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OPINION REVIEW

- 2149 Alteration of the esophageal microbiota in Barrett's esophagus and esophageal adenocarcinoma
Lv J, Guo L, Liu JJ, Zhao HP, Zhang J, Wang JH

REVIEW

- 2162 Inflammatory bowel diseases and spondyloarthropathies: From pathogenesis to treatment
Fragoulis GE, Liava C, Daoussis D, Akriviadis E, Garyfallos A, Dimitroulas T

MINIREVIEWS

- 2177 Harnessing the potential of gene editing technology using CRISPR in inflammatory bowel disease
Limanskiy V, Vyas A, Chaturvedi LS, Vyas D
- 2188 Diversity of *Saccharomyces boulardii* CNCM I-745 mechanisms of action against intestinal infections
Czerucka D, Rampal P

ORIGINAL ARTICLE**Basic Study**

- 2204 Characteristics of mucosa-associated gut microbiota during treatment in Crohn's disease
He C, Wang H, Liao WD, Peng C, Shu X, Zhu X, Zhu ZH

Retrospective Study

- 2217 Role of abdominal ultrasound for the surveillance follow-up of pancreatic cystic neoplasms: a cost-effective safe alternative to the routine use of magnetic resonance imaging
Morelli L, Guadagni S, Borrelli V, Pisano R, Di Franco G, Palmeri M, Furbetta N, Gambaccini D, Marchi S, Boraschi P, Bastiani L, Campatelli A, Mosca F, Di Candio G

Clinical Trials Study

- 2229 Ombitasvir/paritaprevir/ritonavir + dasabuvir +/- ribavirin in real world hepatitis C patients
Loo N, Lawitz E, Alkhoury N, Wells J, Landaverde C, Coste A, Salcido R, Scott M, Poordad F

Observational Study

- 2240 Nested case-control study on risk factors for opportunistic infections in patients with inflammatory bowel disease
Gong SS, Fan YH, Han QQ, Lv B, Xu Y

META-ANALYSIS

- 2251** Effect of prophylactic clip placement following endoscopic mucosal resection of large colorectal lesions on delayed polypectomy bleeding: A meta-analysis
Ayoub F, Westerveld DR, Forde JJ, Forsmark CE, Draganov PV, Yang D

CASE REPORT

- 2264** Recurrent renal cell carcinoma leading to a misdiagnosis of polycystic liver disease: A case report
Liang C, Takahashi K, Kurata M, Sakashita S, Oda T, Ohkohchi N

ABOUT COVER

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Harnessing the potential of gene editing technology using CRISPR in inflammatory bowel disease

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Abstract

The molecular scalpel of clustered regularly interspersed short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) technology may be sharp enough to begin cutting the genes implicated in inflammatory bowel disease (IBD) and consequently decrease the 6.3 billion dollar annual financial healthcare burden in the treatment of IBD. For the past few years CRISPR technology has drastically revolutionized DNA engineering and biomedical research field. We are beginning to see its application in gene manipulation of sickle cell disease, human immunodeficiency virus resistant embryologic twin gene modification and IBD genes such as Gatm (Glycine amidinotransferase, mitochondrial), nucleotide-binding oligomerization domain-containing protein 2, KRT12 and other genes implicated in adaptive immune convergence pathways have been subjected to gene editing, however there are very few publications. Furthermore, since Crohn's disease and ulcerative colitis have shared disease susceptibility and share genetic gene profile, it is paramount and is more advantageous to use CRISPR technology to maximize impact. Although, currently CRISPR does have its limitations due to limited number of specific Cas enzymes, off-target activity, protospacer adjacent motifs and crossfire between different target sites. However, these limitations have given researchers further insight on how to augment and manipulate enzymes to enable precise gene excision and limit crossfire between target sites.

Key words: Clustered regularly interspersed short palindromic repeats; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Gene excision; Gene editing; Gene therapy; Financial impact of inflammatory bowel disease on healthcare; Clustered regularly interspersed short palindromic repeats crossfire

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Core tip: Using clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) to harness the potential of gene editing technology implicated in inflammatory bowel disease. This revolutionary way of editing genes and its application of gene manipulation is only gaining momentum. Genes such as *Gatm* (Glycine amidinotransferase, mitochondrial) and nucleotide-binding oligomerization domain-containing protein 2 have been utilized to show CRISPR is able to manipulate these genes precisely, and this is just the beginning.

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INTRODUCTION

Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology has drastically revolutionized DNA engineering and biomedical research in the past few years. It has enabled researchers to model disease and advance therapeutic intervention techniques in a pragmatic and clinically efficacious way. The **Figure 1** below demonstrates the exponential growth in publications and interest in CRISPR technology over the past few years. **Figure 2** demonstrating use of CRISPR technology in inflammatory bowel disease (IBD). Strong interest in CRISPR has driven gene therapy publications down in the past few years as seen in **Figure 3**. Application of CRISPR is gaining momentum and is seen in sickle cell disease gene modification of hematopoietic stem cells, embryonic stem cells, gene-based therapy for HIV-1 infected individuals and other disease gene modification^[1-6]. It is a new era in biomedical research for use of CRISPR technology in IBD (**Figure 4**). These advancements in biomedical research with the use of CRISPR will enable researchers to begin treating IBD and other gene modifying diseases, thereby relieving the healthcare financial burden of IBD.

IBD affects an estimated 1.5 million people in North America^[7,8]. These patients have considerable morbidity and the associated financial burden on healthcare system is estimated to be 6.3 billion dollars annually with two thirds of that due to hospitalizations and pharmaceutical therapy alone^[9-11]. This does not take into account the financial implications of work and disability of patients and impact on daily life. Even though gene therapy remains expensive in any disease treatment due to pharmaceutical patents, considerable thought should be taken to propel CRISPR use in IBD because the use of this novel technology is less taxing than previously used epigenetic modification^[12].

Prior to the discovery of CRISPR/Cas9 enzyme, RNA sequencing^[7,8], modular protein recognition DNA such as zinc finger nuclease or TAL effector nuclease were used in epigenetic modification and disease modeling, which are complex and require very specific protein engineering and programming^[13]. However, with the revolutionary CRISPR technology, researchers now may edit genomes in a much simpler and precise way as modeled by bacteria^[14,15].

CRISPR/Cas9 was found in *Streptococcus pyogenes*, which uses this mechanism as defense against invading viruses^[14]. Upon infection by a virus the bacteria stores the viral DNA sequence in between sections of regularly interspaced palindromic repeated segments that are closely associated with genes that encode CRISPR-associated proteins. In order for the system to confer underlying efficacy and specificity, the CRISPR and the viral DNA is converted to two RNA moieties: TracrRNA and crRNA, the tracrRNA confers enzymatic activity and the crRNA determines substrate specificity^[13,16]. Once the moieties bind together, they traverse the cell seeking any genetic material that matches the crRNA. Upon sequence matching, the tracrRNA part of Cas enzyme clips the target DNA in specific nucleotide bases disabling its replication. This incredibly precise system can be utilized in therapeutic advances and programmed to target any sequence in the cell. This novel mechanism will be assessed for potential application and targeting of genes implicated in IBD.

CRISPR DNA engineering has advanced quickly and is evolving rapidly. It has

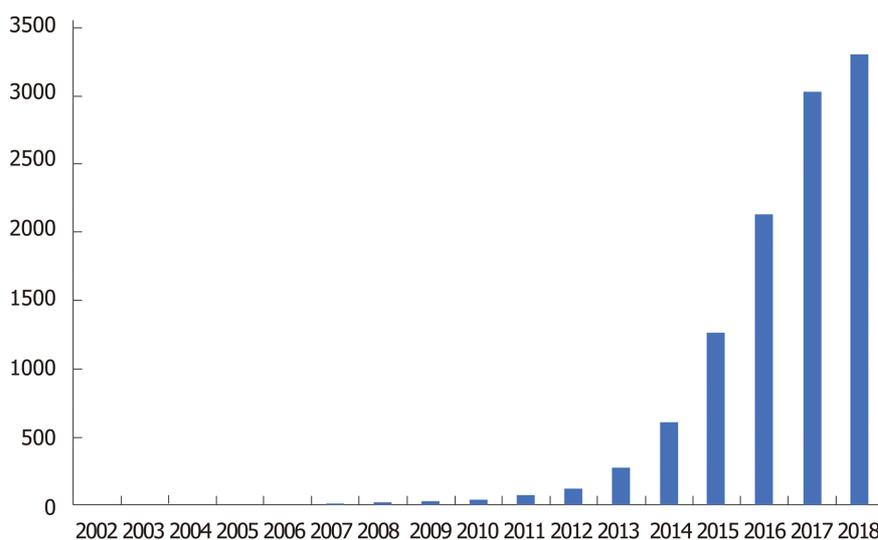


Figure 1 Number of CRISPR publications by year.

already been used in diseases such as sickle cell, beta-thalassemia or HIV resistant human embryos^[1,2,6]. Given its exponential application in disease variants, it may suggest that it's an optimal time to further our understanding of IBD pathogenesis and treatment. Primarily IBD refers to two major categories of chronic relapsing inflammatory intestinal disorders: Ulcerative colitis (UC) and Crohn's disease (CD). With the advent of genetic research both diseases have seen notable success culminating in the discovery of over 160 susceptible genes, among which, many potentially may be targeted by the CRISPR technology^[17,18]. Around one-third of these loci/genes described, confer susceptibility to both CD and UC, which may make targeting and gene editing a viable option^[18,19]. The genetic architecture of IBD has shed much light on central themes in IBD and the level of cellular process that the pathogenesis emerges. A putative CD-susceptibility locus has been mapped to chromosome 16 around locus D16S409 and D16S419 which may shed more light etiology of IBD^[20]. In CD a common genetic theme is seen in defective processing of intracellular bacteria, autophagy and innate immunity. In UC, genetic evidence demonstrates genes that are responsible for proper barrier function are important in preventing UC. However, when analyzing genetic data in more detail, CD and UC have shared disease susceptibility and shared genetic gene profile that may be targeted^[18]. **Table 1** demonstrates common genes implicated and their strength of role in IBD^[18].

Recent research has identified and linked a gene, *Gatm*, to IBD using CRISPR/Cas9 gene-editing technology. When the *Gatm* gene is activated, it induces synthesis of creatine which helps in intestinal mucosal barrier, which helps protect the intestinal wall against inflammation that's caused by bacteria^[21]. When inducing a frame-shift mutation in the *Gatm* gene *via* CRISPR/Cas9 there was signs of inflammatory response in the intestinal wall. This demonstrated its important role in mucosal barrier protection and potential manipulation of this gene using CRISPR. Protection from inflammation by an intact barrier is vital to decrease immune response, which is suppressed or increased by enhancers in the immune activation cascade. In the inflammatory response pathway, IL2RA plays a role in signaling T cells to hamper or increase the response. If the enhancers that switch on IL2RA are defective the T cells won't suppress inflammation and chronic inflammation is associated with 15%-20% of all human malignancies^[8,22]. Inflammation also results in autoimmune disorders such as IBD and inflammatory-induced colon cancer mediated by NF- κ B pathway^[22-24]. Previously it has been demonstrated that single nucleotide polymorphisms -SNPs' mutation of IL2RA leads to improper activation of T cells and subsequently resulting in autoimmune disorders. These SNP's may be targeted by CRISPR/Cas9 and repaired with non-homologous end-joining repair. This has been demonstrated in KRT12 mutations-specific targeting of SNP's as well^[25]. Recent advancements in CRISPR/Cas9 specificity and potency of targeted genes demonstrate that SNP's or genes that have point mutations may be targeted and editing may be attempted.

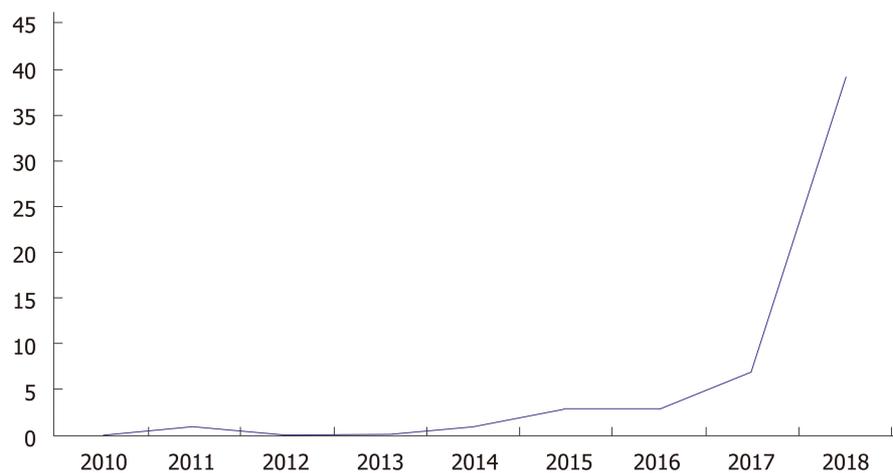


Figure 2 Publications on inflammatory bowel disease using CRISPR.

POTENTIAL THERAPEUTIC TIMING

CD and UC in general have three stages of disease progression, mild-moderate; moderate-severe; and severe. Currently there are no studies indicating potential therapeutic timing when to target affected genes using CRISPR in IBD, however several multicenter trials conducted administering human recombinant IL-10 during active mild/moderate stage of CD or during refractory CD as well as patients undergoing curative ileal or ileocolonic resection^[26,27]. However, results did not show significant clinical improvement or higher remission rates secondary to too low IL-10 dose and adverse effects of medication. In addition, IL-10 alone failed to effectively suppress variety of dysregulated pro-inflammatory cytokines^[26,27]. In later stages of disease process, significant dysregulation of pro-inflammatory cytokines and redundant pathways occur, such as NF- κ B receiving activation from different pathways^[28] thus single target impact is futile. Given that CRISPR can simultaneously multiplex several genes, it will aid researchers to devise appropriate intervention timing^[29]. We also suggest early intervention is optimal to prevent progression of disease and reduce complications. It is imperative to conduct studies to best identify role of CRISPR in various stages of disease.

CURRENT STATUS

Currently, CRISPR is applied in many fields of scientific study. In biotechnology it is used to modify Maize genome in protoplasts. In drug development, it is used to understand modes of drug resistance and drug-target interactions. In epigenetics, it has taken the place of zinc finger nuclease and TALEN in epigenetic modification because the indel frequency is more superior^[6]. Since the CRISPR debut, researchers are improving and enhancing the specificity and accuracy of the Cas9. Currently the Cas9 not only cuts the DNA, but can be altered to perform desired functions. The Cas9 protein has a deaminase region that may be altered to increase highly specific alternation of genome sequence, which will allow for broader specific DNA bases manipulation^[13]. It can also promote gene transcription using enzyme by deactivating the endonuclease activity and add transcriptional activator to increase transcription. The Cas9 can silence domains that recruit factors so that genes are blocked and they are not transcribed. In general targeting studies, Cas9 can be tagged with fluorescent dye so genes can be followed. Furthermore, Cas9 can be multiplexed with multiple guide RNAs to generate multiple breaks in order to cut out large sequences of DNA in one experiment^[29]. This limits time and repetitiveness of experiments conducted and time is of an essence in this race to invent even-more versatile or efficient variations of this powerful enzyme, which greatly simplifies the editing of DNA. Furthermore, very recently successful attempts were made to edit CCR5 gene in human embryos to enable resistance to HIV^[30]. Although, ethics and implications of such studies are currently widely debated. Also, recently KRAS oncogenic alleles were modified leading to decreased cancer cell growth without disturbing wild type alleles^[31]. Since major oncogenic mutations occur on codon-12 of KRAS exon-2, direct targeting of oncogenic KRAS single-nucleotide missense substitution c.35G>T mutation using

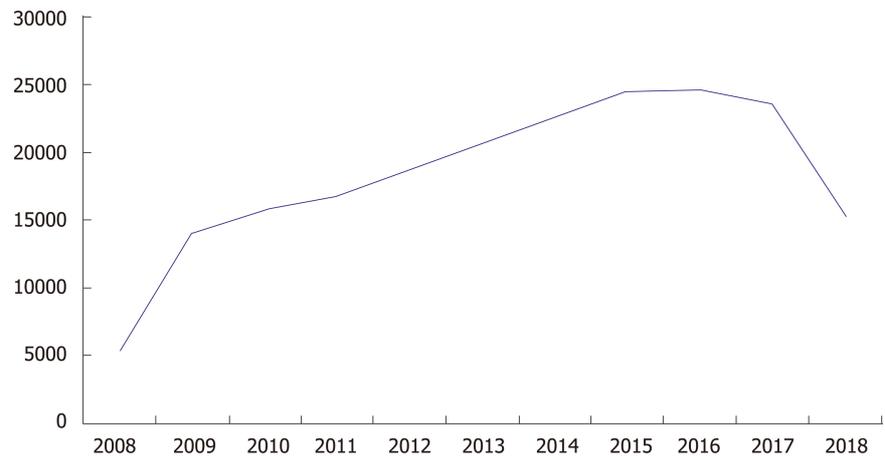


Figure 3 Total publications using gene therapy.

CRISPR/Cas9 system inhibited cancer cell growth and is dependent on efficient target cell transduction^[31].

LIMITATIONS

The excitement generated by the new CRISPR technology in the science community is reflected by exponential publications and application in various fields of study, including agriculture, drug development and epigenetic control but its revolutionary genome editing method is far from perfect. The CRISPR is an RNA based DNA recognition system. It is dependent on a guide molecule composed of RNA to recognize a sequence in the DNA that has specific molecular features. The problem is that the original Cas9 can only land on genome segments that have a trio of 'NGG' (N being any nucleotide) nucleotide base pairs and cut only limited fraction of the genome. The human genome contains 3.2 billion-bases and only one-sixteenth of the genome where Cas9 can land. This limits the specific target genes of interest and leads to "off-target" mutations however to limit these effects a highly specific guide RNA sequence is selected^[15,32]. Also, recently researchers modified the enzyme and developed an xCas9s that has a broader range of three-base landing pads, referred to as protospacer adjacent motifs or PAMs. It works best with NGN sequence, which occurs in one-fourth of the genome. It allows for researchers to perform gene knockout studies, which help them, determine what gene is implicated in disease. However, since majority of diseases are associated with "point mutations", it is difficult to target and repair a mutated gene. Not only that, once the enzyme clips the desired DNA gene, the repair mechanism of the cell is wobbly and during repairs it tends to insert or delete DNA bases. Furthermore, Cas9 enzyme only cuts DNA and only 2% of the genome codes directly from DNA to protein and 98% of genome is regulatory gene sequences. This poses a challenge for precisely modifying RNA. Although, very recently a new enzyme was characterized called Cpf1, which was found in *Staphylococcus aureus*, and is capable of cleaving both DNA and RNA^[33]. This will allow targeting of RNA gain-of-function mutations such as NOD2 or other mutations that can be edited. Furthermore, Cpf1 is also smaller which makes transfection much easier^[33].

Although some of these limitations of the Cas9 are quickly becoming insightful opportunities and researchers are drastically improving it by altering its capabilities. The new enzymes are more precise than the original Cas9 and now there is xCas9, dCas9 and dCas13 which are capable of editing specific base pairs. For example, dCas13 can convert base A to I in RNA and I is a universal base^[34]. Such manipulation of bases is a very appealing target for therapies, particularly inflammation. Furthermore, because RNA is around in the cell for a short period of time before it is degraded repeated administration of RNA base editors would need to be given^[34]. Also, sequencing RNA is problematic and laborious^[35]. On the other hand this may seem disadvantageous but working with RNA may limit some off target genome mutations. In addition to off target genome mutations, crossfire between different cells may occur. Intended target may be either gain of function mutation or loss of function mutation, either way, altering alleles may have their own detrimental effect.

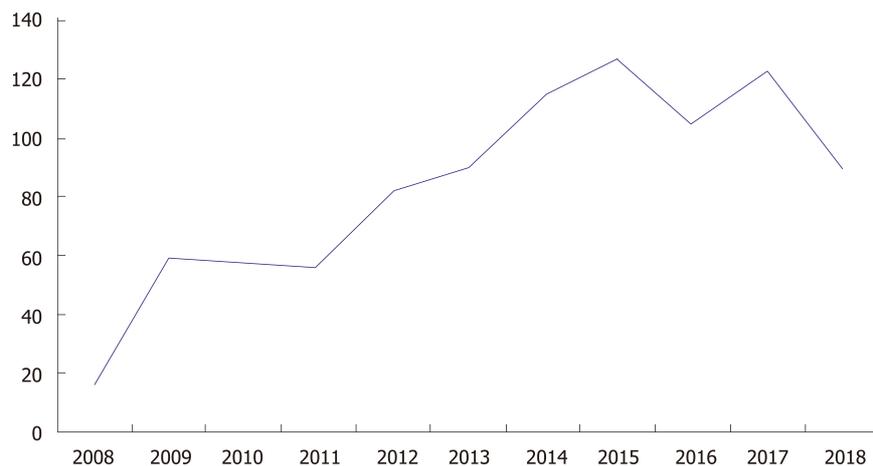


Figure 4 Publications on inflammatory bowel disease using gene therapy.

For example, as indicated in [Figure 5](#), NOD2 loss of function leads to Crohn's and gain of function causes endothelial to mesenchymal transition of glomerular endothelial cells causing diabetic nephropathy^[36]. However, some of these cross reactions may not be as detrimental. In [Figure 6](#), altering ATG16L1 allele to T300A ATG16L1 only incases risk for CD type, but not disease onset^[37]. [Figures 5-7](#) below demonstrate positive and negative effects of altering main genes implicated in IBD; NOD2, STAT3 ATG16L1, IL23R genes using CRISPR technology^[37-41].

CONCLUSION

Despite CRISPR/Cas technology limitations, as new innovative techniques such as anti-CRISPR proteins and new Cas proteins are developed to advance precise DNA editing, application of this revolutionary mechanism is at its prime time to hone in on genes implicated in IBD. Implementation of CRISPR in IBD research will lead to better outcomes and may decrease financial burden on the health care system.

Table 1 Genes to target for optimal impact using CRISPR

Gene	Strength of role in inflammatory bowel disease	Pathway	Function
NOD2 ^[42]	+++++	Cellular Innate Immunity	Intermediate in MDP to NFkB and IL1B production
IRGM	++		
LRRK2	+		
ATG5	+	Autophagy	Phagophore to autophagosome to autophagolysosome creation
ATG16L1 ^[42]	++++		
IRGM	++		
Gatm	+++	Defective Barrier	Leaky lamina propria allowing antigen entry and T cell response
ECM1	+		
CDH1	+		
LAMB1	+		
HNF4A	+		
GNA12	+		
IL10	+++++	Adaptive Immune	Convergence of STAT3, IL10RB, NOD2, ATG16L1 pathway
CARD9/15	++		
CCR6	++		
IL2RA	+		
MST1	+		
TNFSF15	+++		
REL	+		
STAT3	+++++	Th17 Mediated	Activation of Th17 adaptive immunity
IL23R ^[42]	+++++		
IL12B	++		
FUT2	+		

Genes in bold have publications using CRISPR technology. Strength of role is based on + of publications and relationship to inflammatory bowel disease: > 5 +; > 10 ++; > 20 +++; > 40 ++++; > 80 +++++. NOD2: Nucleotide-binding oligomerization domain-containing protein 2; IRGM: Immunity related GTPase M; LRRK2- leucine-rich repeat kinase 2; ATG5: Autophagy related 5; ATG16L1: Autophagy-related protein 16-1; Gatm: Glycine amidinotransferase, mitochondrial; ECM1: Extracellular matrix protein 1; CDH1: Cadherin 1; LAMB1: Laminin subunit beta 1; HNF4A: Hepatocyte nuclear factor 4 alpha; GNA12: G protein subunit alpha 12; IL-10: Interleukin 10; CARD9/15: Caspase-associated recruitment domain; CCR6: Chemokine receptor 6; IL2RA: Interleukin 2 receptor subunit alpha; MST1: Macrophage-stimulating protein; TNFSF15: TNF superfamily member 15; REL: Rel protein; STAT3: Signal transducer and activator of transcription 3; IL23R: Interleukin 23 receptor; IL12B: Interleukin 12; FUT2: Fucosyltransferase 2.

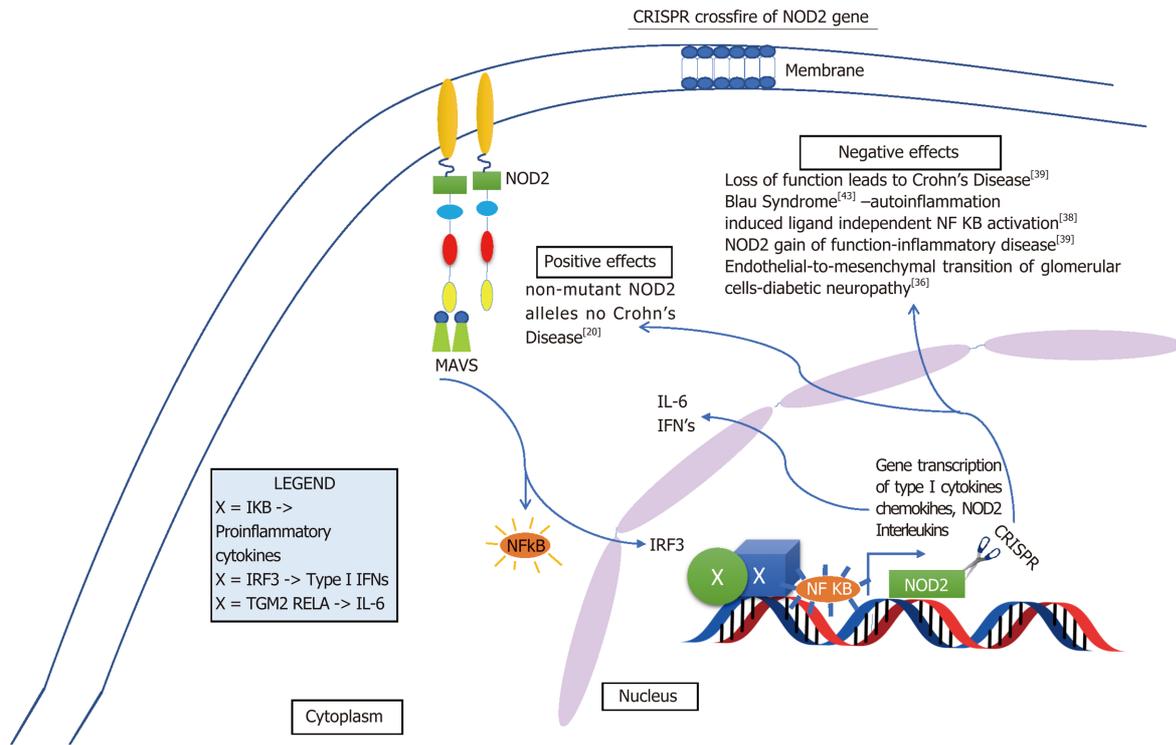


Figure 5 Positive and negative effects of CRISPR when targeting NOD2 gene.

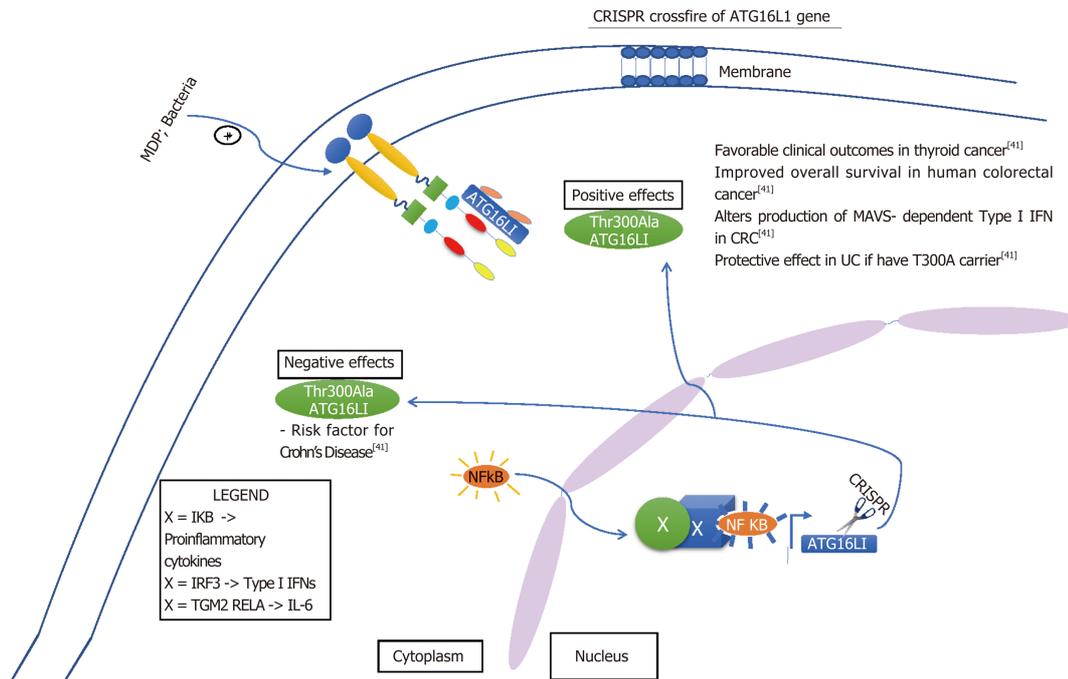


Figure 6 Positive and negative effects of CRISPR when targeting gene ATG16L1. UC: Ulcerative colitis; CRC: Colorectal cancer.

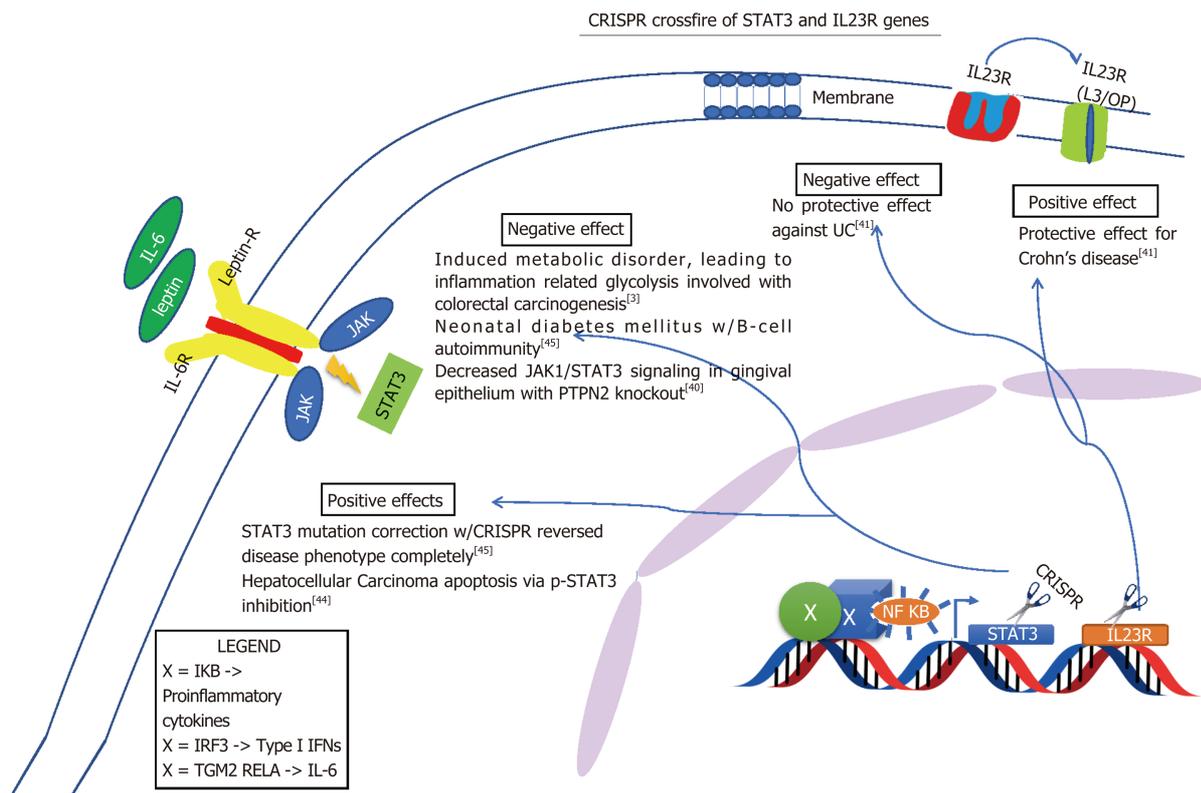


Figure 7 Positive and negative effects of CRISPR when targeting STAT3 and IL23R genes. UC: Ulcerative colitis.

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