Evaluation of Toll-like Receptors in Children with Inflammatory Bowel Disease

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Abstract

Background:Inflammatory bowel disease is a chronic inflammation of gastro- intestinal tract (GIT), it include two forms, Ulcerative Colitis (UC) and Crohn's disease. Including ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory disorders of the gastrointestinal tract that begin most commonly during adolescence and young adulthood. In patients with IBD, host genetic, environmental, and microbial influences converge and result in a dysregulated mucosal immune response against the commensal intestinal microbiota. Cellular receptors in the innate immune system are fundamental and recognize pathogenic molecules to trigger immune responses. Additionally, some toll-like receptor (TLR) polymorphisms/mutations have been identified and directly linked to IBD. Genetic alterations of these receptors might change the composition of microbiota in the gut. Therefore, receptors of the innate immune system, such as TLRs, impact many aspects of IBD etiology, including immune responses, genetics, and microbiota

Keywords:Inflammatory bowel disease (IBD),Toll-like Receptor (TLR).

Inflammatory Bowel Disease in Children:

Including ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory disorders of the gastrointestinal tract that begin most commonly during adolescence and young adulthood **(1).**

Epidemiologic Features

Approximately 25% of patients with IBD present before age 20 years **(1).**
Among children with IBD, 4% present before age 5 years and 18% before age 10 years, with the peak onset in adolescence. The incidence of pediatric IBD is approximately 10 per 100 000 children per year in the United States and Canada and is rising with a prevalence of 100 to 200 per 100 000 children in the United States **(2).**

Pathogenesis

In patients with IBD, host genetic, environmental, and microbial influences converge and result in a dysregulated mucosal immune response against the commensal intestinal microbiota. Recent technologic advances have led to an explosion of discovery of the genetic and microbial influences on IBD. Genome-wide association studies have identified common variants in more than 150 genes that confer risk for IBD.Risk variants can be grouped into biological pathways that

shed light on IBD pathogenesis, including innate and adaptive immunity and epithelial function. No difference exists in the common risk genes between pediatric-and adult-onset IBD; however, early-onset IBD may be associated with a higher burden of common risk variants and rarer variants with high penetrance(3).

Histologic features common to CD and UC include evidence of active inflammation (ie, neutrophils) and chronicity (ie, crypt loss or branching, mucin depletion, and/or lamina propria lymphocytosis). Although the inflammation and injury in UC is limited to the mucosa, CD can be a transmural process. Noncaseating granulomas are observed in as many as 60% of pediatric patients with CD and, in the right clinicopathologic setting, can distinguish CD from UC. Crohn's disease involving the colon only is more common in children than adults, which makes it difficult to distinguish CD and UC in some patients. The term *IBD unspecified* (previously called *indeterminate colitis*) is reserved for patients who cannot be classified definitively as having UC or CD **(4).**
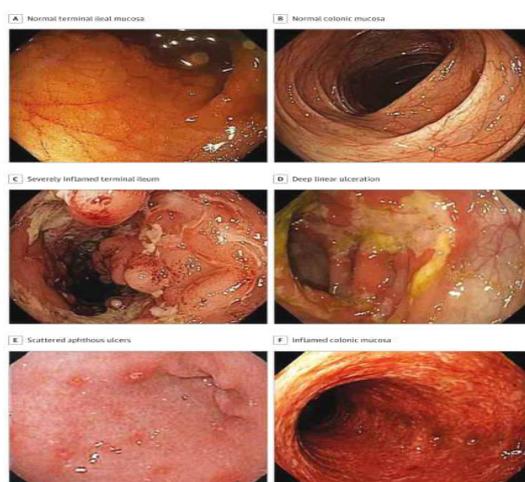

FIG :(1) Representative Endoscopic Images of Normal and Inflamed Gastrointestinal Mucosa From Pediatric Patients With and Without Inflammatory Bowel Disease **(5)**

Toll-like receptors

Toll-like receptor are a class of proteins that play a key role in the innate immune system. They are single, membrane-spanning, non-catalytic receptors usually expressed on sentinel cells such as macrophages and dendritic cells, that recognize structurally conserved molecules derived from microbes. Once these microbes have breached physical barriers such as the skin or intestinal tract mucosa, they are recognized by TLRs, which activate immune cell responses. The TLRs include TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, though the last three are not found in humans **(25).** TLR's received their name from their similarity to the protein coded by the toll gene identified in Drosophila in 1985 by Christiane Nüsslein-Volhard and Eric Wieschaus**(6).**

Function

The ability of immune system to recognize molecules that are broadly shared by pathogens is, in part, due to the presence of Immune receptors called toll-like receptors (TLRs) that are expressed on the membranes of leukocytes including dendritic cells, macrophages, natural killer cells, cells of the adaptive immunity (T and B lymphocytes) and non-immune cells (epithelial and endothelial cells, and fibroblasts) **(7).**

The binding of ligands - either in the form of adjuvant used in vaccinations or in the form of invasive moieties during times of natural infection - to the TLR marks the key molecular events that ultimately lead to innate immune responses and the development of antigen-specific acquired immunity.

Upon activation, TLRs recruit adapter proteins (proteins that mediate other protein-protein interactions) within the cytosol of the immune cell in order to propagate the antigen-induced signal transduction pathway. These recruited proteins are then responsible for the subsequent activation of other downstream proteins, including protein kinases (IKKi, IRAK1, IRAK4, and TBK1) that further amplify the signal and ultimately lead to the upregulation or suppression of genes that orchestrate inflammatory responses and other transcriptional events. Some of these events lead to cytokine production, proliferation, and survival, while others lead to greater adaptive immunity. **(8).**

If the ligand is a bacterial factor, the pathogen might be phagocytosed and digested, and its antigens presented to CD4+ T cells. In the case of a viral factor, the infected cell may shut off its protein synthesis and may undergo programmed cell death (apoptosis). Immune cells that have detected a virus may also release anti-viral factors such as interferons.

TLR3
TLR3 does not use the MyD88 dependent pathway. Its ligand is retroviral double-stranded RNA (dsRNA), which activates the TRIF dependent signalling pathway. Stimulation of TLR3 causes great changes in chromatin remodeling and nuclear reprogramming, and activation of inflammatory pathways is required for these changes, induction of pluripotency genes and generation of human induced pluripotent stem cells (iPSC) colonies **(9).**

Toll-like receptor 2 (TLR2)
TLR2 is a membrane protein, a receptor, which is expressed on the surface of certain cells and recognizes foreign substances and passes on appropriate signals to the cells of the immune system. In the intestine, TLR2 regulates the expression of CYP1A1which is a key enzyme in detoxication of carcinogenic polycyclic aromatic hydrocarbons such as benzo(a)pyrene **(10).**

Toll-like receptor 4 (TLR4)
is a protein that in humans is encoded by the TLR4 gene. TLR4 is a transmembrane protein, member of the toll-like receptor family, which belongs to the pattern recognition receptor (PRR) family. Its activation leads to an intracellular signaling pathway NF-κB and inflammatory cytokine production which is responsible for activating the innate immune system. It is most well-known for recognizing lipopolysaccharide (LPS), a component present in many Gram-negative bacteria (e.g. Neisseria spp.) and select Gram-positive bacteria. Its ligands also include several viral proteins, polysaccharide, and a variety of endogenous proteins such as low-density lipoprotein, beta-defensins, and heat shock protein. TLR4 has also been designated as CD284 (cluster of differentiation 284). The molecular weight of TLR4 is approximately 95 kDa**(11).**
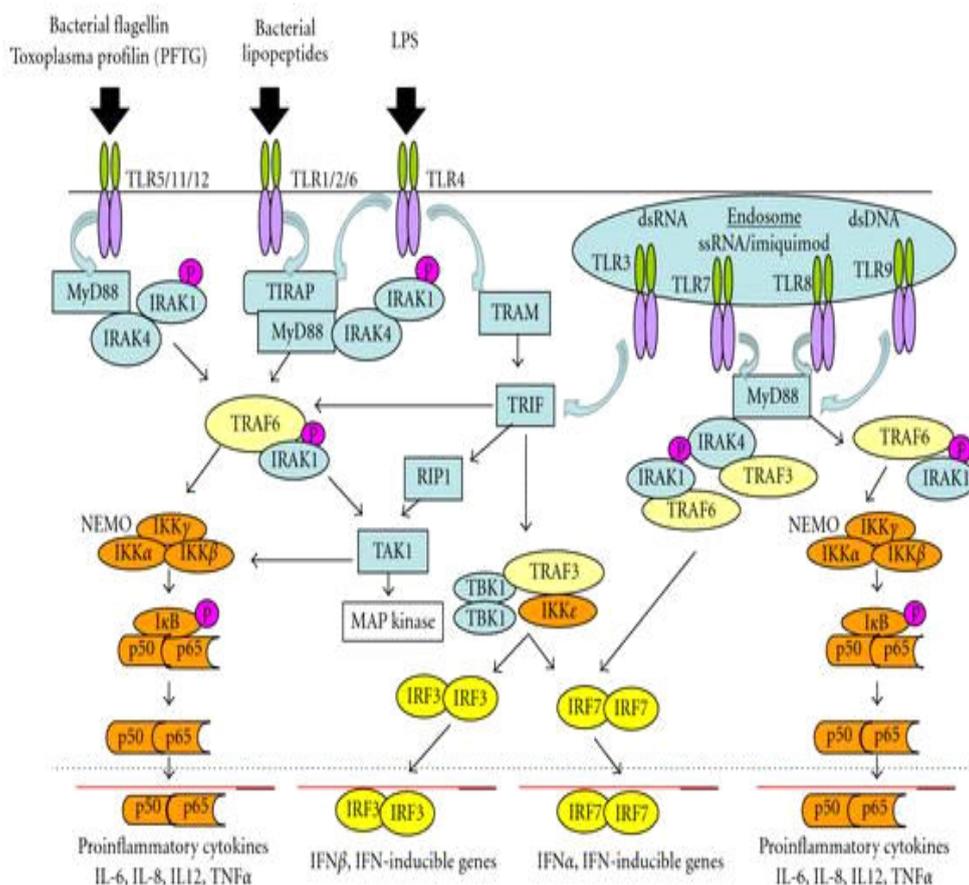
FIG (2) Signaling pathway of toll-like receptors(**12).**

Toll-like Receptors and Inflammatory Bowel Disease

G As is well acknowledged, IBD results from an inappropriate response of a dysfunctional mucosal immune system to the resident microbiota and other noxious antigens. In intestinal ecology, trillions of indigenous commensal microorganisms, including bacteria, fungi, and viruses , maintain homeostasis along with the elements of the host immune system . However, the introduction of an innate immune defect, for example, epithelial barrier leak and/or mucosal destruction can harm the otherwise beneficial host–microbe balance **(3).** Furthermore, the intestine's access to an outside environment facilitates invasion by extrinsic pathogens, thereby inciting a host immune response to contribute to diseases.

Recently TLRs serve as the hub of immune responses to microbes in the gut, thereby inciting IBD. The related research led to this summary of advances in current studies about the relationship between TLR signaling pathways and IBD and our perspective about potential therapeutic strategies for alleviating IBD **(13).**

TLRs are Key Immune Sensors of Microbiota in the Gut

As the body's initial defense weapon, innate immunity plays a critical role in recognizing pathogens and maintaining intestinal homeostasis. TLRs are pattern recognition receptors that work as the immune system's protective sentries whose job is to sense and recognize pathogen-associated molecular patterns (PAMPs). PAMPs are highly conserved structures of microbes, such

as unmethylated double-stranded DNA, single-stranded RNA, lipopolysaccharide (LPS), lipoproteins, and flagellin. Upon activation, TLRs become dimerized and trigger the subsequent activation of downstream signaling cascades, such as inducing a variety of inflammatory cytokines through transcription by mediating the phosphorylation of IκB to activate NF-κB. Furthermore, TLR activation regulates the maturation of dendritic cells (DCs) and induces the proliferation and differentiation of Th1 and Th2 T cells. **(14).**

Because the TLR signaling involves in many life-threatening diseases, TLRs have been the subject of intense study. IBD is only one of these devastating conditions; nevertheless, such patients exhibit far different expressions of TLRs in comparison with healthy controls. Most TLR signaling pathways participate in the progression of IBD, performing sometimes beneficial and other times harmful functions. As an essential adaptor of TLR pathways, myeloid differentiation primary response 88 (MyD88) is a proven actor in the breakdown of immune tolerance. As mentioned above, TLRs not only control innate immunity but also critically regulate adaptive immunity, such as T cell activation. By restraining the TLR-induced over immune responses, T regulatory cells (Tregs) inhibit other T cells from functioning effectively to maintain immune tolerance and have a key role in promoting tolerance at host–microbial interfaces. The balance between Tregs and effector T cells is disturbed in patients with IBD. That is, when Tregs' function of providing immune tolerance is suppressed and effector T cells like Th1, Th2, Th17, and NKT cells are activated, producing and releasing inflammatory cytokines, the progression of an inflammatory disease like IBD grows out of control.**(15).**

TLRs are a Potential Molecular Mechanism of IBD

Toll-like receptors, as sensors of gut microbiota, play a critical role in maintaining the gut's homeostasis, controlling the immune responses and shaping the microbiota. In a mouse model, Inoue et al. found that the postnatal expression of TLR2 and TLR4 in intestinal epithelial cells (IECs) is dynamic and depends on the presence of commensal intestinal microbiota. In reporting about the relationship between intestinal TLRs and commensal microbiota, others showed that oral antibiotic treatment resulted in the upregulation of TLR4, TLR5, and TLR9 in the ileum and TLR3, TLR4, TLR6, TLR7, and TLR8 in the colon; meanwhile the expression of TLR2, TLR3, and TLR6 in the ileum as well as TLR2 and TLR9 in the colon diminished. Additionally, the diversity and total amount of microbiota decreased. Those results confirmed that the microbiota could regulate TLR expression. Although TLRs' ability to exert a protective effect against IBD has been noted, controversy remains. Thus, the actual contribution of TLRs to inflammation and commensal dysbiosis remains uncertain, although the activation of TLR signaling triggers a serial cascade of downstream events that clearly play an important role in the development of IBD. **(16)**

The TLR signaling pathway is very similar with the interleukin (IL)-1R family, characterized by the requirement for a Toll-IL 1 receptor (TIR) domain-containing adaptor protein (TIRAP), protein kinase, and a transcriptional factor to transfer the signal. Except for TLR3, other TLR signaling pathways depend on MyD88 to activate NF-κB and MAPK (mitogen-activated protein kinase) to control the inflammatory response.

The domain containing TIRAP lying downstream of TLR1, 2, 4, and 6 recruits MyD88. Meanwhile, TRIF (TIR domain-containing adaptor inducing IFNβ) participates in TLR3 and TLR4 signaling pathways. Thus, TLR signaling is divided into two types of pathways: one of which is MyD88-dependent and the other MyD88-independent but TRIF-dependent. Downstream

of the TLR signaling pathways, activated NF-κB and IRF [interferon (IFN) regulatory factor] control their target genes   to produce an abundance of inflammatory cytokines and IFNs, which improve resistance to and clearance of pathogens from the body, and can also promote inflammation. Other functions of the latter factors are to upregulate the expression of related genes responsible for phagocytosis and possessing the ability to enhance phagocytic function and to kill microbes. Apart from these functions, TLR signaling recruits activated natural killer cells (NK cells) and DCs. DCs are prompted by TLRs to present antigens to T cells and initiate T cell responses, thus providing a bridge between innate immunity and adaptive immunity. TLRs and the signaling pathway also exist in T cells and, once intrinsic TLR signaling in T cells is lost, it results in significant changes in the gut's microbial composition. Follicular helper T cells are abundantly produced in germinal center and interact with B cells, a process that is also mediated by T cell-intrinsic TLR signaling. TLRs additionally regulate B-cell responses for the purpose of producing monospecific IgM, IgG, and IgA antibodies, which involved in adaptive immunity that can mediate intestinal homeostasis and regulate microbiota content **(17).**
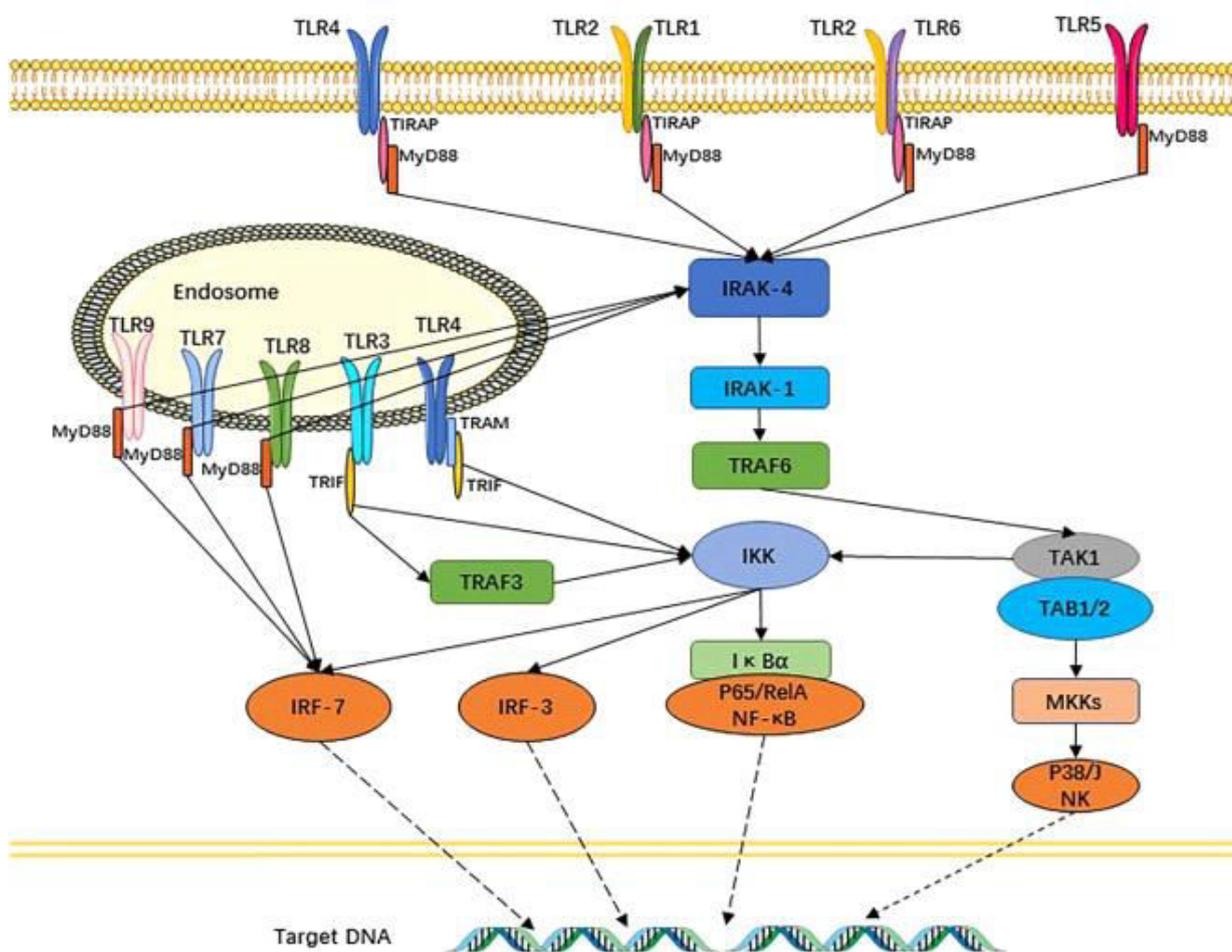

Fig (3) Toll-like receptors (TLRs) and their signaling pathways. TLR1 and TLR6 recognize their ligands as heterodimers with TLR2. For TLR4, MD2, and CD14 are required for lipopolysaccharide recognition and signaling. TLR3, TLR4, TLR5, TLR7, and TLR9 are currently thought to deliver their signal by forming homodimers after interacting with their ligands. TLR3, TLR7/8, and TLR9 are intracellular TLRs and recognize nucleic acids **(18).**

TLRs are Associated with IBD
Toll-like receptors have been detected in both IECs and stromal tissue cells of the gastrointestinal tract and TLR1, 2, 3, 4, 5, and 9 are expressed in the small and large intestines of mice and humans However, TLR6, 7, and 8 are present only in the human colon and murine small intestine .

With regard to patients suffering UC, though, individual TLRs are differentially expressed in the intestinal epithelium. That is, the expression of TLR2, 4, 8, and 9 genes are upregulated in patients with active UC, whereas TLR5 tends to be upregulated in those with active UC (compared to quiescent UC disease) and downregulated in quiescent UC (compared to controls with healthy colonic mucosa) **(19).**

TLR4

TLR4 is the first identified TLR in mammalian system and recognizes LPS in Gram-negative bacteria. Under physiologic conditions, TLR4 is expressed at a low level in IECs. Although the primary role of TLR4 is beneficial for induction of an inflammatory response providing protection from invading bacteria and promoting mucosal integrity; in some experiments, TLR4 proved to be maladaptive, actually causing tissue destruction and ulceration. **(20).**

Considering that TLR4 gene expression was upregulated in the intestinal epithelia of patients with active UC, TLR4 might be a participant in UC disease development. One study showed that stimulated enteric glial cells (EGCs) could release nitric oxide *via* TLR4, leading to the liberation of pro-inflammatory cytokines, which would aggravate gut inflammation. Elsewhere, LPS (a TLR4 ligand) stimulation of monocytes and conventional DCs elicited high levels of pro-inflammatory cytokines, which would intensify the DSS-induced colitis. Afterward, a nutritional activator of innate immunity, wheat amylase-trypsin inhibitor (ATI), was found capable of interacting with TLR4 on myeloid cells. Oral ATIs then proved to induce intestinal myeloid cell infiltration and activation as well as release of inflammatory mediators in the colon, mostly through the TLR4 pathway **(21).**

The foregoing conclusions had important implications for the onset and severity of inflammatory intestinal disease. Much evidence supports the theory that the TLR4 signaling pathway has a negative role in IBD. Interestingly,
(22).

obtained experimental results denoting that TLR2 could protect against many noxious agents. In addition, TLR2 cyto-protective responses from tissue-resident cells maintained mucosal integrity against the lethal TLR4-dependent inflammatory responses of hematopoietic cells. Thus, the role of TLR4 during colitis could be either protective or damaging **(23).**

Effector Factors Downstream of TLR Signaling Pathways in IBD

The TLRs lying upstream of the signaling pathways seem like the scouts, whereas infantries of the army are the effective cytokines secreted by target cells, such as epithelial cells, DCs, and T cells. In this section, we will analyze and discuss the roles of effectors involved in the TLR signaling pathways, whether beneficial or noxious **(24)**

ConflictofInterest: Noconflictofinterest.

References

1. **Benchimol, E. I., Fortinsky, K. J., Gozdyra, P., Van den Heuvel, M., Van Limbergen, J., &Griffiths, A. M. (2015).** Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. Inflammatory bowel diseases, 17(1), 423-439.

2. **Sýkora, J., Pomahačová, R., Kreslová, M., Cvalínová, D., Štych, P., & Schwarz, J. (2018).** Current global trends in the incidence of pediatric-onset inflammatory bowel disease. World journal of gastroenterology, 24(25), 2741.

3. **Knights, D., Lassen, K. G., & Xavier, R. J. (2016).** Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. Gut, 62(10), 1505-1510.

4. **Muise, A. M., Snapper, S. B., &Kugathasan, S. (2016).** The age of gene discovery in very early onset inflammatory bowel disease. Gastroenterology, 143(2), 285-288.

5. **Gevers, D., Kugathasan, S., Denson, L. A., Vázquez-Baeza, Y., Van Treuren, W., Ren, B., ...& Xavier, R. J. (2014).** The treatment-naive microbiome in new-onset Crohn's disease. Cell host & microbe, 15(3), 382-392.

6. **Shim J. O. (2019).** Recent Advance in Very Early Onset Inflammatory Bowel Disease. Pediatric gastroenterology, hepatology & nutrition, 22(1), 41–49. https://doi.org/10.5223/pghn.2019.22.1.41

7. **Kugathasan, S., Judd, R. H., Hoffmann, R. G., Heikenen, J., Telega, G., Khan, F., ...&Binion, D. G. (2017).** Epidemiologic and clinical characteristics of children with newly diagnosed inflammatory bowel disease in Wisconsin: a statewide population-based study. The Journal of pediatrics, 143(4), 525-531.

8. **Griffiths, A. M. (2014).** Specificities of inflammatory bowel disease in childhood. Best Practice & Research Clinical Gastroenterology, 18(3), 509-523.

9. **Henderson, P., Anderson, N. H., & Wilson, D. C. (2014).** The diagnostic accuracy of fecal calprotectin during the investigation of suspected pediatric inflammatory bowel disease: a systematic review and meta-analysis. American Journal of Gastroenterology, 109(5), 637-645.

10. **Minar, P., Haberman, Y., Jurickova, I., Wen, T., Rothenberg, M. E., Kim, M. O., ...& Denson, L. A. (2014).** Utility of neutrophil Fcγ receptor I (CD64) index as a biomarker for mucosal inflammation in pediatric Crohn's disease. Inflammatory bowel diseases, 20(6), 1037-1048.

11. **Vaughn, B. P., Shah, S., &Cheifetz, A. S. (2014).** The role of mucosal healing in the treatment of patients with inflammatory bowel disease. Current treatment options in gastroenterology, 12(1), 103–117. https://doi.org/10.1007/s11938-013-0008-1

12. **Sandborn, W. J., Travis, S., Moro, L., Jones, R., Gautille, T., Bagin, R., ...& Ballard II, E. D. (2018).** Once-daily budesonide MMX® extended-release tablets induce remission in patients with mild to moderate ulcerative colitis: results from the CORE I study. Gastroenterology, **143(5), 1218-**1226.

13. **Feagan, B. G., & MacDonald, J. K. (2016).** Oral 5-aminosalicylic acid for induction of remission

in ulcerative colitis. Cochrane database of systematic reviews, (10).

14. **Dulai, P. S., Thompson, K. D., Blunt, H. B., Dubinsky, M. C., & Siegel, C. A. (2014).** Risks of serious infection or lymphoma with anti–tumor necrosis factor therapy for pediatric inflammatory bowel disease: a systematic review. Clinical Gastroenterology and Hepatology, 12(9), 1443-1451.

15. **Lichtenstein, G. R., Loftus, E. V., Isaacs, K. L., Regueiro, M. D., Gerson, L. B., & Sands, B. E. (2018).** ACG clinical guideline: management of Crohn's disease in adults. Official journal of the American College of Gastroenterology| ACG, 113(4), 481-517.

16. **Hyams, J., Damaraju, L., Blank, M., Johanns, J., Guzzo, C., Winter, H. S., ...& T72 Study Group. (2018).** Induction and maintenance therapy with infliximab for children with moderate to severe ulcerative colitis. Clinical Gastroenterology and hepatology, 10(4), 391-399.

17. **Hyams, J. S., Griffiths, A., Markowitz, J., Baldassano, R. N., Faubion Jr, W. A., Colletti, R. B., ... & Lazar, A. (2018).** Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. Gastroenterology, 143(2), 365-374.

18. **Hyams, J., Crandall, W., Kugathasan, S., Griffiths, A., Olson, A., Johanns, J., ...& REACH Study Group. (2017).** Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. Gastroenterology, 132(3), 863-873.

19. **Huguet, M., Pereira, B., Goutte, M., Goutorbe, F., Dubois, A., Bommelaer, G., & Buisson, A. (2018).** Systematic review with meta-analysis: anti-TNF therapy in refractory pouchitis and Crohn's disease-like complications of the pouch after ileal pouch-anal anastomosis following colectomy for ulcerative colitis. Inflammatory bowel diseases, 24(2), 261-268.

20. **Liu, W., Chen, Y., Golan, M. A., Annunziata, M. L., Du, J., Dougherty, U., Kong, J., Musch, M., Huang, Y., Pekow, J., Zheng, C., Bissonnette, M., Hanauer, S. B., & Li, Y. C. (2015).** Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. The Journal of clinical investigation, 123(9), 3983–3996. https://doi.org/10.1172/JCI65842

21. **Yangyang, R. Y., &Rodriguez, J. R. (2017, December).** Clinical presentation of Crohn's, ulcerative colitis, and indeterminate colitis: Symptoms, extraintestinal manifestations, and disease phenotypes. In Seminars in pediatric surgery (Vol. 26, No. 6, pp. 349-355). WB Saunders.

22. **Paganelli, M., Albanese, C., Borrelli, O., Civitelli, F., Canitano, N., Viola, F., Passariello, R., & Cucchiara, S. (2017).** Inflammation is the main determinant of low bone mineral density in pediatric inflammatory bowel disease. Inflammatory bowel diseases, 13(4), 416–423. https://doi.org/10.1002/ibd.20039

23. **Castaño-Milla, C., Chaparro, M., &Gisbert, J. P. (2014).** Systematic review with meta-analysis: the declining risk of colorectal cancer in ulcerative colitis. Alimentary pharmacology & therapeutics, 39(7), 645–659. https://doi.org/10.1111/apt.12651

24. **Mackner, L. M., Greenley, R. N., Szigethy, E., Herzer, M., Deer, K., &Hommel, K. A. (2017).** Psychosocial issues in pediatric inflammatory bowel disease: report of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition. Journal of pediatric

gastroenterology and nutrition, 56(4), 449–458. https://doi.org/10.1097/MPG.0b013e3182841263

25. **Lu, Y., Li, X., Liu, S., Zhang, Y., & Zhang, D. (2018).** Toll-like Receptors and Inflammatory Bowel Disease. Frontiers in immunology, 9, 72. https://doi.org/10.3389/fimmu.2018.00072.