METHODS OF USING SMAD3 AND JAK2 GENETIC VARIANTS TO DIAGNOSE AND PREDICT INFLAMMATORY BOWEL DISEASE

Abstract: Disclosed are methods of diagnosing Inflammatory Bowel Disease by determining the presence or absence of genetic variants at SMAD3 and/or JAK2 loci. Provided is a method of diagnosing a Crohn's Disease subtype in an individual by determining the presence or absence of a risk variant at the SMAD3 and/or JAK2 loci.
METHODS OF USING SMAD3 AND JAK2 GENETIC VARIANTS TO DIAGNOSE AND PREDICT INFLAMMATORY BOWEL DISEASE

BACKGROUND

All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

Crohn's disease (CD) and ulcerative colitis (UC), the two common forms of idiopathic inflammatory bowel disease (IBD), are chronic, relapsing inflammatory disorders of the gastrointestinal tract. Each has a peak age of onset in the second to fourth decades of life and prevalences in European ancestry populations that average approximately 100-150 per 100,000 (D.K. Podolsky. N Engl J Med 347, 417 (2002); E.V. Loftus, Jr., Gastroenterology 126, 1504 (2004)). Although the precise etiology of IBD remains to be elucidated, a widely accepted hypothesis is that ubiquitous, commensal intestinal bacteria trigger an inappropriate, overactive, and ongoing mucosal immune response that mediates intestinal tissue damage in genetically susceptible individuals (D.K. Podolsky, N Engl J Med 347, 417 (2002)). Genetic factors play an important role in JBD pathogenesis, as evidenced by the increased rates of IBD in Ashkenazi Jews, familial aggregation of IBD, and increased concordance for IBD in monozygotic compared to dizygotic twin pairs (S. Vermeire, P. Rutgeerts, Genes Immun 6, 637 (2005)). Moreover, genetic analyses have linked IBD to specific genetic variants, especially CARD15 variants on chromosome 16q12 and the IBD5 haplotype (spanning the organic cation transporters, SLC22A4 and SLC22A5, and other genes) on chromosome 5q31 (S. Vermeire, P. Rutgeerts, Genes Immun 6, 637 (2005); J.P. Hugot et al., Nature 411, 599 (2001); Y. Ogura et al., Nature 411, 603 (2001); J.D. Rioux et al., Nat Genet 29, 223 (2001); V.D. Peltekova et al., Nat Genet 36, 471 (2004)).

CD and UC are thought to be related disorders that share some genetic susceptibility loci but differ at others. The replicated associations between CD and variants in CARD15 and the IBD5 haplotype do not fully explain the genetic risk for CD. Thus, there is need in the art to determine
other genes, allelic variants and/or haplotypes that may assist in explaining the genetic risk, diagnosing, and/or predicting susceptibility for or protection against inflammatory bowel disease including but not limited to CD and/or UC.

SUMMARY OF THE INVENTION

Various embodiments include a method of diagnosing susceptibility to Inflammatory Bowel Disease (IBD) in an individual, comprising obtaining a sample from the individual, assaying the sample to determine the presence or absence of a risk haplotype at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus, and diagnosing susceptibility to IBD in the individual based on the presence of a risk haplotype at the JAK2 genetic locus and/or SMAD3 genetic locus. In another embodiment, the IBD comprises Crohn’s disease. In another embodiment, the risk haplotype at the JAK2 genetic locus comprises JAK2 Block 1 Haplotype 1, JAK2 Block 2 Haplotype 1, and/or JAK2 Block 3 Haplotype 3. In another embodiment, the risk haplotype at the JAK2 genetic locus comprises SEQ. ID. NO.: 1, SEQ. ID. NO.: 2, SEQ. ID. NO.: 3, SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6 and/or SEQ. ID. NO.: 7. In another embodiment, the risk haplotype at the SMAD3 genetic locus comprises SMAD3 Block 2 Haplotype 4, SMAD3 Block 5 Haplotype 1 and/or SMAD3 Block 6 Haplotype 1. In another embodiment, the risk haplotype at the SMAD3 genetic locus comprises SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13 and/or SEQ. ID. NO.: 14. In another embodiment, the risk haplotype at the SMAD3 genetic locus comprises SEQ. ID. NO.: 15 and/or SEQ. ID. NO.: 16. In another embodiment, the risk haplotype at the SMAD3 genetic locus comprises SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, SEQ. ID. NO.: 20, SEQ. ID. NO.: 21, SEQ. ID. NO.: 22 and/or SEQ. ID. NO.: 23.

Other embodiments include a method of determining a low probability of developing Crohn's disease in an individual, relative to a healthy subject, comprising obtaining a sample from the individual, assaying the sample to determine the presence or absence of a protective haplotype at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus, and diagnosing a low probability of developing Crohn's disease in the individual, relative to a healthy subject, based upon the presence of the protective haplotype at the JAK2 and/or SMAD3 genetic locus. In another embodiment, the protective haplotype at the JAK2
genetic locus comprises JAK2 Block 1 Haplotype 3, JAK2 Block 2 Haplotype 2, and/or JAK2 Block 3 Haplotype 1. In another embodiment, the protective haplotype at the SIV1AD3 genetic locus comprises SMAD3 Block 4 Haplotype 1, SMAD3 Block 5 Haplotype 2, and/or SMAD3 Block 6 Haplotype 2.

Other embodiments include a method of diagnosing a Crohn’s disease subtype in an individual, comprising determining the presence of one or more risk variants at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus, and diagnosing the Crohn’s disease subtype in the individual based upon the presence of the one or more risk variants at the JAK2 and/or SIV1AD3 genetic locus. In another embodiment, the one or more risk haplotypes at the JAK2 genetic locus comprises SEQ. ID. NO.: 1. In another embodiment, the one or more risk variants at the JAK2 genetic locus comprises JAK2 Block 1 Haplotype 1, JAK2 Block 2 Haplotype 1, and/or JAK2 Block 3 Haplotype 3. In another embodiment, the one or more risk variants at the SMAD3 genetic locus comprises SMAD3 Block 2 Haplotype 4, SMAD3 Block 5 Haplotype 1, and/or SMAD3 Block 6 Haplotype 1.

Other embodiments include a method of treating Crohn’s disease in an individual, comprising determining the presence of a risk variant at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus, and treating the individual based upon the presence of the risk variant at the JAK2 genetic locus and/or SMAD3 genetic locus.

Various embodiments include a method of determining the prognosis of Crohn’s disease in an individual, comprising determining the presence or absence of one or more risk variants at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus, and prognosing a complicated case of Crohn’s disease if the individual demonstrates the presence of one or more risk variants at the JAK3 genetic locus and/or SMAD3 genetic locus. In another embodiment, the one or more risk variants at the JAK2 genetic locus comprises JAK2 Block 1 Haplotype 1, JAK2 Block 2 Haplotype 1, and/or JAK2 Block 3 Haplotype 3. In another embodiment, the one or more risk variants at the SMAD3 genetic locus comprises SMAD3 Block 2 Haplotype 4, SMAD3 Block 5 Haplotype 1, and/or SMAD3 Block 6 Haplotype 1.

Other embodiments include a method of treating Crohn’s Disease in an individual, comprising determining the presence of a risk variant at the janus kinase 2 (JAK2) genetic locus in the individual, and treating the individual by inhibiting the JAK2 signaling pathway. In another embodiment, the risk variant at the JAK2 genetic locus comprises SEQ. ID. NO.: 1.
Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various embodiments of the invention.

**BRIEF DESCRIPTION OF THE FIGURES**

Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive. Figure 1 depicts, in accordance with an embodiment described herein, a haplotype map and structure of SMAD3, including SMAD3 Blocks 1 - 3 and corresponding SNPs.

**DESCRIPTION OF THE INVENTION**

All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology 3rd ed.*, J. Wiley & Sons (New York, NY 2001); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure 5th ed.*, J. Wiley & Sons (New York, NY 2001); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual 3rd ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2001), provide one skilled in the art with a general guide to many of the terms used in the present application.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. indeed, the present invention is in no way limited to the methods and materials described.

“SMAD3” as used herein refers to SMAD family member 3.

"JAK.2" as used herein refers to Janus kinase 2 (a protein tyrosine kinase).

“Haplotype” as used herein refers to a set of single nucleotide polymorphisms (SNPs) on a gene or chromatid that are statistically associated.

"GWAS’’ as used herein means Genome-Wide Association Study.

"Risk" as used herein refers to an increase in susceptibility to IBD, including but not limited to CD and UC.
"Protective" and "protection" as used herein refer to a decrease in susceptibility to IBD, including but not limited to CD and UC.

"CD" and "UC" as used herein refer to Crohn's disease and ulcerative colitis, respectively.

"F_A" as used herein means frequency in CD.

"F_U" as used herein means frequency in controls.

"P" as used herein means the P value for that association.

"PAR" as used herein refers to population attributable risk, including an estimation of the proportion of cases in the population attributable to the given risk factor.

As used herein, "treatment" or "treating" should be understood to include any indicia of success in the treatment, alleviation or amelioration of an injury, pathology or condition. This may include but not limited to parameters such as abatement, remission, diminishing of symptoms, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating; improving a patient's physical or mental well-being, or preventing the onset of disease, such as Crohn's disease.

As used herein, "diagnose" or "diagnosis" refers to determining the nature or the identity of a condition or disease. A diagnosis may be accompanied by a determination as to the severity of the disease.

As used herein, "prognostic" or "prognosis" refers to predicting the outcome or prognosis of a disease.

As used herein, the term "biological sample" means any biological material from which nucleic acid molecules can be prepared. As non-limiting examples, the term material encompasses whole blood, plasma, saliva, cheek swab, or other bodily fluid or tissue that contains nucleic acid.

The inventors performed a genome-wide association study testing autosomal single nucleotide polymorphisms (SNPs) on the Illumina HumanHap300 Genotyping BeadChip. Based on these studies, the inventors found single nucleotide polymorphisms (SNPs) and haplotypes that are associated with increased or decreased risk for inflammatory bowel disease, including but not limited to CD. These SNPs and haplotypes are suitable for genetic testing to identify at risk individuals and those with increased risk for complications associated with serum expression of Anti-Saccharomyces cerevisiae antibody, and antibodies to OmpC, and CbiR. The
detection of protective and risk SNPs and/or haplotypes may be used to identify at risk individuals predict disease course and suggest the right therapy for individual patients. Additionally, the inventors have found both protective and risk allelic variants for Crohn's Disease and Ulcerative Colitis.

Based on these findings, embodiments of the present invention provide for methods of diagnosing and/or predicting susceptibility for or protection against inflammatory bowel disease including but not limited to Crohn's Disease and ulcerative colitis. Other embodiments provide for methods of prognosing inflammatory bowel disease including but not limited to Crohn's Disease and ulcerative colitis. Other embodiments provide for methods of treating inflammatory bowel disease including but not limited to Crohn's Disease and ulcerative colitis.

The methods may include the steps of obtaining a biological sample containing nucleic acid from the individual and determining the presence or absence of a SNP and/or a haplotype in the biological sample. The methods may further include correlating the presence or absence of the SNP and/or the haplotype to a genetic risk, a susceptibility for inflammatory bowel disease including but not limited to Crohn's Disease and ulcerative colitis, as described herein. The methods may also further include recording whether a genetic risk, susceptibility for inflammatory bowel disease including but not limited to Crohn's Disease and ulcerative colitis exists in the individual. The methods may also further include a prognosis of inflammatory bowel disease based upon the presence or absence of the SNP and/or haplotype. The methods may also further include a treatment of inflammatory bowel disease based upon the presence or absence of the SNP and/or haplotype.

In one embodiment, a method of the invention is practiced with whole blood, which can be obtained readily by non-invasive means and used to prepare genomic DNA, for example, for enzymatic amplification or automated sequencing. In another embodiment, a method of the invention is practiced with tissue obtained from an individual such as tissue obtained during surgery or biopsy procedures.

As disclosed herein, the inventors constructed haplotypes for both the SMAD3 and JAK2 genetic loci and tested for associations in Crohn's Disease subjects. As described in Tables 1-6 herein, various haplotypes and variants were found to have statistically significant associations with Crohn's Disease.
In one embodiment, the present invention provides a method of diagnosing susceptibility to Inflammatory Bowel Disease (IBD) in an individual by determining the presence or absence of a risk variant at the SMAD3 and/or JAK2 genetic locus, where the presence of the risk variant at the SMAD3 and/or JAK2 genetic locus is indicative of susceptibility to IBD in the individual.

In one embodiment, the present invention provides a method of diagnosing a Crohn’s Disease (CD) subtype in an individual by determining the presence or absence of a risk variant at the SMAD3 and/or JAK2 genetic locus, where the presence of the risk variant at the SMAD3 and/or JAK2 genetic locus is indicative of the CD subtype in the individual.

In one embodiment, the present invention provides a method of treating CD in an individual by determining the presence of one or more risk variants at the SMAD3 and/or JAK2 genetic locus, and treating the individual.

A variety of methods can be used to determine the presence or absence of a variant allele or haplotype. As an example, enzymatic amplification of nucleic acid from an individual may be used to obtain nucleic acid for subsequent analysis. The presence or absence of a variant allele or haplotype may also be determined directly from the individual’s nucleic acid without enzymatic amplification.

Analysis of the nucleic acid from an individual, whether amplified or not, may be performed using any of various techniques. Useful techniques include, without limitation, polymerase chain reaction based analysis, sequence analysis and electrophoretic analysis. As used herein, the term "nucleic acid" means a polynucleotide such as a single or double-stranded DNA or RNA molecule including, for example, genomic DNA, cDNA and mRNA. The term nucleic acid encompasses nucleic acid molecules of both natural and synthetic origin as well as molecules of linear, circular or branched configuration representing either the sense or antisense strand, or both, of a native nucleic acid molecule.

The presence or absence of a variant allele or haplotype may involve amplification of an individual’s nucleic acid by the polymerase chain reaction. Use of the polymerase chain reaction for the amplification of nucleic acids is well known in the art (see, for example, Mullis et al. (Eds.), The Polymerase Chain Reaction, Birkhauser, Boston, (1994)).

A TaqmanB allelic discrimination assay available from Applied Biosystems may be useful for determining the presence or absence of a variant allele. In a TaqmanB allelic discrimination assay, a specific, fluorescent, dye-labeled probe for each allele is constructed. The
probes contain different fluorescent reporter dyes such as FAIVI and VICTM to differentiate the amplification of each allele. In addition, each probe has a quencher dye at one end which quenches fluorescence by fluorescence resonant energy transfer (FRET). During PCR, each probe anneals specifically to complementary sequences in the nucleic acid from the individual.

The 5′ nuclease activity of Taq polymerase is used to cleave only probe that hybridize to the allele. Cleavage separates the reporter dye from the quencher dye, resulting in increased fluorescence by the reporter dye. Thus, the fluorescence signal generated by PCR amplification indicates which alleles are present in the sample. Mismatches between a probe and allele reduce the efficiency of both probe hybridization and cleavage by Taq polymerase, resulting in little to no fluorescent signal. Improved specificity in allelic discrimination assays can be achieved by conjugating a DNA minor groove binder (MGB) group to a DNA probe as described, for example, in Kutyavin et al., "3′-minor groove binder-DNA probes increase sequence specificity at PCR extension temperature," Nucleic Acids Research 28:655-661 (2000)). Minor groove binders include, but are not limited to, compounds such as dihydrocyclopyrroloindole tripeptide (DPI).

Sequence analysis also may also be useful for determining the presence or absence of a variant allele or haplotype.

Restriction fragment length polymorphism (RFLP) analysis may also be useful for determining the presence or absence of a particular allele (Jarcho et al. in Dracopoii et al., Current Protocols in Human Genetics pages 2.7.1-2.7.5, John Wiley & Sons, New York; Innis et al., (Ed.), PCR Protocols, San Diego: Academic Press, Inc. (1990)). As used herein, restriction fragment length polymorphism analysis is any method for distinguishing genetic polymorphisms using a restriction enzyme, which is an endonuclease that catalyzes the degradation of nucleic acid and recognizes a specific base sequence, generally a palindrome or inverted repeat. One skilled in the art understands that the use of RFLP analysis depends upon an enzyme that can differentiate two alleles at a polymorphic site.

Allele-specific oligonucleotide hybridization may also be used to detect a disease-predisposing allele. Allele-specific oligonucleotide hybridization is based on the use of a labeled oligonucleotide probe having a sequence perfectly complementary, for example, to the sequence encompassing a disease-predisposing allele. Under appropriate conditions, the allele-specific probe hybridizes to a nucleic acid containing the disease-predisposing allele but does not hybridize to the one or more other alleles, which have one or more nucleotide mismatches as
compared to the probe. If desired, a second allele-specific oligonucleotide probe that matches an alternate allele also can be used. Similarly, the technique of allele-specific oligonucleotide amplification can be used to selectively amplify, for example, a disease-predisposing allele by using an allele-specific oligonucleotide primer that is perfectly complementary to the nucleotide sequence of the disease-predisposing allele but which has one or more mismatches as compared to other alleles (jViullis et al., supra, (1994)). One skilled in the art understands that the one or more nucleotide mismatches that distinguish between the disease-predisposing allele and one or more other alleles are preferably located in the center of an allele-specific oligonucleotide primer to be used in allele-specific oligonucleotide hybridization. In contrast, an allele-specific oligonucleotide primer to be used in PCR amplification preferably contains the one or more nucleotide mismatches that distinguish between the disease-associated and other alleles at the 3' end of the primer.

A heteroduplex mobility assay (HMA) is another well known assay that may be used to detect a SNP or a haplotype. HMA is useful for detecting the presence of a polymorphic sequence since a DNA duplex carrying a mismatch has reduced mobility in a polyacrylamide gel compared to the mobility of a perfectly base-paired duplex (Delwart et al., Science 262:1257-1261 (1993); White et al., Genomics 12:301-306 (1992)).

The technique of single strand conformational, polymorphism (SSCP) also may be used to detect the presence or absence of a SNP and/or a haplotype (see Hayashi, K., Methods Applic. 1:34-38 (1991)). This technique can be used to detect mutations based on differences in the secondary structure of single-strand DNA that produce an altered electrophoretic mobility upon non-denaturing gel electrophoresis. Polymorphic fragments are detected by comparison of the electrophoretic pattern of the test fragment to corresponding standard fragments containing known alleles.

Denaturing gradient gel electrophoresis (DGGE) also may be used to detect a SNP and/or a haplotype. In DGGE, double-stranded DNA is electrophoresed in a gel containing an increasing concentration of denaturant; double-stranded fragments made up of mismatched alleles have segments that melt more rapidly, causing such fragments to migrate differently as compared to perfectly complementary sequences (Sheffield et al., "Identifying DNA Polymorphisms by Denaturing Gradient Gel Electrophoresis" in Innis et al., supra, 1990).
Other molecular methods useful for determining the presence or absence of a SNP and/or a haplotype are known in the art and useful in the methods of the invention. Other well-known approaches for determining the presence or absence of a SNP and/or a haplotype include automated sequencing and RNAase mismatch techniques (Winter et al., Proc. Natl. Acad. Sci. 82:7575-7579 (1985)). Furthermore, one skilled in the art understands that, where the presence or absence of multiple alleles or haplotype(s) is to be determined, individual alleles can be detected by any combination of molecular methods. See, in general, Birren et al. (Eds.) Genome Analysis: A Laboratory Manual Volume 1 (Analyzing DNA) New York, Cold Spring Harbor Laboratory Press (1997). In addition, one skilled in the art understands that multiple alleles can be detected in individual reactions or in a single reaction (a "multiplex" assay). In view of the above, one skilled in the art realizes that the methods of the present invention for diagnosing or predicting susceptibility to or protection against CD in an individual may be practiced using one or any combination of the well known assays described above or another art-recognized genetic assay.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following terms are defined below.

EXAMPLES

The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

Example 1

Table 1 - Significant JAK2 haplotypes and variants
Table 1 describes various JAK2 haplotypes with statistically significant associations. The "B" corresponds with the Block number, and the "H" corresponds with the Haplotype number.

<table>
<thead>
<tr>
<th>JAK2 haplotypes of JAK2 from Cedar's</th>
<th>Case</th>
<th>Control</th>
<th>P</th>
<th>OR</th>
<th>PAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1H1carrier</td>
<td>0.633</td>
<td>0.640</td>
<td>1.8×10^{-2}</td>
<td>1.2</td>
<td>11.30%</td>
</tr>
<tr>
<td>B1H3carrier</td>
<td>0.466</td>
<td>0.509</td>
<td>2.7×10^{-2}</td>
<td>0.8</td>
<td>-10.20%</td>
</tr>
<tr>
<td>B2H1carrier</td>
<td>0.630</td>
<td>0.591</td>
<td>4×10^{-2}</td>
<td>1.2</td>
<td>10.69%</td>
</tr>
<tr>
<td>B2H2homozygotes</td>
<td>0.115</td>
<td>0.150</td>
<td>6.2×10^{-3}</td>
<td>0.7</td>
<td>-4.50%</td>
</tr>
<tr>
<td>B3H1carrier</td>
<td>0.864</td>
<td>0.710</td>
<td>1.1×10^{-2}</td>
<td>0.8</td>
<td>-14.20%</td>
</tr>
<tr>
<td>B3H3carrier</td>
<td>0.496</td>
<td>0.451</td>
<td>2×10^{-2}</td>
<td>1.2</td>
<td>8.30%</td>
</tr>
</tbody>
</table>

Example 2

Table 2 – Haplotype information of JAK2

Table 2.

The JAK2 haplotypes referenced in Table 1 above and herein are defined in Table 2 below, where JAK2 Blocks 1-3 and haplotypes 1-3 are defined by listed SNPs and the corresponding allele. The "B" corresponds with the Block number, and "H" corresponds with the Haplotype number.

<table>
<thead>
<tr>
<th>Block</th>
<th>SNPs</th>
<th>Haplotype 1 Alleles</th>
<th>Haplotype 2 Alleles</th>
<th>Haplotype 3 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rs3808850 [SEQ. ID. NO.: 2]</td>
<td>T</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Rs1887429 [SEQ. ID. NO.: 3]</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>Rs2274471 [SEQ. ID. NO.: 4]</td>
<td>A</td>
<td>A</td>
<td>G</td>
</tr>
</tbody>
</table>
Table 3 - Significant SMAD3 haplotypes and variants

Table 3 describes various SMAD3 haplotypes with statistically significant associations. The "B" corresponds with the Block number, and the "H" corresponds with the Haplotypic number.

<table>
<thead>
<tr>
<th>SMAD3</th>
<th>Case</th>
<th>Control</th>
<th>P</th>
<th>OR</th>
<th>PAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>best SNP from GWAS(meta-analysis)</td>
<td>not identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>haplotypes of SMAD3 from Cedars</td>
<td>B2H4carrier</td>
<td>0.129</td>
<td>0.100</td>
<td>$2 \times 10^2$</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>B4H1carrier</td>
<td>0.753</td>
<td>0.793</td>
<td>$1.5 \times 10^2$</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>B5H1carrier</td>
<td>0.738</td>
<td>0.691</td>
<td>$1.1 \times 10^2$</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>B5H2carrier</td>
<td>0.478</td>
<td>0.531</td>
<td>$4.3 \times 10^3$</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>B6H1carrier</td>
<td>0.504</td>
<td>0.452</td>
<td>$8.2 \times 10^3$</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>B6H2homo</td>
<td>0.051</td>
<td>0.082</td>
<td>$1.2 \times 10^3$</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Example 4

Table 4(a) - 4(d) - Haplotype information of SMAD3

Table 4(a).

Table 4(a) describes haplotype information on Block 2 of SMAD3, specifically for SMAD3 Block 2 Haplotype 4. The "B" corresponds with the Block number, and "H" corresponds with the Haplotype number.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs9972423 [SEQ. ID. NO.: 8]</td>
<td>T</td>
</tr>
<tr>
<td>Rs2118611 [SEQ. ID. NO.: 9]</td>
<td>G</td>
</tr>
<tr>
<td>Rs11071933 [SEQ. ID. NO.: 10]</td>
<td>C</td>
</tr>
<tr>
<td>Rs1438386 [SEQ. ID. NO.: 11]</td>
<td>G</td>
</tr>
<tr>
<td>Rs718663 [SEQ. ID. NO.: 12]</td>
<td>G</td>
</tr>
<tr>
<td>Rs7163381 [SEQ. ID. NO.: 13]</td>
<td>A</td>
</tr>
<tr>
<td>Rs920293 [SEQ. ID. NO.: 14]</td>
<td>A</td>
</tr>
</tbody>
</table>

Table 4(b).

Table 4(b) describes haplotype information on Block 4 of SMAD3, specifically SMAD3 Block 4 Haplotype I. The "B" corresponds with the Block number, and "H" corresponds with the Haplotype number.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs745103</td>
<td>A</td>
</tr>
<tr>
<td>Rs12439792</td>
<td>A</td>
</tr>
</tbody>
</table>
Table 4(c).
Table 4(c) describes haplotype information on Block 5 of SMAD3, specifically SMAD3 Block 5 Haplotype 1 and 2. The “B” corresponds with the Block number, and “H” corresponds with the Haplotype number.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Haplotype 1 Alleles</th>
<th>Haplotype 2 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs893473 [SEQ. ID. NO.: 15]</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Rs2289263 [SEQ. ID. NO.: 16]</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>B5H1:GC 0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B5H2:GA 0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B5H3:AA 0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4(d).
Table 4(d) describes haplotype information on Block 6 of SMAD3, specifically SMAD3 Block 6 Haplotype 1 and 2. The "B" corresponds with the Block number, and "H" corresponds with the Haplotype number.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Haplotype 1 Alleles</th>
<th>Haplotype 2 Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs2033785 [SEQ. ID. NO.: 17]</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Rs11637659 [SEQ. ID. NO.: 18]</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>Rs10152307 [SEQ. ID. NO.: 19]</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>Rs4776900 [SEQ. ID. NO.: 19]</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>
Table 5. Additional associations with JAK2 Haplotypes

<table>
<thead>
<tr>
<th>Locus</th>
<th>Hap</th>
<th>F_A</th>
<th>F_U</th>
<th>CHISQ</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2</td>
<td>CTC</td>
<td>0.3959</td>
<td>0.364</td>
<td>5.885</td>
<td>1</td>
<td>0.01527</td>
</tr>
</tbody>
</table>

SNPs that define the alternative JAK2 haplotype: rsl 0758669|rs3808850)rs 1887429

rs10758669: C is the associated allele, other allele is A
rs3808850: T is the associated allele, other allele is T
rsl 887429: C is the associated allele, other allele is A

Table 6. Additional associations with SMAD3 Haplotypes

<table>
<thead>
<tr>
<th>Locus</th>
<th>Hap</th>
<th>F_A</th>
<th>F_U</th>
<th>CHISQ</th>
<th>DF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2</td>
<td>CTC</td>
<td>0.3959</td>
<td>0.364</td>
<td>5.885</td>
<td>1</td>
<td>0.01527</td>
</tr>
</tbody>
</table>

Table 6. Additional associations with SMAD3 Haplotypes

Table 6 describes information on an additional SMAD3 haplotype association.
SNPS that define the alternative SMAD3 haplotype: rs21 1861 | rsl 1071933
rs21 1 861 1: G is the associated allele, other allele is A
rsl 1071933: C is the associated allele, other allele is G

While the description above refers to particular embodiments of the present invention, it
should be readily apparent to people of ordinary skill in the art that a number of modifications
may be made without departing from the spirit thereof. The presently disclosed embodiments
are, therefore, to be considered in all respects as illustrative and not restrictive.

Various embodiments of the invention are described above in the Description of the
Invention. While these descriptions directly describe the above embodiments, it is understood
that those skilled in the art may conceive modifications and/or variations to the specific
embodiments shown and described herein. Any such modifications or variations that fall within
the purview of this description are intended to be included therein as well. Unless specifically
noted, it is the intention of the inventor that the words and phrases in the specification and claims
be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

The foregoing description of various embodiments of the invention known to the
applicant at this time of filing the application has been presented and is intended for the purposes
of illustration and description. The present description is not intended to be exhaustive nor limit
the invention to the precise form disclosed and many modifications and variations are possible in
the light of the above teachings. The embodiments described serve to explain the principles of
the invention and its practical application and to enable others skilled in the art to utilize the
invention in various embodiments and with various modifications as are suited to the particular
use contemplated. Therefore, it is intended that the invention not be limited to the particular
embodiments disclosed for carrying out the invention.

While particular embodiments of the present invention have been shown and described, it
will be obvious to those skilled in the art that, based upon the teachings herein, changes and
modifications may be made without departing from this invention and its broader aspects and,
therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. Furthermore, it is to be understood that the invention is solely defined by the appended claims. It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least." the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles “a” or "an" limits any particular claim containing such introduced claim recitation to inventions containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (e.g., “a” and/or "an" should typically be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should typically be interpreted to mean at least the recited number (e.g., the bare recitation of "two recitations," without other modifiers, typically means at least two recitations, or two or more recitations).

Accordingly, the invention is not limited except as by the appended claims.
CLAIMS

1. A method of diagnosing susceptibility to Inflammatory Bowel Disease (JBD) in an individual, comprising:
   obtaining a sample from the individual;
   assaying the sample to determine the presence or absence of a risk haplotype at the janus kinase 2 (JAK.2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus; and
   diagnosing susceptibility to IBD in the individual based on the presence of a risk haplotype at the JAK2 genetic locus and/or SMAD3 genetic locus.

2. The method of claim 1, wherein JBD comprises Crohn's disease.

3. The method of claim 1, wherein the risk haplotype at the JAK2 genetic locus comprises JAK2 Block 1 Haplotype 1, JAK2 Block 2 Haplotype 1, and/or JAK2 Block 3 Haplotype 3.


5. The method of claim 1, wherein the risk haplotype at the SMAD3 genetic locus comprises SMAD3 Block 2 Haplotype 4, SMAD3 Block 5 Haplotype 1 and/or SMAD3 Block 6 Haplotype 1.


7. The method of claim 1, wherein the risk haplotype at the SMAD3 genetic locus comprises SEQ. ID. NO.: 15 and/or SEQ. ID. NO.: 16.

9. A method of determining a low probability of developing Crohn's disease in an individual, relative to a healthy subject, comprising:
   - obtaining a sample from the individual;
   - assaying the sample to determine the presence or absence of a protective haplotype at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus; and
   - diagnosing a low probability of developing Crohn's disease in the individual, relative to a healthy subject, based upon the presence of the protective haplotype at the JAK2 and/or SMAD3 genetic locus.

10. The method of claim 9, wherein the protective haplotype at the JAK2 genetic locus comprises JAK2 Block 1 Haplotype 3, JAK2 Block 2 Haplotype 2, and/or JAK2 Block 3 Haplotype 1.

11. The method of claim 9, wherein the protective haplotype at the SMAD3 genetic locus comprises SMAD3 Block 4 Haplotype 1, SMAD3 Block 5 Haplotype 2, and/or SMAD3 Block 6 Haplotype 2.

12. A method of diagnosing a Crohn's disease subtype in an individual, comprising:
   - determining the presence of one or more risk variants at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus; and
   - diagnosing the Crohn's disease subtype in the individual based upon the presence of the one or more risk variants at the JAK2 and/or SMAD3 genetic locus.

13. The method of claim 12, wherein the one or more risk haplotypes at the JAK2 genetic locus comprises SEQ. ID. NO.: 1.
14. The method of claim 12, wherein the one or more risk variants at the JAK2 genetic locus comprises JAK2 Block 1 Haplotype 1, JAK2 Block 2 Haplotype 1, and/or JAK2 Block 3 Haplotype 3.

15. The method of claim 12, wherein the one or more risk variants at the SMAD3 genetic locus comprises SMAD3 Block 2 Haplotype 4, SMAD3 Block 5 Haplotype 1, and/or SMAD3 Block 6 Haplotype 1.

16. A method of treating Crohn's disease in an individual, comprising:
   determining the presence of a risk variant at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus; and
   treating the individual based upon the presence of the risk variant at the JAK2 genetic locus and/or SMAD3 genetic locus.

17. A method of determining the prognosis of Crohn's disease in an individual, comprising:
   determining the presence or absence of one or more risk variants at the janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus; and
   prognosing a complicated case of Crohn's disease if the individual demonstrates the presence of one or more risk variants at the JAK3 genetic locus and/or SMAD3 genetic locus.

18. The method of claim 17, wherein the one or more risk variants at the JAK2 genetic locus comprises JAK2 Block 1 Haplotype 1, JAK2 Block 2 Haplotype 1, and/or JAK2 Block 3 Haplotype 3.

19. The method of claim 17, wherein the one or more risk variants at the SMAD3 genetic locus comprises SMAD3 Block 2 Haplotype 4, SMAD3 Block 5 Haplotype 1, and/or SMAD3 Block 6 Haplotype 1.

20. A method of treating Crohn's Disease in an individual, comprising:
   determining the presence of a risk variant at the janus kinase 2 (JAK2) genetic locus in the individual; and
treating the individual by inhibiting the JAK2 signaling pathway.

21. The method of claim 20, wherein the risk variant at the JAK2 genetic locus comprises
SEQ. ID. NO.: 1.
INTERNATIONAL SEARCH REPORT

A CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2010 01)
USPC - 435/6

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)

IPC(8) - C12Q 1/68 (2010 01)
USPC - 435/6

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

Nature Genetics August 2008, Vol 40
Nature Reviews June 2008, Vol 8

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

WEST (PGPBL, USPT, EPAB, JPAB), espOCenet, Google Scholar inflammatory bowel disease, JNK2, SNP, diagnosis, prognosis, locus, block, haplotype, Crohn's disease NCBI SNP rs3808850, rs187429, rs 10758669
GenCore 6.3 SEQ ID NO 1

C DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>BARRETT et al Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease Nature Genetics August 2008, 40(8) 955 - 962, abstract, pg 955, right col, second para, pg 957, right col, second para, pg 959, left col, second para, pg 960, right col</td>
<td>1-2, 4, 12-13</td>
</tr>
<tr>
<td>A</td>
<td>CHO et al The genetics and immunopathogenesis of inflammatory bowel disease Nature Reviews June 2008, 8 458-466</td>
<td>1-2, 4, 9, 12-13, 16-17, 20-21</td>
</tr>
<tr>
<td>X,P</td>
<td>Wang, et al Diverse genome-wide association studies associate the IL12/IL23 pathway with Crohn Disease Am J Hum Genet Epub 26 Feb 2009, 84(3) 399-405</td>
<td>1-2,12, 16-17, 20</td>
</tr>
</tbody>
</table>

D Further documents are listed in the continuation of Box C

* Special categories of cited documents
A* document defining the general state of the art which is not considered to be of particular relevance
E* earlier application or patent but published on or after the international filing date
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)
O* document referring to an oral disclosure, use, exhibition or other means
P* document published after the international filing date
X* later published document 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
Y* 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
A* document member of the same patent family

Date of the actual completion of the international search
29 April 2010 (29 04 2010)

Date of mailing of the international search report
05 MAY 2010

Name and mailing address of the ISA/US
Mail Stop PCT, Attn ISA/US, Commissioner for Patents
P O Box 1450, Alexandria, Virginia 22313-1450
Facsimile No 571-273-3201

Authorized officer
Lee W Young
**INTERNATIONAL SEARCH REPORT**

**International application No**

PCT/US 10/20921

**Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1 \[\] Claims Nos
   because they relate to subject matter not required to be searched by this Authority, namely

2 \[\] Claims Nos
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3 D Claims Nos
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

**Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows

Group A claims 1-21, drawn to a method of method of diagnosing susceptibility to Inflammatory Bowel Disease (IBD) in an individual by determining the presence or absence of a risk haplotype at the Janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus The first invention (claims 1-4, 9-21) encompasses determining a risk haplotype at the JAK2 locus that comprises SEQ ID NO 1 Should an additional fee(s) be paid, Applicant is invited to elect an additional genetic loci and/or SEQ ID NO(s) to be searched The exact claims searched will depend on the specifically elected SEQ ID NO(s)

[Note claims 3, 10-11, 14-15, 18-19 were excluded from Group A, because they are drawn to a non-elected subject matter]

--- Please see continuation on attached additional sheet ---

1 \[\] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2 \[\] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees

3 \[\] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos

4 X No required additional search fees were timely paid by the applicant Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos Claims 1-2, 4, 9, 12-13, 16-17, 20-21 limited to SEQ ID NO 1

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation
- No protest accompanied the payment of additional search fees

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)
Continuation of Box (III) - Lack of Unity

The inventions listed as Group I do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I share the technical feature of diagnosing susceptibility to Inflammatory Bowel Disease (IBD) in an individual by determining the presence or absence of a risk haplotype at the Janus kinase 2 (JAK2) genetic locus and/or SMAD family member 3 (SMAD3) genetic locus. However, this shared technical feature is obvious over prior art. Specifically, a paper titled "The genetics and immunopathogenesis of inflammatory bowel disease" by Cho (Nat Rev Immunol, June 2008, 8(6):458-466) discloses that "[in a large meta-analysis of [Genome-wide association] studies of cohorts of European and North American ancestry, more than 30 independent genomic regions showed significant and repeated evidence for association with Crohn's disease. Of the over 30 regions showing the highest level of association, four have a role in IL23R signaling - IL23R, IL12B, STAT3 and JAK2" (pg 461, col. 2). As said association of the JAK2 locus was known at the time of the invention, the claimed method of diagnosing susceptibility to Inflammatory Bowel Disease (IBD) would have been obvious, and thus cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Group I is the specific nucleic acid sequence recited therein. The inventions do not share a special technical feature, because 1) nucleic acid sequences of JAK2 and SMAD3 genes were known at the time of the invention, 2) US 20040072154 A1 to Morris, et al. discloses a nucleic acid sequence comprising the claimed SEQ ID NO 1 (nucleotides 6058-6658 of SEQ ID NO 178, wherein a nucleotide corresponding M301 is C6358), 3) US 20070037165 A1 to VENTER, et al. discloses a nucleic acid sequence identical to the claimed SEQ ID NO 8 (SEQ ID NO 60284). Without a shared special technical feature, the inventions lack unity with one another.

Group I therefore lacks unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.