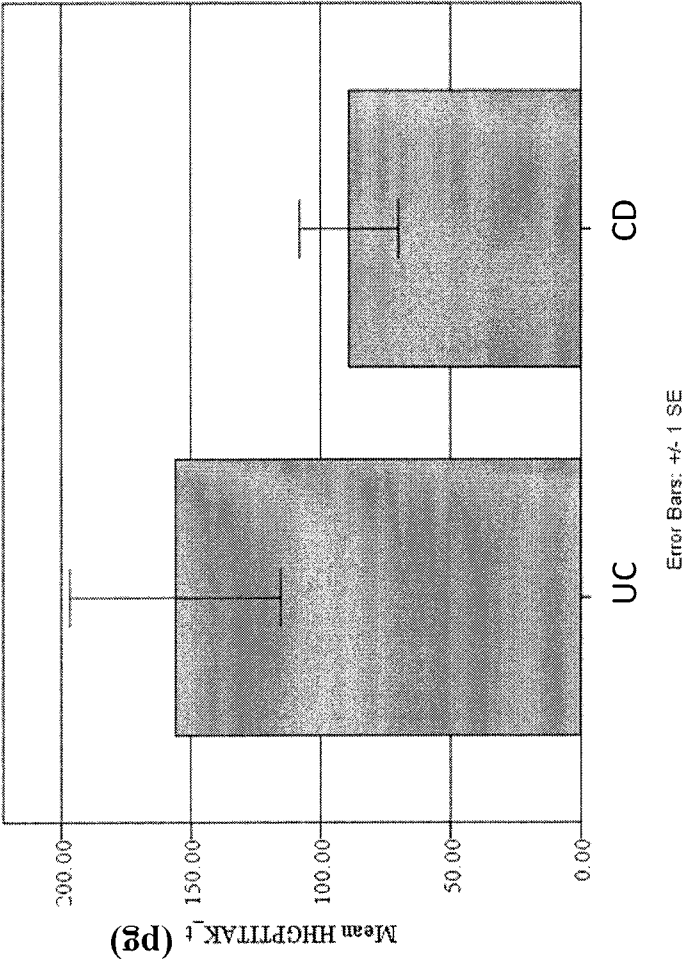


FIGURE 4C

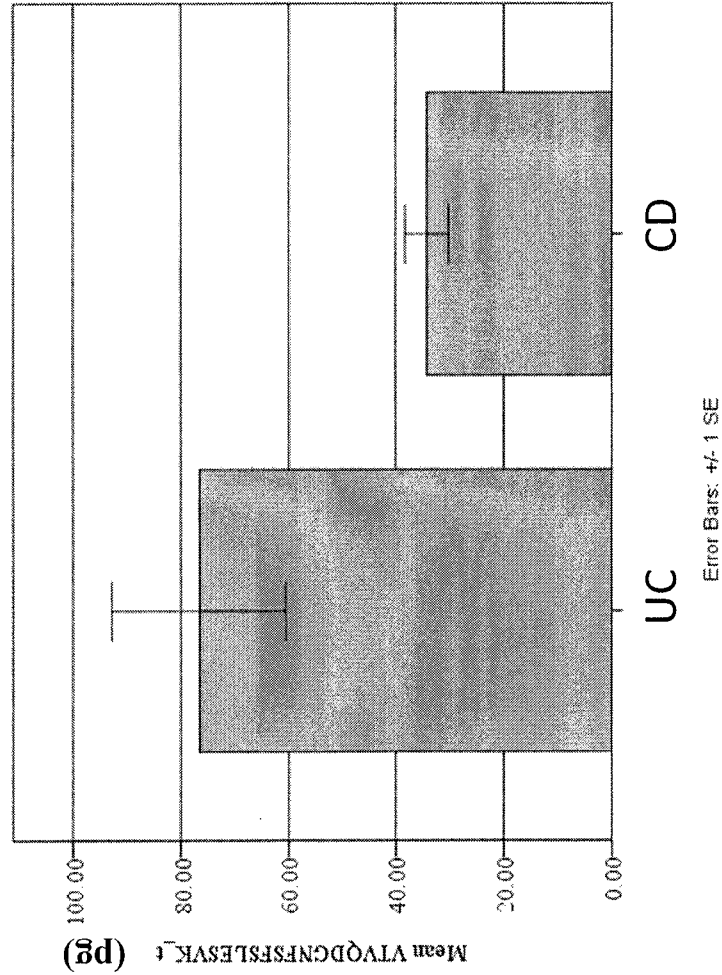


FIGURE 5

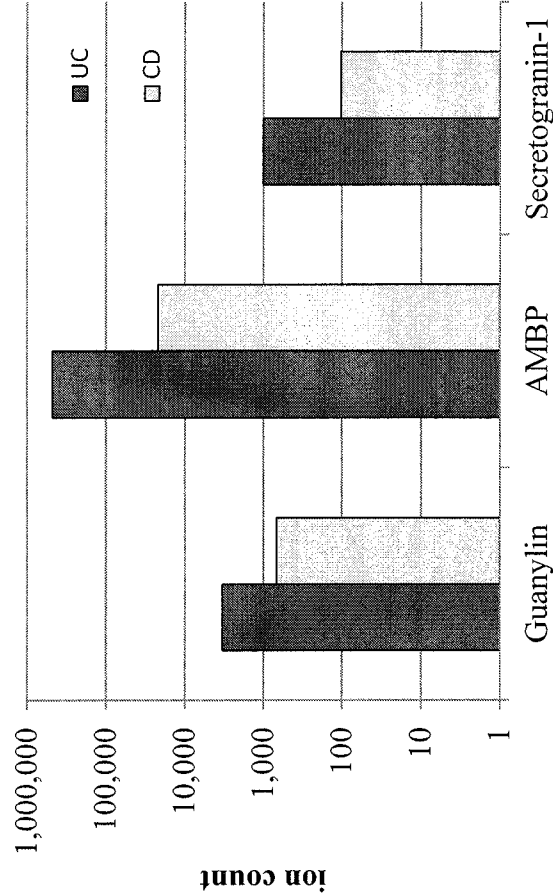


FIGURE 6

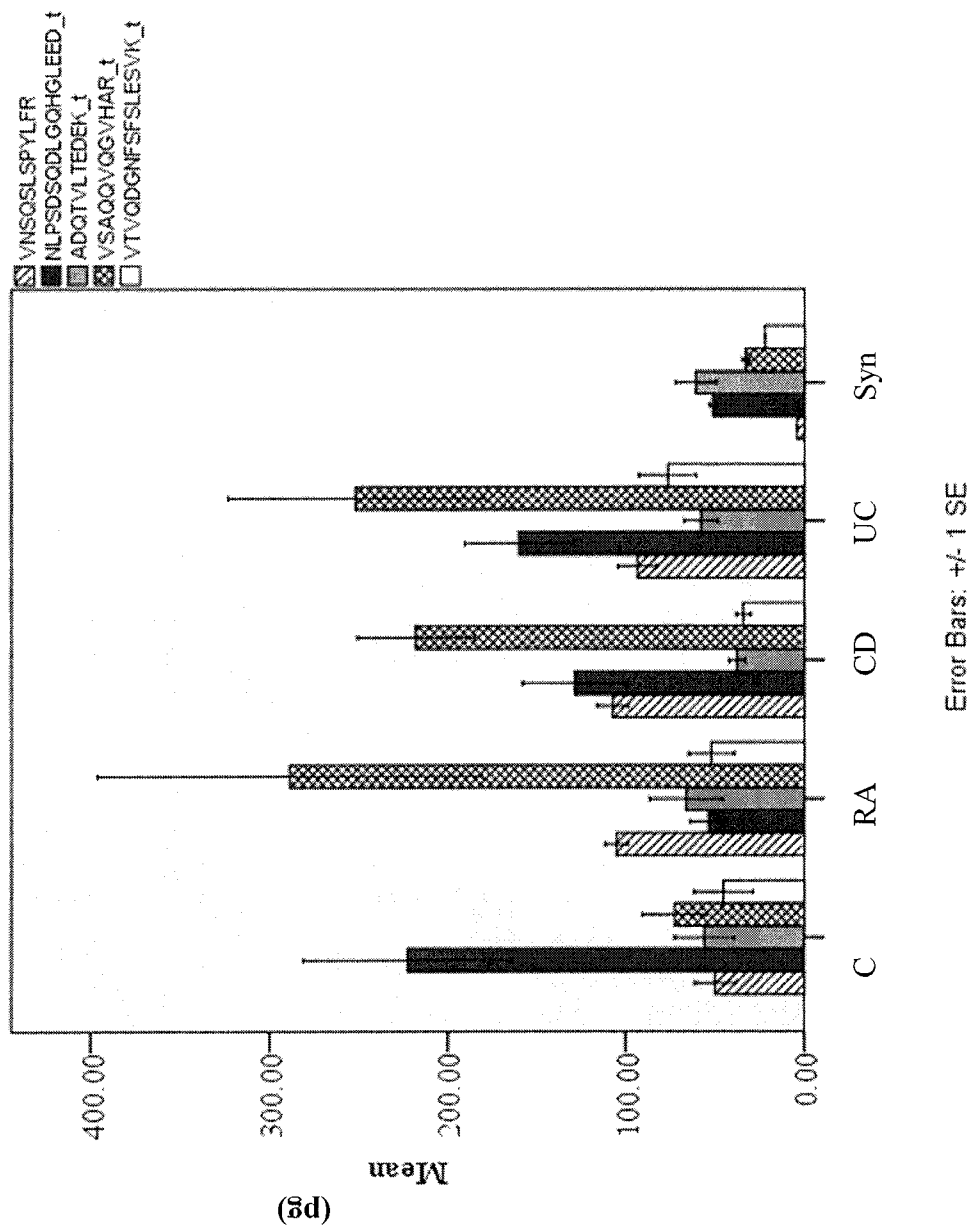


FIGURE 7

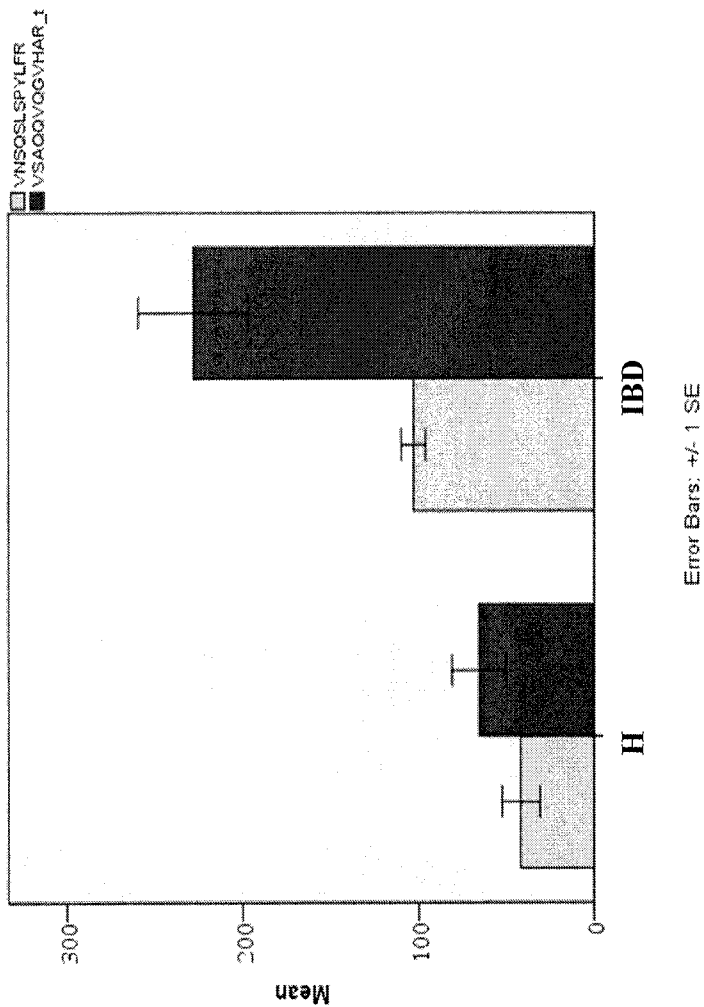


FIGURE 8

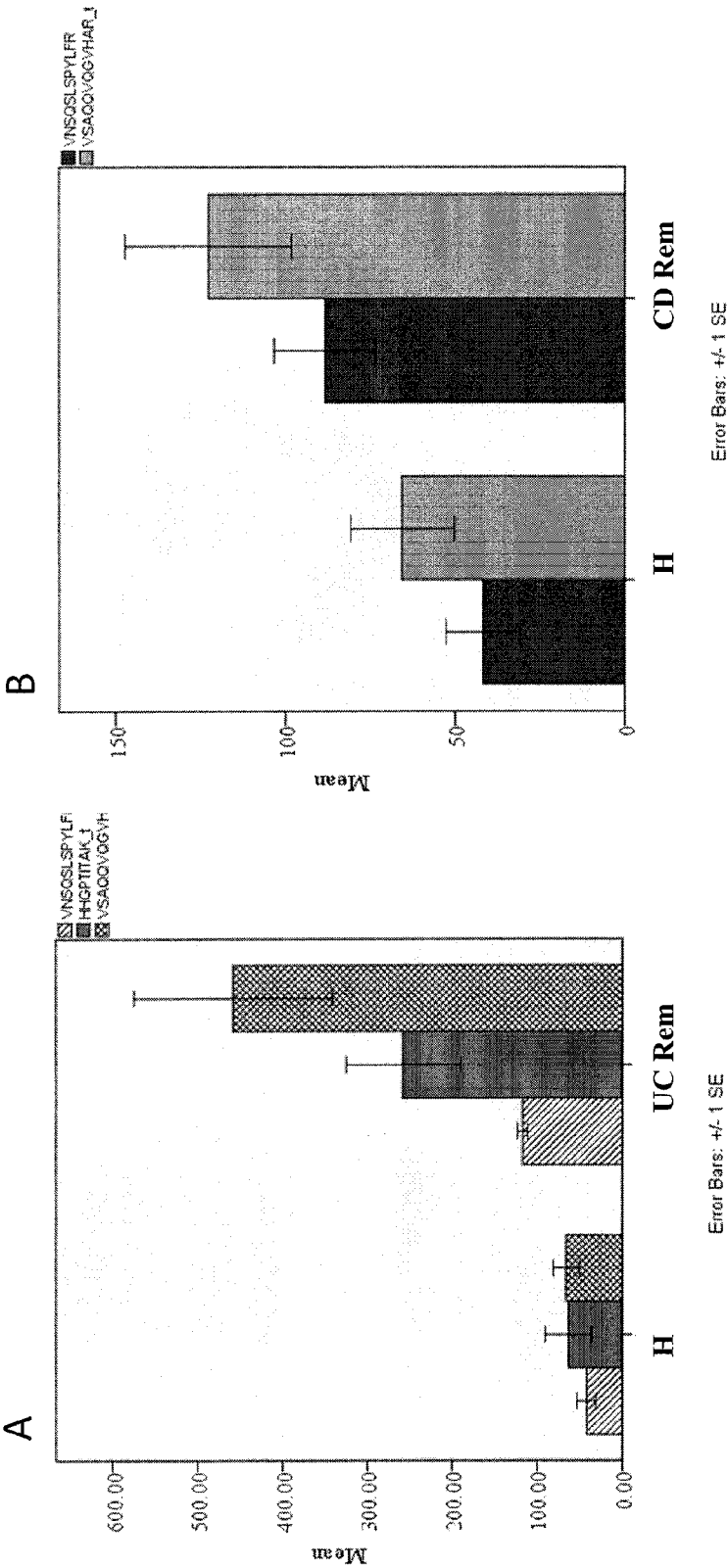


FIGURE 9

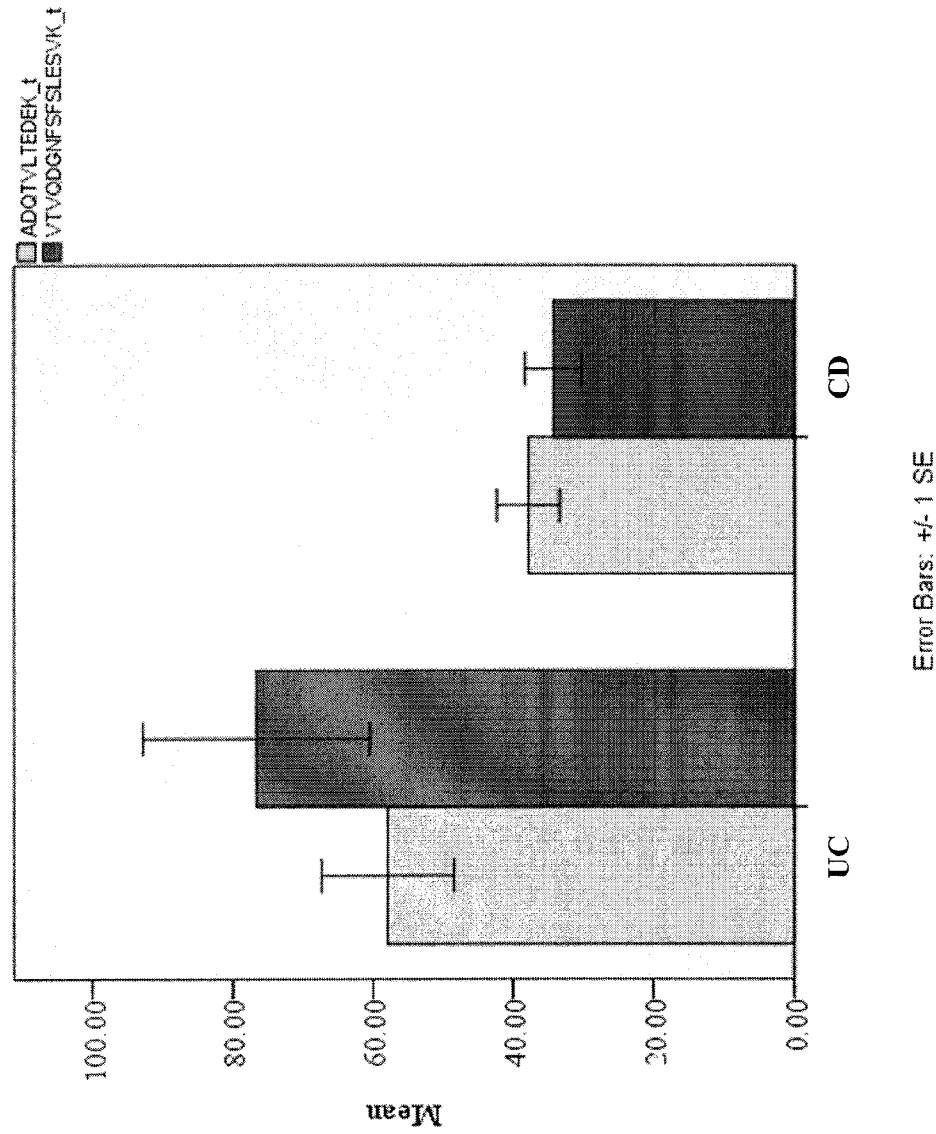


FIGURE 10

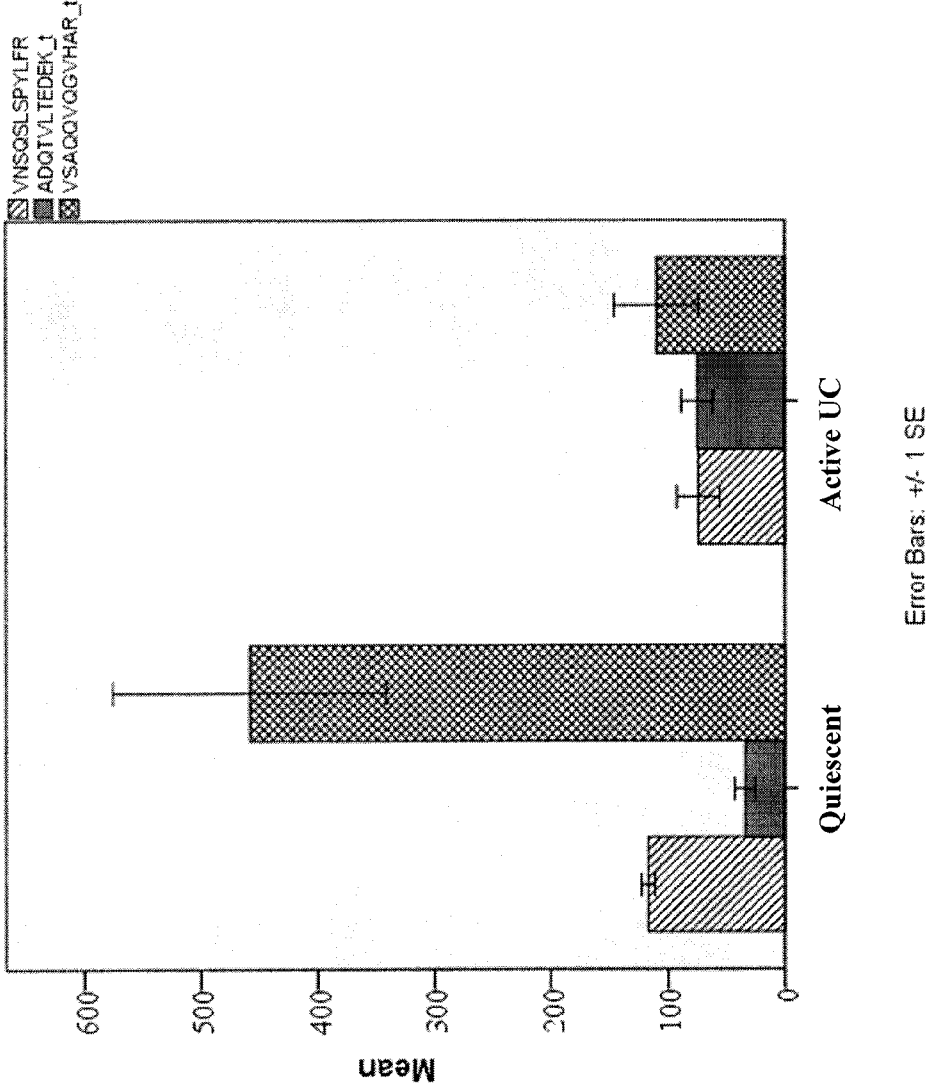


FIGURE 11

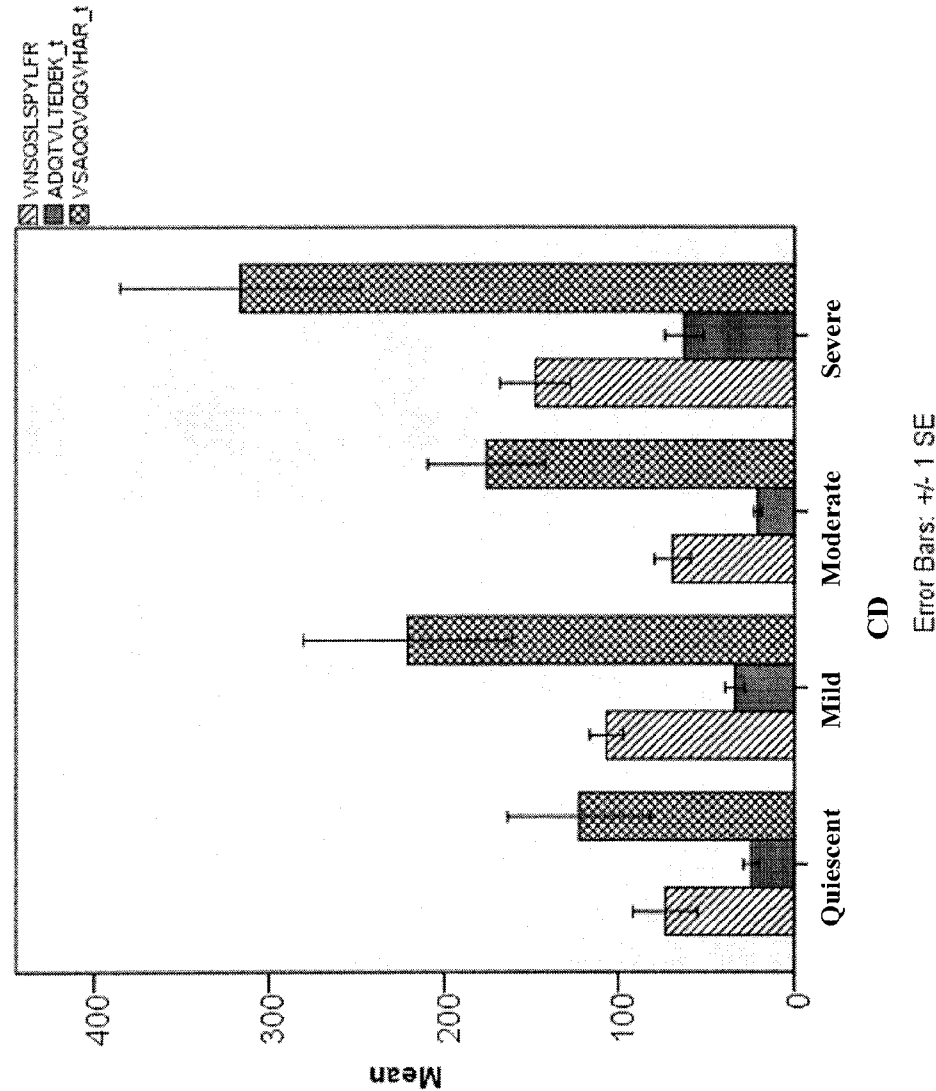
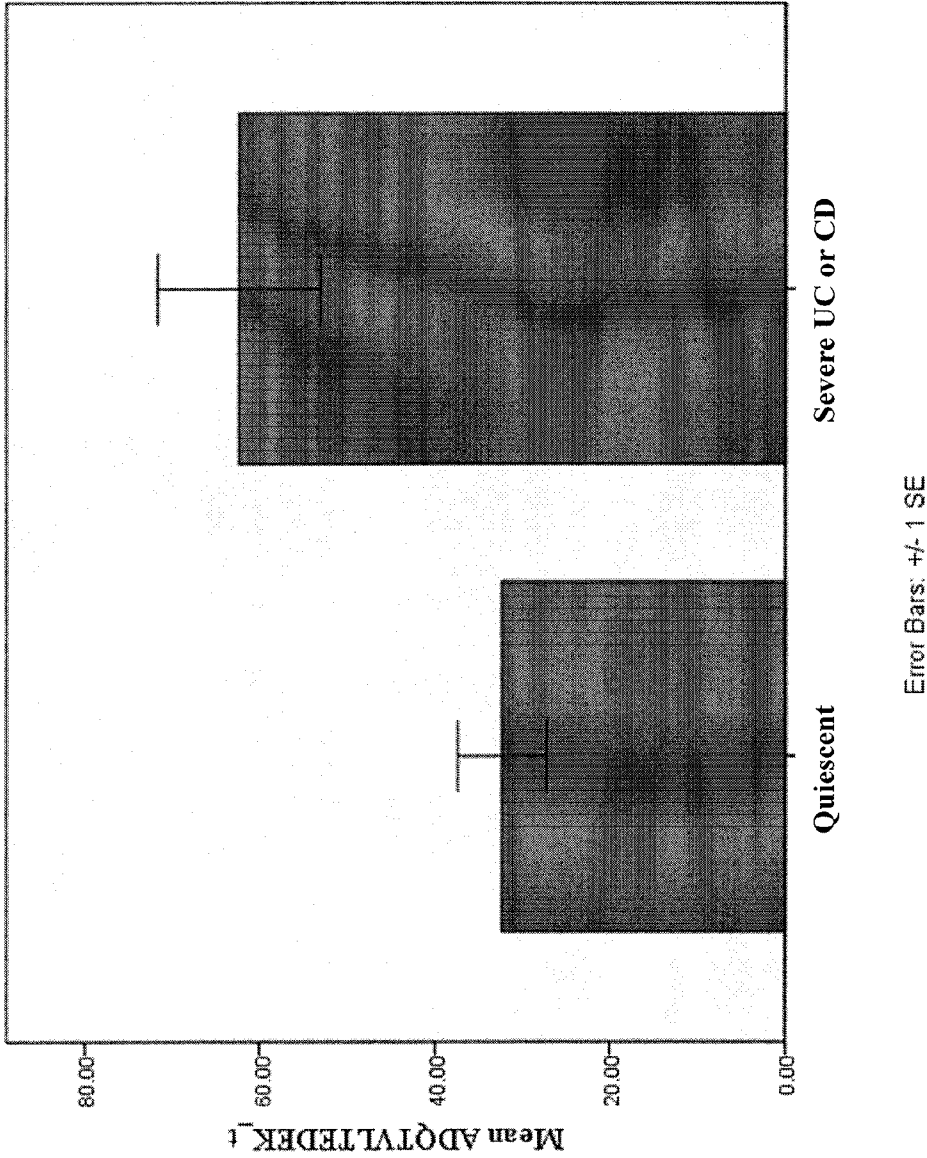


FIGURE 12



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2014/000682

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/50 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, HCAPLUS, BIOSIS, EMBASE, WPI, EPODOC: Ulcerative colitis, inflammatory bowel disease, IBD, colitis, Crohn's, Crohns, Bowel disease, colon disease, disease, Diagnosis, prognosis, biomarker, signature, biosignature, secretogranin-1, chromogranin-B, NM001819, NP001810, CHGB, SCG1, SG1, chromogranin, secretogranin, guanylin, preproguanylin, guanylate cyclase activator, GUCA2A, SPP24, secreted phosphoprotein 2, secreted phosphoprotein 24, NM006944, NP008875, AMBP, alpha-1-microglobulin, bikunin, NM001633, NP001624, alpha-1-microglycoprotein, proteinHC, serglycin, secretory granule proteoglycan core protein, proteoglycan19, Prgl, FLJ12930, Hematopoietic proteoglycan core protein, MGC9289, Platelet proteoglycan core protein, proteoglycan peptide core protein and similar terms and synonyms. GENOMEQUEST SEQUENCE SEARCH: SEQ IDS: 2, 4, 6, 7, 9, 11. Inventor name search performed in Google and Pubmed: WASINGER V C, WASINGER VALERIE CHRISTINE, LEONG RUPERT WING-LOONG, WASINGER AND LEONG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 16 September 2014		Date of mailing of the international search report 16 September 2014	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer Susan MacKenzie AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832690	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2014/000682
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STRID, H. et al. 'Fecal chromogranins and secretogranins are increased in patients with ulcerative colitis but are not associated with disease activity', Journal of Crohn's and Colitis, 2013, Vol. 7, e615-e622 See abstract	1, 2, 11-13, 20-22, 24, 25
X	US 2009/0258848 A1 (CHAKRAVARTI et al) 15 October 2009 See page 4, Table 5, Marker 151	1, 3
X	DERICI, U. et al. 'Does the urinary excretion of α 1-microglobulin and albumin predict clinical disease activity in ulcerative colitis?', Advances in Therapy, 2008, Vol. 25, pages 1342-1352 see Fig. 1, abstract, Table 2	1, 6, 24
X	WO 2001/029269 A2 (CASE WESTERN RESERVE UNIVERISTY) 26 April 2001 See page 56	1, 7, 24
X	WO 2008/021290 A2 (HOMESTEAD CLINICAL CORPORATION et al) 21 February 2008 SEQ ID NOS: 71099, 71100, 665560, 2312, 6011 comprise SEQ IDs: 7, 6, 11, 9 and 2 respectively	24
A	HAN, N.Y. et al., 'Quantitative proteomic approaches in biomarker discovery of inflammatory bowel disease', Journal of Digestive Diseases, 2012, Vol. 13, pages 497-503 Whole document	1-26
A	M'KOMA, A.E. et al., 'Proteomic profiling of mucosal and submucosal colonic tissues yields protein signatures that differentiate the inflammatory colitides', Inflammatory Bowel Disease, 2011, Vol. 17, pages 875-883 Whole document	1-26

Form PCT/ISA/210 (fifth sheet) (July 2009)

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- (1) Claims 1, 2, 21 (all in full), 11-13, 15, 16, 17, 19, 20, 22, 24, 26 (all in part). A method of diagnosing an IBD comprising comparing the level of secretogranin-1 to a reference value and related device.
- (2) Claims 1, 3, 9, 18 (all in full), 13, 15, 16, 17, 24, 25, 26 (all in part). A method of diagnosing an IBD comprising comparing the level of guanylin to a reference value and related device.
- (3) Claims 1, 4, 5, 8, 10, 23 (all in full), 11- 15, 19, 20, 22, 24, 26 (all in part). A method of diagnosing an IBD comprising comparing the level of SPP 24 to a reference value and related device.
- (4) Claims 1, 6 (all in full), 13, 15, 16, 24 (all in part). A method of diagnosing an IBD comprising comparing the level of AMBP to a reference value and related device.
- (5) Claims 1, 7 (all in full), 13, 14, 24 (all in part). A method of diagnosing an IBD comprising comparing the level of serglycin to a reference value and related device.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is the concept of the diagnosis of inflammatory bowel disease using protein or peptide biomarkers. However this feature does not make a contribution over the prior art because it is disclosed in many documents in the art including the X category documents cited in the International Search Report, in addition to the following documents:

HAN, N.Y. et al., 'Quantitative proteomic approaches in biomarker discovery of inflammatory bowel disease', Journal of Digestive Diseases, 2012, Vol. 13, pages 497-503

M'KOMA, A.E. et al., 'Proteomic profiling of mucosal and submucosal colonic tissues yields protein signatures that differentiate the inflammatory colitides', Inflammatory Bowel Disease, 2011, Vol. 17, pages 875-883

Each of these documents disclose methods of diagnosing or assessing inflammatory bowel disease comprising analysing protein or peptide biomarkers in samples and comparing to control reference samples.

Therefore in the light of the prior art this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2014/000682	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
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End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			