80300_Auto_Edited..revised.docx

Modern drug discovery for Inflammatory Bowel Disease: the role of computational methods

Computational methods for IBD drug development

Titilayo Omolara Johnson, Augustina Oduje Akinsanmi, Stephen Adakole Ejembi, Olugbenga Eyitayo Adeyemi, Jane-Rose Oche, Grace Inioluwa Johnson, Abayomi Emmanuel Adegboyega

Abstract

Inflammatory bowel diseases (IBDs) comprising ulcerative colitis, Crohn's disease and microscopic colitis are characterized by chronic inflammation of the gastrointestinal tract. IBD has spread around the world and is becoming more prevalent at an alarming rate in developing countries whose societies have become more westernized. Cell therapy, intestinal microecology, apheresis therapy, exosome therapy and small molecules are emerging therapeutic options for IBD. Currently, it is thought that low-molecular-mass substances with good oral bio-availability and the ability to permeate the cell membrane to regulate the action of elements of the inflammatory signaling pathway are effective therapeutic options for the treatment of IBD. Several small molecule inhibitors are being developed as a promising alternative for IBD therapy. The use of highly efficient and time-saving techniques, such as computational methods, is still a viable option for the development of these small molecule drugs. The computer-aided (*in silico*) discovery approach is one drug development technique that has mostly-proven efficacy. Computational approaches when combined with traditional drug development methodology dramatically boost the likelihood of drug discovery in a sustainable and cost-effective manner. This review focuses on the modern drug discovery approaches for the design of novel IBD drugs with an emphasis on the role of computational methods. Some computational approaches to IBD genomic studies, target identification, and virtual screening for the discovery of new drugs and in the repurposing of existing drugs are discussed.

Core Tip: For the design of small molecule drugs to treat inflammatory bowel disease (IBD), highly effective and time-saving approaches, such as computational methods, are still a viable choice. By complementing experimental studies with computational approaches, the probability of successful drug discovery is increased while simultaneously reducing associated costs. This article provides a summary of the current drug discovery pipeline for IBD, with special emphasis on the part played by computational methods. The use of *in silico* genomic studies, target identification, and virtual screening to find new drugs and repurpose existing drugs for the treatment of IBD are discussed.

INTRODUCTION

Inflammatory bowel disease (IBD) refers to a group of disorders characterized by chronic inflammation of the gastrointestinal tract that is oftentimes triggered by unknown causes. Crohn's disease (CD) and ulcerative colitis (UC) are the two most prevalent IBDs[1]. The clinical courses are characterized by consistent relapses and recoveries, linked to destructive idiopathic inflammation of the digestive tract. Despite the fact that the precise causes of IBD are still being investigated, it is believed to be the result of an intricate relationship between various parameters, including immune system dysfunction, microbial dysbiosis, a genetic susceptibility, and some environmental factors[2,3].

As the etiology of IBD continues to be a "crossword" to solve, its prevalence and significant global impact continue to rise[4-6]. Globally, the prevalence of IBD increased by 85.1% between 1990 and 2017, and the number of people diagnosed with the disease increased by 95%, as reported by the Global Burden of Disease ("GBD") 2017 Inflammatory Bowel Disease Collaborators. Since 1990, the incidence of IBD has risen in newly industrialized countries in Africa, Asia and South America, including Brazil, to the extent that at the turn of the 21st century this idiopathic disease[7] has become a global disease with accelerating incidence in countries whose societies have become more westernized[8]. Also of interest is the increasing prevalence of microscopic colitis, a

subtype of IBD characterized by chronic watery diarrhea caused by colon inflammation. The disease has recently gained more attention due to the availability of more information about its pathogenesis, diagnosis and therapy. The incidence of microscopic colitis is progressively on the rise, approaching that of CD and UC in some populations[9].

Treatments options for IBDs, especially CD and UC, have made significant progress in the last few years[5]. A wide range of anti-inflammatory and symptom-relieving drugs is available for patients with UC. Recent treatments aim to improve the patient's quality of life by alleviating symptoms like abdominal pain and diarrhea and bringing the disease under control as a whole[10]. Conventional treatments include aminosalicylates, corticosteroids, immunomodulators and biologics as part of the pharmacotherapy to control symptoms. When necessary, they also include some general procedures, including surgery. The advent of biologics and other therapeutic advancements in recent years has altered not only the treatment modalities but also the perception of IBD therapy. While symptom remission, complication avoidance and complication treatment are all important, mucosal healing is also a major target. Healing of the mucosa occurs when inflammation in that area subsides and the mucosal lining is returned to its normal structure[11]. Emerging evidence suggests that mucosal healing is linked to improved long-term outcomes, including lower rates of clinical recurrence, hospitalization, surgery, and disability[12,13].

Many patients with IBD have seen a dramatic improvement in their long-term outcomes, both regarding disability and quality of life since the introduction of monoclonal antibodies targeting tumor necrosis factor (TNF) about 20 years ago. However, despite these developments, there are still many unfulfilled needs. For instance, less than a third of treated patients who begin a biologic therapy achieve and maintain drug remission at 1 year. Even in cases when clinical and endoscopic criteria for remission have been met, symptoms such as diarrhea, stomach discomfort, joint problems, and exhaustion may still be experienced [14]. Also, the lack of responsiveness to biologic therapies, which can be caused in part by the protein's immunogenicity

upon administration, and the need to discontinue drugs because of intolerance or side effects show that a better generation of therapeutic alternatives is still needed. To achieve the desired results of immune homeostasis restoration and better symptom control, additional progress is still required. Emerging treatment options for IBD include cell therapy, intestinal microecology, apheresis therapy, exosome therapy, and small molecules[15]. Low-molecular-mass compounds with excellent oral bioavailability and the capacity to cross the cell membrane to modulate the function of parts of the inflammatory signaling pathway are now regarded to be promising therapeutic alternatives for the treatment of IBD. Several small molecule inhibitors are being explored as a possible treatment option for IBD, such as inhibitors of Janus kinase [JAK] enzymes[1]. For the development of these small molecule drugs, the use of highly effective and time-saving techniques, such as computational methods, remain a viable option.

Owing to the intricate nature of the molecular mechanisms involved in disease pathogenesis, the process of developing small molecule drugs is complicated.[16,17]. The traditional methods of drug design and development require time-consuming, costly and laborious scientific procedures. On the other hand, computational tools hold great promise as a viable option for the design and development of new small molecules of biomedical relevance[18]. Computer-aided drug design (CADD) employs computer processing power, statistics, mathematics and three-dimensional (3D) graphics to elucidate the affinity and binding mode of small molecules against therapeutic targets.

As a whole, computational methods aid in the identification of candidate target proteins for drug screening and design by searching through large amounts of genomic data for the most important genes[19,20]. In combination with experimental data, protein structures can be predicted with some accuracy[21]. In order to find drug binding sites and understand how drugs work, it is necessary to conduct research into the structural and thermodynamic features of target proteins, both of which can be accomplished with biomolecular simulations using multiscale models[22]. Next, chemical libraries are explored using virtual screening to identify potential drug candidates based on the

ligand binding sites on target proteins[23]. With a significantly reduced number of potential drug candidates, *in vitro* and/or *in vivo* experiments can be used to further assess the effectiveness of these molecules. Another CADD approach, besides virtual screening, is provided by *de novo* drug design techniques, which produce highly specific small molecules with good synthetic accessibility[24].

Medicinal chemists use CADD methods to help in rationalizing the selection of hit compounds and in hit-to-lead optimization. CADD has been used as an efficient method for identifying potential lead compounds toward the development of drugs for a wide range of diseases, including IBDs. A variety of computational approaches, such as molecular docking, molecular dynamic (MD) simulation, quantitative structure-activity relationship (QSAR) and pharmacophore modeling, are used to rationally design potent therapeutics with higher efficacies and fewer side effects[25]. The availability of more information about the molecular basis of IBD pathogenesis has further enhanced the use of computational methods in the design of small molecule drugs for IBD treatment. In the pathogenesis of IBD, there is an interplay between various factors such that any stimulus can lead to a cascade of events or even a vicious cycle. This gives a variety of therapeutic targets for which IBD drugs can be designed.

MOLECULAR ASPECTS OF IBD PATHOGENESIS

An interplay of genetic, epithelial and immune factors in the presence of intestinal microbiota are believed to be responsible for the development of the IBD[26].

Genetic factors

The genetic factors causing IBD were initially established through family and twin studies. The disease was observed to cluster in families, with a relative risk of 13-36 for siblings of CD patients and 7-17 for UC patients[27]. Hence, the prevalence of IBD is much higher among first-degree relatives of those with IBD than it is among the general population, suggesting a role for genetic factors in the pathogenesis of the condition. Monozygotic twins exhibit a much higher rate of disease concordance than dizygotic

twins, and there are significant differences in the incidence and prevalence of IBD among various populations, according to the twin studies[27]. In CD, the concordance rate for monozygotic twins is approximately 50% compared to 15% for dizygotic twins. Meanwhile, the concordance of monozygotic twins for UC is only 16%, suggesting that genetic factors are less dominant in this form of IBD. In general, there is a growing number of genetic markers linked with the pathogenesis of IBD at various levels including innate immunity, epithelial integrity, antigen presentation, cell adhesion, and drug transporter. Along with environmental and immune system factors, the genetic markers at the different levels play a major role in genetic susceptibility to IBD[28].

Epithelial defects

Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is a cytoplasmic protein that is induced in epithelial cells[29] and is constitutively expressed in neutrophils, macrophages, and dendritic cells[30]. Specifically, the leucine-rich repeat domain of NOD2 is necessary for recognition of muramyl dipeptide, a fragment of peptidoglycan present in bacterial cell walls. Ultimately, muramyl dipeptide causes nuclear factor kappa B activation and the induction of proinflammatory cytokines[31,32]. Activation of the innate and adaptive mucosal immune responses as a result of NOD2 polymorphism causes the synthesis of cytokines, metalloproteinases and enzymes, which subsequently results in tissue destruction and epithelial barrier defect.

Evidence from genome-wide association studies (GWAS)[33,34] also shows the involvement of hepatocyte nuclear factor 4α (HNF4α) in the pathogenesis of UC. HNF4α is a member of the nuclear receptor superfamily of ligand-dependent transcription factors that is highly conserved and well expressed in liver and gastrointestinal organs. Several studies have linked HNF4α to intestinal epithelial differentiation, lipid metabolism and epithelial junctions[27,35], which are important components in colon development[36]. A zebrafish model suggests that the gut microbiota negatively regulates expression levels of HNF4α[37]. There is evidence linking alterations in HNF4 expression and activity, as well as germline variants, to IBD

and colorectal cancer[38,39]. In UC, polymorphisms of HNF4α result in defects of epithelial tight junctions and intestinal permeability. Due to these epithelial barrier defects, there is transepithelial influx of bacteria, activation of the immune responses, release of cytokines, and further increase in epithelial junction permeability[40–42].

The development of UC has also been linked to a decrease in fatty acid oxidation in the colonic mucosal epithelium. Carnitine is a necessary cofactor in lipid metabolism and may be transported by the organic cation transporter (OCTN), a family of transporter proteins for organic cations. Carnitine helps shuttle long-chain fats into the mitochondria. Both OCTN1 and OCTN2 have been linked to CD-causing mutations: SLC22A4 1672C/T for OCTN1 and SLC22A5 +207G/C for OCTN2. The TC haplotype, formed by the presence of one or more of these mutations, is linked to the development of ileal, colonic and perineal symptoms and the requirement for surgical treatment of CD[28,43].

A number of tissues including the colon, small intestine, placenta, liver, heart and skeletal muscle contain high levels of Drosophila long disc homologue 5. The Drosophila long disc homologue 5 gene belongs to the membrane-associated guanylate kinase gene family, which encodes cell scaffolding proteins. It is thought to be involved in the maintenance of epithelial cell integrity and in signal transduction. Mutations in this gene have been linked to an increase in intestinal permeability[28]. The D haplotypes were found to be associated with UC and CD in a European cohort[44]. CD patients in Japan also showed another form of this gene (rs37462)[45]. Patients with IBD were found to have an excess transmission of haplotype D, which is defined by the haplotype-tagging single nucleotide polymorphisms (SNPs) G113A[28].

Mucosal immune responses

In normal intestinal mucosal immune response, the presence of gut microbiota has conditioned the dendritic cells present in the epithelium so that there is activation of T regulator cells that produce anti-inflammatory cytokines interleukin (IL)-10 and transforming growth factor-beta (TGF-β) as well as suppression of T effector cells.

However, in IBD, the dendritic cells respond to gut microbiota by activating CD4+ T effector cells that differentiate into T helper (Th1, Th2 and Th17) cells depending on the IBD type[40,42]. Recently, the proinflammatory cytokine IL-12 family, which includes IL-22, IL-23, IL-25, and IL-27, has been linked to the pathophysiology of CD and other immune-mediated disorders[46]. The IL-12 family is responsible for the differentiation of Th cells into Th1 cells. IL-12 and IL-23 are heterodimeric proteins with a unique subunit linked to a shared p40 subunit. Patients with CD have elevated levels of these subunits[47,48]. Ustekinumab, a Food and Drug Administration (FDA)-approved drug for moderate-severe CD, inhibits IL-12 and IL-23 receptors on T cells, natural killer cells, and antigen-presenting cells[49]. In addition, several monoclonal antibodies have been discovered that target the Th1/Th17 pathway via IL-23. However, none of them have progressed to phase 3 trials.[50,51].

The upregulation of IL-13, another crucial cytokine in the Th2 immune response, is considered to be a major trigger of mucosal inflammation in UC patients[52,53]. Research in mice revealed an increase in IL-13 in colitis, and this increase could be mitigated by inhibiting the ability of IL-13 to bind to its signaling receptor[54]. Tralokinumab is a human immunoglobulin G4 monoclonal antibody that binds to IL-13 and inhibits its activity.

Molecular studies have also identified NOD2 as a susceptibility gene in CD. The gene encodes a protein that transduces signals to activate nuclear factor kappa B in monocytes and functions as an intracellular receptor for bacterial products. Muramyl dipeptide activates NOD2, leading to autophagy in dendritic cells. Defective autophagy induction and decreased bacterial localisation in autophagolysosomes are observed in CD patients with susceptibility polymorphisms in the NOD2 gene. Genetic analyses have identified two other genes that are involved in autophagy and intracellular bacteria clearance, namely IRGM (immunity-related GTPase M) and ATG16L1(autophagy-related 16–like-1), demonstrating the importance of autophagy in IBD immune responses. Other genes related with autoimmune disease, such as IL23R and PTPN2, reveal an additional feature of CD pathophysiology.[38,40].

Another major player in IBD pathogenesis is TGF-β1, an essential factor in the healing of intestinal cells and the reduction of inflammation[55]. It is an immunosuppressive cytokine with the ability to inhibit pathogenic T cell activity and antigen-presenting cell responses. Increased levels of SMAD7, an intracellular protein that binds TGF-β1 receptor and prevents TGF-β1- and SMAD-associated signaling, are believed to account for the deficiency in TGF-β1 activity observed in IBD patients. Mongersen, an oral medication that inhibits SMAD7 activity after administration to the terminal ileum and right colon is being investigated for efficacy in CD.[56]. In its current form, the site of action is limited to the terminal ileum and right colon, hence it may not be beneficial for patients with more complicated CD or post-operative recurrence[57].

The involvement of the JAK family of intracellular non-receptor tyrosine protein kinases in the pathogenesis of a number of autoimmune diseases has also been demonstrated[58]. Many autoimmune diseases, including IBD, have been linked to specific SNPs in the genome, and the JAK/STAT signaling pathway has yielded several cytokines and receptors as potential treatment targets[59]. The JAK/STAT pathway interferes with a couple of inflammatory pathways and development of disease as seen in CD and UC patients is characterized by imbalanced effector T cells, leading to increased effector Th cells (Th1, Th2 and Th17 cells), thus mediating the inflammation[60]. Figure 1 shows a schematic description of the JAK/STAT pathway leading to the expression of genes encoding some inflammatory markers. Inhibition of JAK leads to the inhibition of signaling of a specific subset of cytokines, which are implicated in inflammation[61]. Some small molecule inhibitors of the JAK/STAT pathway with good oral bioavailability have been developed. A very good example is tofacitinib. When compared to monoclonal antibodies, JAK inhibitors have the therapeutic advantage of being able to target multiple downstream signaling pathways induced by inflammatory cytokines, whereas monoclonal antibodies can only block a single molecule[62].

The successful development of tofacitinib, as well as the promising results of other JAK inhibitors in both UC and CD (as shown in Figure 2), demonstrate that JAK

inhibition has a role in the treatment of IBD. However, long-term safety studies in people with rheumatoid arthritis and UC who took tofacitinib showed a higher risk of reactivation of herpes zoster, especially at higher doses[63]. This increased risk is likely a class effect of JAK inhibitors and related to IFN and IL-15 inhibition. Also, there might be a possible thrombogenic risk, as shown by patients with rheumatoid arthritis[64]. More selective JAK-1, JAK-3 or tyrosine kinase 2 inhibitors are expected to improve safety while maintaining efficacy. However, these drugs are still systemic; a gut-selective JAK inhibitor with high intestinal exposure and target engagement but no systemic effects are still needed for treating people with IBD. Continued progress is being made in all of these areas[65].

IBD DRUG DISCOVERY AND DEVELOPMENT

The process of identifying a therapeutic agent for extensive study as a potential drug candidate is known as drug discovery[66]. In general, the modern drug discovery process involves identifying a condition to be treated and its unfulfilled medical necessity, selecting a druggable molecular target and validating it, developing in vitro assays followed by high-throughput screening of compound libraries against the target to identify hits and optimizing hits to create lead compounds with acceptable potency and selectivity towards the biological target and demonstrated efficacy in an animal model. Following that, the lead compounds are further refined to improve their effectiveness and pharmacokinetics before moving further with drug development (Figure 3). The discovery and development of innovative drugs is a complex process that takes around 12 years and an average of $1.8 billion to bring a new medication to market[66,67].

The process of IBD drug discovery begins with target discovery and selection followed by biological confirmation in cellular and animal models[68]; this is usually the pre-clinical phase. Several therapeutic targets of IBD have been identified, and some are listed in Table 1. Target identification in IBDs is based on qualitative relevance to the pathogenesis of the disease as well as increased magnitude of expression[68].

Biochemical pathways and specific proteins are targeted, and the drugs are developed based on the understanding of the mechanism of action of these targets. This is followed by pharmaceutical developments such as screening for safety, toxicology, pharmacokinetics and efficacy. At the preclinical stage, drugs are tested with targets to investigate levels of interactions and outcomes. Models and methods used differ between laboratories based on specific targets of interest.

For instance, in IBD, targets such as JAK/STAT and interleukins mediating inflammation such as IL-12, IL-23, IL-6, IL-22Fc[69], tyrosine kinase, toll-like receptors and integrins[70] are explored using biologic agents at different stages of development. Following the *in vivo* studies using murine and human T cells, JAK/STAT inhibition by tofacitinib[71] has led to further application of the drug in several clinical trials. Integrins, a class of receptors that facilitate T lymphocyte trafficking into the gut[72] are targets as well. Integrins facilitate adhesive interactions between lymphocytes and endothelial cells, which leads to the trafficking[73] and can lead to T cell-dependent chronic intestinal inflammation in IBD[70]. Etrolizumab, an anti-adhesion agent approved by the FDA for treatment of IBD was subjected to *in vitro* testing, where it internalized β7 integrin using cell culture models[74]. Etrolizumab selectively inhibits α4β7 and αEβ7, which are involved in T lymphocyte homing in the gut[75]. Although there are challenges involved in the use of *in vivo* and *in vitro* methods in establishing IBD[68], conditions for the studies of potential drugs are optimized. The molecules with desired preclinical effect on the target goes into clinical trial.

In clinical trials, drugs that scale through preclinical studies are expected to maintain remission in long-term mucosal healing. Clinical trial phases are regarded as long and extensive periods that take years from the point of research to the stage of approval of a candidate drug. Prior to these trials, detailed protocols that show the characteristics of the studies are provided[76]. The clinical phase comes after certainty has been established that the drugs/molecules are effective and non-toxic. Clinical trials involve three main phases before approval, known as phase I, phase II, and phase III. The first two phases are early phases.

The phase I is the stage in drug development when testing and pharmacokinetics studies are carried out using healthy or ill subjects[76] to ascertain safety level of the drugs. The pharmacokinetics and pharmacodynamics of the drugs are determined using volunteer patients or patients who are not responsive to previous treatments in order to determine drug safety alongside desired clinical activities and an Absorption, Digestion, Metabolism and Excretion (ADME) profile[68]. For instance, a phase I study of Japanese patients with UC treated with vedolizumab, an anti-integrin antibody, showed an appreciable level of tolerance in the desired clinical activity of the drug as well as a satisfactory level of pharmacodynamics and pharmacokinetics[77]. The outcome of this phase determines whether the drug scales through to the next phase. The phase I studies can last for up to many years and part of the process involves identification of adverse effects or events other than the occurrence(s) observed in the preclinical stage. Foreknowledge of a possible mechanism of action of a drug can also guide expectations in clinical trials.

The phase II is the stage that involves dose ranging. The efficacy of the drugs are determined alongside the optimum dose that would be considered safe[76]. At the point of dose determination, the outcome of the effect of different doses would guide the decision to proceed to the next phase. A phase II study of varied doses of ozanimod, an agent that targets sphingosine-1-phosphate receptors 1 and 5, was used to treat adult patients with moderate to severe CD based on an uncontrolled multicenter trial within 12 wk with effects of the different doses at different intervals and doses observed[78]. A drug that scales through is launched into the third phase.

Phase III is the stage whereby the efficacy of the drug is compared with that of an already established standard. Patients recruited for this phase are usually from the general population compared to phase I and II. The side effects of the drugs are studied as well as their effectiveness comparing them with common treatments to help guide safety and proper use. Currently, etrolizumab, is undergoing a phase III clinical trial for treatment of IBD using six multicenter prospective randomized controlled trials and two open-label extension studies[75]. Upon conclusion of the studies, the drugs are

considered for approval based on FDA standards. Drugs are reviewed by the FDA based on the evidence of preclinical and clinical outcomes and approved for marketing based on the terms of the FDA.

ROLE OF COMPUTATIONAL METHODS IN IBD DRUG DISCOVERY

The use of computational methods has become a crucial part of the drug discovery process[18]. A number of commercially available medications as well as several clinical candidates have been discovered or improved with the help of molecular modeling techniques. CADD combines multiple chemical-molecular and quantum approaches to the discovery, design and development of drugs. Structure-activity relationships form the basis of many CADD methods. The objectives of CADD are multidisciplinary in nature, with the end goal being the modification of bioactive molecules, the formation of therapeutic alternatives and the knowledge of biological events at the molecular level[79].

From drug identification to pharmacological role discovery and from preclinical testing to drug marketing, computers have played a fundamental role in the entire drug discovery and development process[15]. In addition, CADD opens the door to the possibility of drug repurposing, which is the systematic identification of new potential uses from drugs that have already been approved for other indications[79]. According to the results of a computational drug repurposing study conducted by Bai *et al*[80], atorvastatin shows promise as a novel treatment approach for improving symptoms in patients with UC. They developed a framework for systematically integrating publicly accessible heterogeneous molecular data with clinical data on a large scale in order to repurpose FDA-approved drugs for a wide variety of human diseases. Computational methods are useful in various aspects of modern drug discovery for IBD. These include but are not limited to genetic studies for the identification of pathways linked to IBD pathogenesis, target identification, and virtual screening.

IBD genetic studies

Computational methods have made tremendous contributions to the field of IBD genetic studies. As mentioned earlier, genetic factors contribute to the pathogenesis of IBD and the understanding of the molecular events are important for target identification. The advent of GWAS is a turning point in the history of genetic research into complex human disease. GWAS is a technique for identifying genetic markers that are linked to an increased probability of developing a disease or exhibiting a certain trait. The strategy entails analyzing the genomes of a large number of people to identify genetic variations that are more common in people who have a disease or a particular trait than in those who do not. Following the discovery of such genomic variants, researchers often use GWAS to search for other potentially causal variants located in close proximity[81]. IBD is a prime example of the usefulness of GWAS and related analyses. Using GWAS based on SNPs, researchers were able to identify 163 genetic loci and numerous signaling pathways that are linked to IBD[82]. Many of these loci have pleiotropic effects, and risk prediction models were developed using a wide variety of genetic variations. Key contributions of the gut microbiome and important interactions between genes and the environment are emerging as a result of these studies[83].

The development of computational methods has contributed significantly to the identification of the vast quantity of genetic determinants. There are a number of different software packages available for use in GWAS research[84], and a common example is PLINK. In addition to standard GWAS features like quality control filtering and SNP association testing, PLINK also includes more advanced tools like gene-based analysis, annotation and epistasis testing in a compact, user-friendly package. PLINK initially discovered the actin-related protein 2/3 complex subunit 2[85], IL10, HNF4a, cadherin-3, cadherin-1 and laminin subunit beta-1[33] loci and recently discovered 163 IBD loci[82]. In the majority of GWAS-based genetic studies of IBD, PLINK has served as the primary analytical tool. A linear mixed model, which accounts for both fixed and random effects, has been used in GWAS in recent years. FaST[86], EMMAX[87] and GEMMA[88] are all examples of linear mixed model-based software. They are able to

adjust for both overt and covert connections within a population simultaneously. Multiple correlated phenotypes can be analyzed with GEMMA as well.

There is a plethora of pathway analysis software packages available, many of which are based on GWAS[89]. The development and implementation of GWAS-based pathway software has frequently been illustrated with CD as an example. To examine the CD dataset from the Wellcome Trust Case Control Consortium, Wang *et al*[90] used the GenGen software to find significant associations of the IL12/IL23 pathway with CD status in multiple cohorts genotyped with different SNP chips and from different ethnic backgrounds[91]. This was among the first analyses of IBD pathways based on GWAS data. IL3 activation and signaling pathway were also linked to CD, based on the research by Torkamani *et al*[92]. They used the MetaCore program, which is a for-profit product created by GeneGo Inc. (St. Joseph, MI, United States).

Using the statistical technique of Simes/false discovery rate, Peng *et al*[93] discovered significant enrichment of the JAK/STAT signaling pathway, as well as the cytokine-cytokine receptor interaction. With a model-based strategy, Carbonetto and Stephens[94] found a similar result for a number of cytokine signaling pathways. Holmans *et al*[95] used the software ALIGATOR to discover the involvement of MHC genes in addition to IL/cytokine signaling. Similarly, Jostins *et al*[82] examined the largest IBD GWAS to date for enrichment of canonical pathways or Gene Ontology terms and discovered significant enrichment of Gene Ontology terms pertaining to the regulation of cytokine production, lymphocyte activation, and the JAK/STAT signaling pathway.

It is possible that the differences between the studies can be attributed to the different statistical methods and/or the different pathway databases used in their studies. However, all of these pathways were related to interleukins and the immune system. Torkamani *et al*[92] found a significant association between calcium signaling and the ChREBP regulation pathway; Peng *et al*[93] found a significant association between ABC transporters and the extracellular matrix receptor interaction. Numerous

user-friendly web-based software programs, such as i-GSEA4GWAS[96] and GSEA-SNP[97], have been developed for pathway analysis using GWAS data.

IBD target identification

Following the discovery of the biological basis of a disease, the next step in drug discovery is the identification of potential drug targets. The most promising targets for drug discovery are hypothesized to be highly prevalent in the disease-affected population, to have a well-established function in the underlying pathology and to be directly linked to the disease of interest. Potential drug targets are defined as disease-modifying rather than disease-causing. In the present day, a wide range of methods, both experimental and computational, are used to identify drug targets. The experimental methods rely heavily on comparative genomics, with phenotype and gene association analysis serving as complementary tools. All experimental methods yield credible findings, but they have significant drawbacks, including the high cost and extensive scientific labor needed to experimentally probe the entire space of chemical compounds to identify viable drug targets[98]. In light of these drawbacks, researchers and pharmaceutical companies increasingly rely on computational methods for initial investigations before turning to experimental approaches for validation and other purposes.

Various bioinformatics resources are available for the identification of drug targets as illustrated in Table 2. These programs efficiently process a large volume of data from genomic, transcriptomic and proteomic databases and ultimately provide potential drug targets in a short period of time and at a low cost. Several computational methods are currently accessible, each of which makes use of a unique type of molecular information, such as a gene or genomic sequence, molecular interaction data or the 3D structure of a protein[98]. There are strong connections between most of these methods.

Mohan *et al*[99] reported using genetic databases to find new molecular targets for IBD. They used four different genetic databases to categorize the protein-coding genes

associated with UC (3783 genes), CD (3980 genes), uveitis (1043 genes), arthritis (5583 genes), primary sclerosing cholangitis (1313 genes), and pyoderma gangrenosum (119 genes). The databases used were Genecards: The Human Gene Database, DisGeNET, the Comparative Toxicogenomics Database, and the Universal Protein Resource. Then, they used Network Data Exchange to map a distinct signal pathway based on the identified common genes underlying the aforementioned diseases. Across UC, CD, uveitis, arthritis, pyoderma gangrenosum and primary sclerosing cholangitis, they identified a distinct set of 20 genes with the highest probability of overlap. The interactome of these 20 coding genes for proteins is shown in Figure 4. Different disease processes were linked to some unique immune modulators. IL-25 and monensin-resistant homolog 2 were observed in UC, CD, pyoderma gangrenosum, and arthritis. Arachidonate 5-lipoxygenase was found to contribute to the development of UC, CD, and arthritis. The involvement of SLCO1B3 is unique to pyoderma gangrenosum, UC, and CD. TNF was found to be involved in the pathogenesis of UC, CD, psoriatic spondylitis, and arthritis.

Virtual screening

Drug discovery relies heavily on the physical screening of large chemical libraries for biological targets in order to find new lead compounds. High-throughput screening is a method for finding active molecules in experiments by analyzing more than a million compounds biochemically. However, developing and deploying this technology takes a long time and a sizable investment. Therefore, virtual high-throughput screening was developed as a more affordable and effective calculation method. This technique has seen extensive use in the earliest stages of drug discovery. The goal is to search through huge compound libraries to find the structure of a novel, active small molecule. To some extent, this supports the goals of high-throughput screening. Virtual screening saves money by reducing the number of compounds used to measure pharmacological activity, while high-throughput screening uses all compounds in the database[18,24,100]. In

this sub-section, we will go over the various virtual screening techniques that are commonly used in IBD drug discovery.

Molecular docking: Molecular docking is commonly used in the drug discovery and development process because of its ability to predict interaction patterns between proteins and small molecules as well as proteins[18,101,102]. The principle behind this phenomenon proposes that ligand and receptor recognition is predicated on a similarity in spatial shape and energy. Understanding the action mechanism of a drug requires first establishing its binding conformation to a specific protein receptor[24]. The goal of docking is to accurately assess the strength of binding by fitting the structure of a ligand within the requirements of a receptor binding site[103]. The most frequently cited molecular docking software packages for use in drug discovery are summarized in Table 3.

Keretsu et al[104] used computational methods to design new JAK1 inhibitors from a series of pyrrolopyridine derivatives. Autodock 4.2 was utilized to predict the protein-ligand binding. Tofacitinib, an established ligand, which was co-crystallized with the structure of JAK1 (protein data bank ID: 3EYG) obtained from the protein data bank (www.rcsb.org), was used as a reference compound to validate the docking procedure. The docked conformation of tofacitinib closely matched that of one obtained in the crystal structure. The binding affinity of compound 42 for JAK1 was found to be -10.2 kcal/mol. The F958 and L959 residues of the protein formed H-bond interactions with the pyrrolopyridine moiety. As a result, the methyl group of the methyl piperidine moiety protruded beyond the binding pocket and into the bulk solvent. The chlorobenzyl group slid into the hydrophobic pocket made by the residues of the activation loop, the α-helix and the P-loop. The selected binding conformation was prepared for further MD simulation study. The finding of the study was presented as a possible guide in the design of more effective JAK1 inhibitors[104].

In order to identify the bioactive compounds of the *Phyllanthus nivosus* leaf responsible for its activity against UC for further drug design, Johnson

et al[101] combined molecular docking with an *in vivo* study. Levels of TNF-α, IL-6, nitric oxide, malondialdehyde, reduced glutathione, superoxide dismutase and catalase in the serum of rats experimentally induced with UC and treated with *Phyllanthus nivosus* were monitored. The bioactive ingredients of the most active fraction were identified using gas chromatography-mass spectrometry followed by molecular docking against IL-1b converting enzyme (caspase-1), beta-2 adrenergic receptor, cyclooxygenase-2 and TNF-α. The study led to the identification of ethyl iso-allocholate cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate and alpha-cadinol as promising compounds for further development into drugs for the treatment of UC[101].

In another study, Halder *et al*[105] used molecular docking in combination with other *in silico* techniques for the repurposing of FDA-approved medications and provided a framework for drug exploration and computational methods in the discovery of drugs for the treatment of IBD. After being imported and processed using Protein Preparation Wizard and LigPrep, respectively, the molecular target TNF (protein data bank ID: 2AZ5) with a small molecule inhibitor and the FDA-approved drugs (from the Zinc database) were subjected to molecular docking, ADMET analysis and binding free-energy calculation (MMGBSA). Following that, the medications were ranked based on docking score, ADMET parameters and MMGBSA dG binding score. The selected drugs were then subjected to an induced-fit docking study. The MD simulation study was conducted on the two most promising compounds, iopromide (ZINC000003830957) and deferoxamine (ZINC000003830635). Finally, the bioisosteric substitution was applied to enhance the ADMET properties of these compounds[105].

Pharmacophore modelling: Virtual screening of databases with the pharmacophore model has become one of the most important ways to find new lead compounds as compound databases and computing power have advanced. A pharmacophore is a conceptual description of the molecular features required for molecular recognition of a ligand by a biological macromolecule, which describes how structurally distinct ligands can bind to the same receptor site. To achieve therapeutic efficacy, drug molecules

adopt an active conformation that is both geometrically and energetically complementary to that of the target macromolecule.

Medicinal chemists have discovered that modifications to specific chemical groups in drug molecules greatly affect the interaction between drugs and targets, while modifications to other groups have little to no effect[106]. Additionally, it was discovered that molecules exhibiting the same activity share common properties. Accordingly, Ehrlich proposed the idea of pharmacophores in 1909[107], which referred to the molecular framework of atoms with active essential characteristics. In 1977, Gund[108] provided further elaboration on the concept of pharmacophores as a class of molecules that recognize receptors and form structural features of molecular biological activity.

Pharmacophores can be discovered using one of two common approaches. If the structure of the target molecule is known, then the structure of the pharmacophore can be inferred using techniques like conformational analysis and molecular folding[109]. The pharmacophore recognition procedure will then choose an active compound that can be used to create the model. Conversely, pharmacophore studies are conducted on a number of compounds when either the structure of the target or its action mechanism is still unknown; this allows for a summary of data on certain groups that are crucial to the activity of the compound[24].

Babu *et al*[110] combined ligand-based pharmacophore modeling with virtual screening and molecular docking to find JAK1 inhibitors with high potency and selectivity. In the first step, they developed ligand-based pharmacophore models and checked them for accuracy with potency and selectivity validation techniques. A pharmacophore-based virtual screening was carried out on eight selected pharmacophore models using six different databases. ADME prediction and molecular docking were used to narrow down the hits found during screening. Docking results were verified using the binding free-energy calculation and induced fit docking techniques. A cross docking analysis was then performed to determine which lead compounds are selective for JAK1. In the end, five promising compounds were chosen

and subjected to further investigation using MD and density functional theory. T5923555 and T5923531 were identified as the most promising leads among the five compounds and will be pursued for additional validation *via in vitro* and *in vivo* techniques.

QSAR: QSAR is frequently used in the drug discovery process to find compounds with desirable inhibitory effects on target proteins, with minimal side effects (nonspecific activity). The QSAR model is a quantitative investigation into the relationships that exist between small organic molecules and large biological macromolecules. The calculated properties of molecules (such as the absorption, distribution and metabolism of small organic molecules in living organisms) are correlated with their biological activity, as determined experimentally[111]. QSAR is the most precise and efficient approach to drug design when the structure of the receptor being targeted is unknown. In the 1980s, 3D structural information was incorporated into the quantitative structure-activity relationship to form the 3D-QSAR method. Since the 1990s, structure-based drug design has increasingly replaced QSAR in the area of drug design due to the increase in computational power and the availability of the 3D structure of many biomolecules. However, QSAR with its advantages of small amount of calculation and good predictive ability[112], continues to play an important role in pharmaceutical studies.

3D-QSAR enables the investigation of the three-dimensional structure of bioactive compounds and the correct representation of energy changes and interaction patterns between bioactive molecules and receptors. Different drugs are evaluated by fitting their physicochemical and 3D structural parameters to the quantitative relationship. The structures of the newly created compounds are subsequently predicted and improved upon. 3D-QSAR analysis is a research method that integrates QSAR with computational chemistry and molecular graphics. It is an effective method for determining the nature of drug-target interactions, creating hypothetical images of simulated targets, determining the correlation between drug structure and activity and

developing new medications. In addition, predictive 2D-QSAR models, 3D-QSAR models and 3D target and ligand-based approaches have all been developed for the purpose of finding IBD drugs[24].

Yang *et al*[113] incorporated molecular docking and QSAR study in the design of a series of TNF-α converting enzyme (TACE) inhibitors with the ability to bind in the S1' pocket of the enzyme. A total of 12 analogues were synthesized by altering the chain length and alkylation pattern on the aromatic ring of the side chain. Most compounds inhibited cellular TNF-α production and TACE *in vitro*. The most promising compound from *in vitro* and *in vivo* pharmacokinetic studies had a moderate systemic clearance and a good oral bioavailability of 42%. It was also tested in a rat model carrageenan-induced paw edema and found to be effective at reducing edema in the animal's paws. The series of α-alkoxyaryl alkyl substituted chromen-based analogues was then validated by means of a QSAR study and docking. Coumarin core TACE inhibitors with long, bulky α-substituent groups are able to enter the S1' and S3' pockets, where they form van der Waals interactions, with increased inhibitory activity. The docking study of the compound demonstrated its dual-function inhibitory activity toward TACE and matrix metallopeptidase-3. Based on the resulting QSAR descriptors, new α-substituted chromen-based TACE inhibitors with enhanced TACE inhibitory activity can be developed.

MD simulation in IBD drug discovery: MD simulation[102,114–116] is another popular approach to studying biomolecules; it is based on Newtonian mechanics and applies empirical molecular mechanics force fields. The drug discovery process can be aided by using explicit/implicit solvent models, which allow for simulations of time and space, and all-atom, united-atom and coarse-grained MD simulations[117,118]. MD simulations have typically been used to identify potential drug binding sites on target proteins, calculate the binding free energy between proteins and ligands, determine the mechanism of action of drug molecules, and more[119,120].

Taldaev *et al*[121] used MD to create structural representations of the arrangement of binding sites for the JAK family enzymes in order to elucidate the selectivity of upadacitinib for JAK1 among other isoforms. They found that the high affinity of upadacitinib was due to its ability to form four hydrogen bonds with amino acid residues in the hinge region of JAK1 as opposed to just two with other JAK isoforms. Structural features of the JAK1 binding site, including the unique residues S963 and E966, are responsible for stabilizing the molecule at the hinge region, as proposed by the authors. Hydrogen bonding with the JAK1 (E883) and JAK2 (N859) amino acid residues in the glycine loops was reported to increase the affinity. The research findings were presented as having the potential to direct the creation of more selective and effective next-generation JAK inhibitors, thereby enhancing the treatment of a wide range of cytokine-mediated diseases.

In another MD simulation research conducted by Du *et al*[122], the interaction mechanism between oncostatin M (OSM) and its receptor (OSMR) at the atomic level was predicted. Binding of OSM to OSMR is said to be implicated in the pathogenesis of IBD. The OSMR interaction domain was built using the homology modeling approach. Docking was used to establish the near-native structure of the OSM-OSMR complex, and long-time scale MD simulation in an explicit solvent was used to sample the conformations when OSM binds to OSMR. Following the equilibration of the simulated system, the per-residue energy contribution was determined to describe the key residues for the formation of the OSM-OSMR complex. Premised on these key residues, eight residues (OSM: Arg100, Leu103, Phe160, and Gln161; OSMR: Tyr214, Ser223, Asp262 and Trp267) were identified as "hot spots" by computational alanine mutagenesis analysis and confirmed by further MD simulation of the R100A (one of the discovered "hotspots") mutant. Furthermore, the FTMap analysis revealed six cavities at the OSM-OSMR interface, which were proposed as key binding sites. The predicted 3D structure of the OSM-OSMR complex and the discovered "hotspots" provide useful information in understanding the OSM-OSMR interactions, and the identified locations serve as potential targets in designing small molecules to inhibit the interactions[122].

CONCLUSION

The global prevalence of IBD is increasing, with developing countries experiencing an increase due to modernization. There is still a need for a new generation of alternative therapies due to the loss of response to biologic drugs, which can be caused in part by the immunogenicity of the administered protein as well as the need to discontinue drugs due to intolerance or side effects. The development of small molecule drugs is difficult because of the complexity of the molecular pathways involved in the progression of disease. The conventional drug design and development processes are lengthy, expensive and filled with arduous scientific procedures. However, computational tools show considerable promise as a practical means of creating new small molecules with biomedical application. There have been a number of groundbreaking successes with drugs for several diseases developed using CADD. Many of these drugs are either FDA-approved or under clinical trials. However, computational methods appear to be underutilized for IBD drug research, as observed during this literature search. To hasten efforts in finding treatments for IBD, more scientists need to turn to computational methods. The modern drug discovery process for IBD makes use of a wide range of computational tools, some of which are geared toward specific tasks such as genomic studies, target identification, and virtual screening.

80300_Auto_Edited..revised.docx

ORIGINALITY REPORT

25%

SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	404 words —	5%
2	bsdwebstorage.blob.core.windows.net Internet	268 words —	3%
3	www.mdpi.com Internet	155 words —	2%
4	pubmed.ncbi.nlm.nih.gov Internet	115 words —	1%
5	pure.rug.nl Internet	113 words —	1%
6	Jee Sun Yang, Kwangwoo Chun, Jung Eun Park, Misun Cho et al. "Structure based optimization of chromen-based TNF-α converting enzyme (TACE) inhibitors on S1' pocket and their quantitative structure–activity relationship (QSAR) study", Bioorganic & Medicinal Chemistry, 2010 Crossref	80 words —	1%
7	www.wjgnet.com Internet	76 words —	1%
8	test.dovepress.com Internet	69 words —	1%

9   Jin Li. "Application of computational methods in genetic study of inflammatory bowel disease", World Journal of Gastroenterology, 2016
Crossref

57 words — 1%

10   Debojyoti Halder, Subham Das, Alex Joseph, R. S. Jeyaprakash. " Molecular docking and dynamics approach to drug repurposing for inflammatory bowels disease by targeting TNF alpha ", Journal of Biomolecular Structure and Dynamics, 2022
Crossref

55 words — 1%

11   Amir Taldaev, Vladimir R. Rudnev, Kirill S. Nikolsky, Liudmila I. Kulikova, Anna L. Kaysheva. "Molecular Modeling Insights into Upadacitinib Selectivity upon Binding to JAK Protein Family", Pharmaceuticals, 2021
Crossref

42 words — 1%

12   academic.oup.com
Internet

40 words — < 1%

13   www.pubfacts.com
Internet

38 words — < 1%

14   www.tandfonline.com
Internet

33 words — < 1%

15   Lawrence Bai, Madeleine K D Scott, Ethan Steinberg, Laurynas Kalesinskas, Aida Habtezion, Nigam H Shah, Purvesh Khatri. "Computational drug repositioning of atorvastatin for ulcerative colitis", Journal of the American Medical Informatics Association, 2021
Crossref

28 words — < 1%

16   Sandeep Sinha, Divya Vohora. "Drug Discovery and Development", Elsevier BV, 2018
Crossref

26 words — < 1%

17   med.sums.ac.ir   26 words — < 1%
Internet

18   static.cigna.com   24 words — < 1%
Internet

19   Grainne Holleran, Loris Lopetuso, Valentina   19 words — < 1%
Petito, Cristina Graziani et al. "The Innate and
Adaptive Immune System as Targets for Biologic Therapies in
Inflammatory Bowel Disease", International Journal of
Molecular Sciences, 2017
Crossref

20   "Pediatric Inflammatory Bowel Disease", Springer   18 words — < 1%
Science and Business Media LLC, 2017
Crossref

21   Julian Panés, Severine Vermeire. "JAK Inhibitors: 18 words — < 1%
Back to Small Molecules for the Treatment of
IBD", Journal of Crohn's and Colitis, 2020
Crossref

22   Orry, A.J.W.. "Structure-based development of   18 words — < 1%
target-specific compound libraries", Drug
Discovery Today, 200603
Crossref

23   wjes.biomedcentral.com   18 words — < 1%
Internet

24   journals.sagepub.com   17 words — < 1%
Internet

25   "Treatment of Inflammatory Bowel Disease with   16 words — < 1%
Biologics", Springer Science and Business Media
LLC, 2018
Crossref

| 26 | pubag.nal.usda.gov | 15 words — < 1% |
| | Internet | |

| 27 | Arthur Kaser, Sebastian Zeissig, Richard S. Blumberg. "Inflammatory Bowel Disease", Annual Review of Immunology, 2010 | 14 words — < 1% |
| | Crossref | |

| 28 | "Current Drug Synthesis", Wiley, 2022 | 13 words — < 1% |
| | Crossref | |

| 29 | Titilayo Omolara Johnson, Kenneth Daniel Odoh, Charles Obiora Nwonuma, Augustina Oduje Akinsanmi, Abayomi Emmanuel Adegboyega. "Biochemical evaluation and molecular docking assessment of the anti-inflammatory potential of Phyllanthus nivosus leaf against ulcerative colitis", Heliyon, 2020 | 13 words — < 1% |
| | Crossref | |

| 30 | mediatum.ub.tum.de | 13 words — < 1% |
| | Internet | |

| 31 | "Abstracts from 8th International ISSX Meeting", Drug Metabolism Reviews, 2010 | 12 words — < 1% |
| | Crossref | |

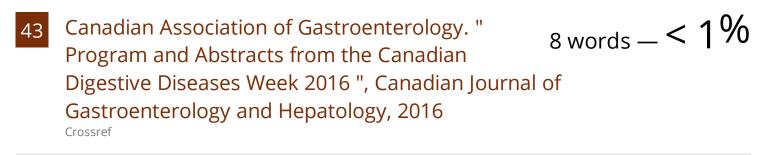| 32 | Andrés Arango-Restrepo, Daniel Barragán, J. Miguel Rubi. "A Criterion for the Formation of Nonequilibrium Self-Assembled Structures", The Journal of Physical Chemistry B, 2021 | 12 words — < 1% |
| | Crossref | |

| 33 | d-nb.info | 12 words — < 1% |
| | Internet | |

| 34 | studentsrepo.um.edu.my | 12 words — < 1% |
| | Internet | |

| 35 | www.scielo.br
Internet | 12 words — < 1% |
| --- | --- | --- |
| 36 | storage.googleapis.com
Internet | 11 words — < 1% |
| 37 | www.dovepress.com
Internet | 11 words — < 1% |
| 38 | Mengjiao Guo, Hui Zheng, Tengfei Ji, Hu Fa, Jing He. "A Triangle Framework among Subgraph Isomorphism, Pharmacophore and Structure-function Relationship", Companion Proceedings of the Web Conference 2022, 2022
Crossref | 10 words — < 1% |
| 39 | M. Z. Hernandes, F. J.de S. Pontes, L. C.D. Coelho, D. R.M. Moreira, V. R.A. Pereira, A. C.L. Leite. "Recent Insights on the Medicinal Chemistry of Metal-Based Compounds: Hints for the Successful Drug Design", Current Medicinal Chemistry, 2010
Crossref | 9 words — < 1% |
| 40 | orbi.uliege.be
Internet | 9 words — < 1% |
| 41 | rss.globenewswire.com
Internet | 9 words — < 1% |
| 42 | Arun Bahadur Gurung, Kripamoy Aguan, Sivaprasad Mitra, Atanu Bhattacharjee. "Identification of molecular descriptors for design of novel Isoalloxazine derivatives as potential Acetylcholinesterase inhibitors against Alzheimer's disease", Journal of Biomolecular Structure and Dynamics, 2016
Crossref | 8 words — < 1% |

43 Canadian Association of Gastroenterology. " Program and Abstracts from the Canadian Digestive Diseases Week 2016 ", Canadian Journal of Gastroenterology and Hepatology, 2016
Crossref
8 words — < 1%

44 N. -G. Li, Z. -H. Shi, Y. -P. Tang, Wei-Li, Lian-Yin, J. -A. Duan. "Discovery of Selective Small Molecular TACE Inhibitors for the Treatment of Rheumatoid Arthritis", Current Medicinal Chemistry, 2012
Crossref
8 words — < 1%

45 dataverify.creative-biolabs.com
Internet
8 words — < 1%

46 pharmaceuticalchemistry.conferenceseries.com
Internet
8 words — < 1%

47 research.bioinformatics.udel.edu
Internet
8 words — < 1%

48 www.science.gov
Internet
8 words — < 1%

49 www.semanticscholar.org
Internet
8 words — < 1%

50 Crohn s Disease and Ulcerative Colitis, 2012.
Crossref
7 words — < 1%

51 Vipul Yadav, Felipe Varum, Roberto Bravo, Esther Furrer, Daniela Bojic, Abdul W. Basit. "Inflammatory bowel disease: exploring gut pathophysiology for novel therapeutic targets", Translational Research, 2016
Crossref
7 words — < 1%

52 Yamamoto-Furusho, Jesus K. "Genetic factors associated with the development of inflammatory bowel disease", World Journal of Gastroenterology, 2007.
Crossref

6 words — < 1%