Inflammatory Bowel Disease
Innovation & Changing Paradigms

Reza Malekzadeh M.D, AGAF
Professor of Medicine DDRI/TUMS
IAGH May 2017
Historical timelines of Crohn’s disease and ulcerative colitis throughout the world

- Wilks introduces ulcerative colitis into the medical vernacular (1875)
- Paper on regional ileitis published in JAMA by Crohn (1909)
- Ulcerative colitis is more common than Crohn’s disease (1932)
- Incidence of ulcerative colitis stabilizes, Crohn’s disease still rising (1950)
- IBD is a global disease with increasing prevalence (1960)
- Over 300 patients with ulcerative colitis hospitalized in London (1970)
- IBD recognized throughout North America and Europe (1980)
- IBD is a disease of Westernized nations with rising incidence (1990)
- The incidence of IBD rises in newly industrialized countries (2000)
IBD in Iran

- 1985 Mirmajlessi First Paper describing CUC in Iran in English
- 2000 Malekzadeh First Paper describing CD in Iran in English
Historical timelines of Crohn’s disease and ulcerative colitis throughout the world

- 1875: Wilks introduces ulcerative colitis into the medical vernacular
- 1909: Paper on regional ileitis published in *JAMA* by Crohn
- 1932: Ulcerative colitis is more common than Crohn’s disease
- 1950: Incidence of ulcerative colitis stabilizes, Crohn’s disease still rising
- 1960: IBD is a global disease with increasing prevalence
- 1970: Over 300 patients with ulcerative colitis hospitalized in London
- 1980: IBD recognized throughout North America and Europe
- 1990: IBD is a disease of Westernized nations with rising incidence
- 2000: The incidence of IBD rises in newly industrialized countries
Events Culminating in Intestinal Inflammation

- Genetic polymorphism
- Mechanisms of genetic modification
  - DNA methylation
  - microRNA
- Colonization with intestinal flora
- Environmental factors
  - Diet
  - Antibiotic use
  - Mucosal disruption (eg, NSAID use)
  - Pathogens
  - Stressors (eg, smoking)
Etiologic Theories in IBD

Genetic Predisposition

Mucosal Immune System
(immunoregulatory defect)

Environmental Triggers
(lumenal bacteria, infection)

IBD
Chronic Inflammation: Imbalance Between Mediators

Pro-inflammatory

Anti-inflammatory

TNFα
IL-1β
IL-8
IL-12
IFNγ

IL-4/IL-13
IL-1Ra
TGFβ
IL-10
At least 50,000 Iranian have IBD

Inflammatory Bowel Disease (IBD)

- Chronic intestinal inflammation
- No known cause
- No cure

~1.6 million Americans have IBD

At least 50,000 Iranian have IBD
$9.9 Billion\textsuperscript{4,5} \\
\text{Direct and indirect costs}

\textbf{Substantial Burden}\textsuperscript{6-10}
\begin{itemize}
\item Increased morbidity and mortality
\item Reduced labor productivity
\item Social stigma
\item Increased cancer risk
\item Impaired quality of life
\item Increased medical costs
\item Difficulty with physical intimacy
\item Limited choices in career and travel
\item Reduction in ability to work
\item Reduction in leisure time
\end{itemize}
The Rise of IBD Prevalence

• The rise of IBD in newly industrialized countries parallels its growth in the Western world 30 to 40 years ago.

• Genetic and environmental studies in these countries may provide new clues to the pathogenesis of IBD but also add another layer of complexity since risk factors and gene-environment interactions may vary by continents and ethnicities.

• A series of therapeutic interventions with the ultimate goal of cutting the incidence of IBD by half by the year 2032 is going on by scientists across the globe.
Traditional (Historical) IBD Treatment Strategies

- Symptom based
- Patient fails before moving to another therapy
- Complications need to occur before treatments are changed
- Unable to change natural history of disease
- Imprecise ("dirty therapy")
Modern Goals of IBD Management

**Induction of Clinical Remission**
- Turning “off” the inflammation
- Feeling well
- Normalization of laboratory parameters, growth, development, and nutrition

**Maintenance of Clinical Remission**
- Stable disease control and optimization of therapy
- **NO STEROIDS**
- Prevention of relapse over time (sustained and durable)
- Changing the natural course of the disease

**Disease Monitoring, Prevention**
- Monitoring for early relapse
- Monitoring therapies
- Prevention of infections
- Cancer prevention
Early Intervention

• In a post-hoc analysis of the SONIC trial, 65% of patients with early-stage CD (18 months after diagnosis, according to the Paris definition) who received combination therapy with infliximab and azathioprine achieved deep remission at week 26 compared with 25% in the infliximab monotherapy group and 10% in the azathioprine group.

• Surgery could also be considered for early complicated CD, depending on disease extent.

• A trial is underway to compare the effects of ileocolic resection vs infliximab for noncomplicated distal ileitis in adults.
Detection and treatment of IBD before symptoms.
Eight items independently associated with a diagnosis of CD

• Nonhealing or complex perianal fistula, abscess, or perianal lesions (apart from hemorrhoids);
• Nocturnal diarrhea;
• First-degree relative with confirmed IBD;
• Weight loss (5% of usual body weight) in the last 3 months;
• Chronic abdominal pain (>3 months);
• Mild fever in the last 3 months;
• No abdominal pain 30–45 minutes after meals, predominantly after vegetables;
Treat to Target

• Switching the target from clinical remission to endoscopic healing has mainly been supported by post-hoc analysis of clinical trials.

• In the step-up/top-down study of patients with CD, complete endoscopic healing (defined as a simple endoscopic score of 0) after 2 years of therapy was the only factor that predicted sustained, steroid-free remission at 3 and 4 years after therapy was initiated.
Early effective treatment during this window of opportunity could slow disease progression and prevent damage.
Response to Biologic Therapy in Crohn's Disease Is Improved With Early Treatment: An Analysis of Health Claims Data\textsuperscript{37}

- Lower risk of concomitant corticosteroid use
- Less dose escalation of anti-TNF agent
- Less discontinuation or switch of anti-TNF therapy
- Fewer CD-related surgeries
Tight Control and Monitoring

• Levels of fecal calprotectin (FCP) better-predicted relapse than levels of CRP, but combined measurement of both markers most accurately predicted relapse of patients with CD. Pharmacokinetic evaluations have been used mostly for patients losing response to maintenance therapy.

• Panel of international experts recommended the assessment of anti-TNF drug and antibody concentrations at the end of induction therapy in primary nonresponders, in secondary nonresponders, and after a drug holiday.
(Left) Summary of recommendations from STRIDE Program for CD. (Right) Summary of recommendations from STRIDE Program for UC.

Treating a patient with Crohn’s disease AND Treating intestinal Inflammation

Resolution of abdominal pain and normalization of bowel habits should be the target. Endoscopic or cross-sectional imaging assessment should be performed within 6 to 9 months after the start of therapy. Absence of ulceration is the target.

Outcome assessment
- Every 3 months until symptom resolution
- Every 6 - 12 months after symptom resolution

When endoscopy cannot adequately evaluate inflammation, resolution of inflammation as assessed by cross-sectional imaging is a target.

Biomarkers including CRP and fecal calprotectin are not targets but adjunctive measures of inflammation for monitoring.
De-escalation

• Many researchers have tried to identify factors that predict relapse, mostly in patients with CD.
• Subclinically active disease (i.e., increased levels of biomarkers, endoscopic lesions), prognostic factors associated with poor disease course (i.e., extensive disease, young age at diagnosis).
• A prior disease course with complications are associated with higher risk of relapse.
• Low trough levels of anti-TNF agents at the time of withdrawal have also been associated with lower risk of relapse.
When Discontinue anti-TNF

• In patients who discontinue anti-TNF therapies, close follow-up with regular biomarker assessment may allow early recognition of relapse and therefore early reintervention.

• A reassuring message is that if relapse occurs, retreatment may be successfully and safely reinstituted
In patients with CD:

- In patients with CD, disease location, young age at diagnosis, disease behavior, perianal disease, use of steroids at diagnosis, and smoking are consistently identified prognostic factors.
- Ileal and ileocolonic disease have been associated with development of complications.
- Patients with upper gastrointestinal disease or proximal small bowel disease are more likely to require hospitalization.
- Rectal involvement is strongly associated with the development of perianal complications.
The behavior of CD

- The behavior of CD can also predict complications, as intra-abdominal penetrating and stricturing disease has been linked to more severe disease and a higher risk of surgery.99,100 Perianal disease increases higher risk of surgery, permanent stoma, and poor outcomes.

- Younger age at diagnosis is correlated with more severe and extensive disease, including a higher risk of disability.

- Finally, smoking is not only a risk factor for CD, but has also been associated with development of fistulizing and stricturing complications, as well as increased need for surgical resection.
In patients with UC

• In patients with UC, young age at diagnosis, need for frequent courses of steroids, and disease extent are probably the strongest predictive factors for complications.

• Extensive colitis has been associated with higher risk of colectomy, colon cancer, and mortality, compared with patients with left-sided disease or proctitis.

• Endoscopic lesions can also predict outcomes because deep ulcers have been associated with higher risk of colectomy for patients with either CD or UC.
Cutting edge translational research in IBD

• The key mechanism underpinning the pathogenesis of CD and UC is a dysregulated immune response to commensal microbiota in a genetically susceptible host.
Paneth cell Phenotype key insight for Stratification of Crohn’s Disease

• A recent specific discoveries — the phenotype of Paneth cells, which is altered in association with the CD risk alleles ATG16L1 and NOD2.

• Analysis of Paneth cell phenotypes may provide key insights into stratification of CD patients with respect to risk of disease progression and response to biologics, offering a clinical application to progress made in the genetics of IBD since the discovery of NOD2 in 2001.
Dysbiosis and IBD

• A new understanding of how composite genes (metagenome) and metabolic products (metabolome) of bacteria mediate mucosal homeostasis in the gut and how alterations of this composition and metabolic function contribute to aggressive immune responses in IBD and experimental colitis.

• The potential role of fungi has been and viral communities are altered in IBD and could aberrantly interact with potential bacterial pathogens.
The knowledge of gut microbiota and dysbiosis in IBD can be used for therapeutic purposes as demonstrated in the most recent clinical trial using fecal microbiota transplant (FMT) to treat UC.
Intestinal Fibrosis.

- Fibrosis is a manifestation of irreversible damage in CD (and perhaps in UC as well) and as such is an important component of the Lemann index.
- The diagnosis and management of fibrosis, including cross-sectional imaging, endoscopic techniques and surgery, has been standardized in the last few years to minimize the need for interventions, such as surgical resections, that lead to short bowel syndrome and intestinal failure.
- So far, no specific anti-fibrotic drug is available to
- prevent or treat this stage of the disease but this may change
- in the near future as a better understanding of the mechanisms
- of fibrosis may lead to development of anti-fibrotic
- therapies as in liver disease
Most recent progress in the treatment of IBD

• The goal of therapies has now shifted from mere control of symptoms towards blocking disease progression and preventing intestinal damage and disability.
New concepts have emerged in the management of IBD such as:

- Treat to target.
- Early intervention,
- Tight control
- Disease monitoring.
- Personalization.
- Prevention.
Monitoring disease progression

• Endoscopy.
• Cross-sectional imaging.
• Biomarkers (C-reactive protein and calprotectin)
• Practical algorithms
Anti-TNF Biologics

- Infliximab: Chimeric, Monoclonal antibody
  - IgG1 Fc

- Adalimumab: Human, Monoclonal antibody
  - Fab'

- Golimumab: Chimeric, Monoclonal antibody
  - Fab'

- Certolizumab pegol: PEGylated humanized Fab' fragment
  - 2 × 20 kDa PEG
Anti-integrins

• Natalizumab
  • Binds to the α4 integrins, ubiquitous in inflammatory cells in circulation
  • Can bind to VCAM throughout the body

• Vedolizumab
  • Binds to α4β7, and inhibits binding of α4β7 to gut-specific MAdCAM, and prevents inflammatory cells from entering gut tissue
Evaluation of Efficacy & Safety of CinnoRA® in IBD
Comparative Effectiveness of Infliximab and Adalimumab for Crohn’s Disease

- Retrospective cohort study by using U.S medicare data from 2006-2010

- Patients with CD; IFX (1459) or ADA (871) after January 31, 2007

- After 26 weeks of treatment, 49% of patients receiving IFX remained on drug, compared with 47% of those receiving ADA

- Fewer patients treated with IFX underwent surgery than those treated with ADA, but this difference was not statistically significant (5.5 vs 6.9 surgeries per 100 person-years)

- *We observed similar effectiveness of IFX and ADA for CD on the basis of 3 clinically important outcome measures*
Therapeutic armamentarium is rapidly expanding in IBD

• Ustekinumab (third class of biologics targeting IL-23) recently approved by FDA for the treatment of CD.

• Emergence of oral small molecules new therapy:
  ✓ Tofacitinib in UC.
  ✓ Mongersen in CD
Therapeutic armamentarium is rapidly expanding in IBD

• Ustekinumab (third class of biologics targeting IL-23) recently approved by FDA for the treatment of CD.

• Emergence of oral small molecules new therapy:
  - Tofacitinib in UC.
  - Mongersen in CD
Nontargeted IBD therapies

• **A. Induction:** Corticosteroids, 5-aminosalicylates, cyclosporine, and tacrolimus.

• **B. Maintenance:** Thiopurines, methotrexate, 5-aminosalicylates, and tacrolimus.
Targeted therapies

• **A. Monoclonal antibodies** (induction and maintenance): Tumor necrosis factor inhibitors, vedolizumab, *other integrin/adhesion molecule inhibitors, interleukin-12/23 inhibitors, and interleukin-6 inhibitors.*

• **B. Oral synthetic** (induction and maintenance or stop and start): *Jakinibs, integrin blockers, sphingosine-1-phosphate (S1P) regulators, and SMAD7 antisense oligonucleotide.*
Integrins

Integrins are transmembrane glycoproteins involved in interactions between cells and between cells and the extracellular matrix.

Integrins are heterodimers formed by an $\alpha$ and a $\beta$ chain.

Two highly homologous $\beta_3$-containing integrins have been identified: $\alpha V \beta 3$ and $\alpha IIb \beta 3$.

$\alpha V \beta 3$ is present on the surface of numerous cell types (endothelial cells, smooth muscle cells, leukocytes, platelets, osteoclasts, mesangial cells),

$\alpha IIb \beta 3$ is found only on platelets and their bone marrow precursors, the megakaryocytes.
Integrin Structure, Activation, and Interactions

Integrin activation

- Chemokines
- GPCRs

CD44, CD47, CD98, and tetraspanins regulate the conformational switch of integrins → ability to microcluster and anchor to actin cytoskeleton.

Abul K. Abbas, et al. Cellular and Molecular Immunology 7th EDITION
Integrins and chemokines are attractive targets for inhibition by synthetic small molecules

- These have synthetic structures that interfere with the molecular interactions between the integrins and their natural ligands.
- These are still challenging to develop with adequate selectivity and bioavailability, but may provide therapeutic agents at lower cost and greater stability than parenterally administered monoclonal antibodies.
- These targets may also be amenable to antisense oligonucleotide strategies with newer generation technologies.
Oral synthetic targeted therapies are emerging as alternatives to monoclonal antibodies

• Sharing the same target such as the integrin blockers, while others having unique targets such as JAK inhibitors (Jakinibs).

• Oral targeted therapies may have some general characteristics, such as much shorter half-lives of drugs, lack of immunogenicity, potential off-target effects, and relatively narrow dosing windows.
The mechanisms of action of the four most promising classes of oral synthetic drugs are:

• a) Tyrosin Kinase Inhibitors: Jakinibs, which reduce the production of several cytokines such as IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, b)

• b) Integrin inhibitors (AJM300), which blocks trafficking of lymphocytes into the lamina propria from the blood vessels,

• c) Sphingosine phosphate Modulator (Ozanimod), which prevents the exit of lymphocytes from lymph nodes by following the S1P gradient via internalization of the receptor,

• d) Antisense Oligonucleotide (Mongersen), which down-regulates SMAD7 and restores transforming growth factor-b1 signaling.
The potential advantages of synthetic targeted oral therapies also include:

• Convenient storage, transport, and the possibility of use as topical therapy.
• Potential disadvantages include daily and sometimes multiple daily dosing and uncertainty about bioavailability at the target site.
Oral integrin inhibitor

- Are among a growing class of therapies for inflammatory bowel disease
- Integrins are secondary adhesion molecules on leukocyte surfaces that are activated by proinflammatory cytokines released by activated T cells.
- Integrins consist of 2 transmembrane glycoprotein subunits, a and b, which bind specific cell adhesion molecules according to their subtype.
- The first leukocyte adhesion molecule inhibitor approved in multiple sclerosis and IBD was natalizumab, an infused humanized immunoglobulin (Ig)G4 monoclonal antibody directed against the a4 integrin subunit.
AJM300, an Oral Antagonist of a4 integrin for UC

- AJM300 was well tolerated and more effective than placebo in inducing clinical response, clinical remission, and mucosal healing in patients with moderately active UC.
Proportion of patients with a clinical response

- **Clinical response**
  - Placebo: 25.5% (13/51)
  - AJM300: 62.7% (32/51)
  - $P = 0.0002$

- **Clinical remission**
  - Placebo: 3.9% (2/51)
  - AJM300: 23.5% (12/51)
  - $P = 0.0099$

- **Mucosal healing**
  - Placebo: 29.4% (15/51)
  - AJM300: 58.8% (30/51)
  - $P = 0.0014$
Jakinibs
“oral biologics”

• Jakinibs are a new class of medication, sometimes called oral biologics.
• The word “biologic” is misleading, however, because jakinibs work in an entirely different way than the biologics that have been used to date.
• Jakinibs are small molecules that work inside cells.
• Traditional biologics such as etanercept (Enbrel), adalimumab (Humira), abatacept (Orencia) and Infliximab (Remicade) block pro-inflammatory cytokines from outside.
• Jakinibs are taken by mouth while traditional biologics are given through infusions or injections.
Inhibition of transcription factor activation via inhibition of JAK2 tyrosine kinase
### JAK2-inhibiting agents in Clinical trial

JAK2-inhibiting agents in clinical trials.

<table>
<thead>
<tr>
<th>Drug</th>
<th>JAK activity</th>
<th>Stage of development</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>INCBO18424</td>
<td>JAK1</td>
<td>Phase III: COMFORT-I (placebo controlled)/COMFORT II (best available oral/parenteral therapy controlled)</td>
<td>Ongoing (Phase I/II published)</td>
</tr>
<tr>
<td></td>
<td>JAK2</td>
<td>[PMF, post-ET/PV MF] Phase III: RESPONSE (best available care controlled) [HU-resistant PV]</td>
<td></td>
</tr>
<tr>
<td>TG101348</td>
<td>JAK2</td>
<td>Phase I/II: PMF, post-PV/ET MF</td>
<td>Published</td>
</tr>
<tr>
<td>CYT387</td>
<td>JAK1</td>
<td>Phase I/II: PMF, post-PV/ET MF (included subjects with prior JAK2 inhibitor therapy)</td>
<td>Reported</td>
</tr>
<tr>
<td></td>
<td>JAK2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>JAK3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEP701</td>
<td>JAK2</td>
<td>Phase II: PV, ET, PMF, post-PV/ET MF</td>
<td>Published</td>
</tr>
<tr>
<td>SB1518</td>
<td>JAK2</td>
<td>Phase I/II: PMF</td>
<td>Reported</td>
</tr>
<tr>
<td>AZD1480</td>
<td>JAK2</td>
<td>Phase I/II: PMF, post-PV/ET MF</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>JAK2</td>
<td>Phase II: PV (JAK2V2617F mutant only)</td>
<td></td>
</tr>
<tr>
<td>AT9283</td>
<td>JAK2</td>
<td>Phase I/II: PMF included</td>
<td>Ongoing</td>
</tr>
<tr>
<td>ITF2357</td>
<td>JAK2</td>
<td>Phase II: GIVINOSTAT (in combination with HU-resistant PV; JAK2V617F mutant only)</td>
<td>Ongoing</td>
</tr>
<tr>
<td>LY2784544</td>
<td>Uncertain</td>
<td>Phase I: PV, ET, PMF (JAK2V617F mutant only)</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>
### Selected JAK2-inhibiting agents in preclinical studies

Selected JAK2-inhibiting agents in preclinical studies.

<table>
<thead>
<tr>
<th>Drug</th>
<th>JAK activity</th>
<th>In vitro/in vivo studies (−no, +yes)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS014</td>
<td>JAK2</td>
<td>+/-</td>
<td>Non-ATP mimetic</td>
</tr>
<tr>
<td>NS018</td>
<td>JAK2</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>CP690550</td>
<td>JAK2, JAK3</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>BMS911543</td>
<td>JAK2</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>TG101209</td>
<td>JAK2</td>
<td>++</td>
<td>Combination with MEK, PIM1, PI3K/mTOR inhibitors or LBH589 tested</td>
</tr>
<tr>
<td>WP1066</td>
<td>JAK2</td>
<td>+/-</td>
<td>PI3K, STAT3 with off-target JAK2</td>
</tr>
</tbody>
</table>

JAK, janus kinase; ATP, adenosine tri-phosphate; MEK, mitogen-activated protein kinase; PIM1, PIM-1 oncogene; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; STAT3, signal transducer and activator of transcription 3.
Jakinibs interrupt the signaling pathway.

- Jakinibs block the enzymes JAK1, JAK2, JAK3, and tyrosine kinase 2, which play a role in the cell-signaling process that leads to the inflammatory and immune responses seen in RA, IBD and other conditions. Jakinibs interrupt the signaling pathway.

- “They are like the first translator. They are the molecules that take that signal from the cell surface and start moving it down the chain of command,”
Tofacitinib, the first of the Jakinibs

- Became the first of the targeted synthetic oral drugs that showed efficacy at clinical and mucosal levels in UC in an 8-week trial.
- Elevation of cholesterol, both low and high density, as well as cytopenia were adverse events of interest.
- In rheumatoid arthritis, the Jakinibs are generally positioned after the failure of alpha-1 anti-tumor necrosis factor monoclonal antibody; it is expected that the strategy may be somewhat different in IBD, with the availability of vedolizumab.
What Will Jakinibs Cost?

• Initially some wondered if jakinibs would be cheaper than biologics, since producing a small molecule drug is not considered as complicated.

• “Tofacitinib is $2,000 a month before insurance.”
What Are The Potential Side Effects of Jakinibs?

• Increased risk of serious infections, tuberculosis, cancers and lymphoma as well as high cholesterol, respiratory tract infections, headache and diarrhea in some patients.
Mongersen, an oral SMAD7 antisense oligonucleotide

• Silencing of gene expression with non-coding nucleic acid (antisense DNA decoys siRNA/shRNA and microRNA)

• Inhibition of proinflammatory gene expression
Genetic therapy

Gene addition

- Substituting delivery of proper version of mutated gene
  - Inherited diseases

- Enhancing delivery of gene which expression is impaired
  - Acquired diseases

- Suppresive delivery of gene killing the cells
  - Acquired diseases

Gene inhibition

- Silencing of gene expression with non-coding nucleic acids (antisense DNA decoys siRNA/shRNA microRNA)

Gene repair

- Correction of the mutated gene
Therapeutic nucleic acids

- DNA
  - Genes (delivered by DNA vectors)
    - Antisense oligonucleotides
  - Non-coding sequences
    - DNA decoys
- RNA
  - Genes (delivered by RNA vectors)
    - Ribozymes
  - Non-coding sequences
    - siRNA
    - microRNA
Hybridisation of nucleic acids as a way to inhibit gene expression
I. Nucleic acids that bind to other nucleic acid

1. **Antisense oligonucleotides**: short, 12-20 nts.
2. **Triple helix-forming oligonucleotides (TFO)**
   pyrimidine oligodeoxynucleotides that specifically bind to a major groove of polypurine region of dsDNA via the formation of triple helices according to recognition rules established by Hoogsten
3. **Ribozymes** – short catalytically active RNSs
4. **Deoxyribozymes (DNAzymes)** – short catalytic DNA that cleave sequence-specifically targeted RNA.
   More stable than RNA, it is easier to synthesize and to modify them.

5. **siRNA/microRNA**

II. Nucleic acids that bind to proteins
Antisense oligonucleotides

Short fragments of single strand, chemically modified DNA nucleotides (oligonucleotides), complementary to a given mRNA.

Paul Zamecnik (b. 1912) discoverer of tRNA died October 2009

Zamecnik and Stephenson developed antisense technology, in which short, synthetic nucleotide sequences can be used to silence the activity of individual genes. They published their results, in which they used a 13-nucleotide sequence to halt production of Rous sarcoma virus in chicken embryos, in 1978. That paper appeared in Proceedings of the National Academy of Sciences,
Antisense oligonucleotides

The diagram illustrates the process of gene expression, showing the steps from DNA transcription to protein synthesis. It includes the following stages:

1. **DNA Transcription**: DNA is transcribed into a pre-mRNA (mRNA).
2. **Splicing**: The pre-mRNA undergoes splicing to remove introns and retain exons.
3. **Translation**: The mRNA is translated into a protein by ribosomes.
4. **Degradation**: The mRNA is degraded by RNase H.

The antisense oligonucleotide hybridizes with the mRNA, preventing it from being translated into a protein.
Antisense oligonucleotides

(2) Splicing arrest

(1) Translational blockade

5′UTR AUG (Translation initiation codon)

5′ mRNA cap

3′UTR

(3) RNase-H degradation of mRNA

60S Ribosomal subunits

40S
Clinical application of antisense oligonucleotides

Vitravene - CMV-induced retinitis

Bcl2 antisense - melanoma
Examples of clinical trials of antisense oligonucleotides

1. Transthyretin amyloidosis (TTA) - familial amyloid polyneuropathy

2. Apolipoprotein C-III inhibition in patients with hypertriglyceridemia - treatment resulted in significant lowering of triglyceride levels

3. Inhibition of SMAD7 - in Crohn’s disease - Mongersen, an oral SMAD7 antisense oligo - in Crohn’s disease there is a reduced activity of the immunosuppressive TGF-beta1 due to the high level of SMAD7, an inhibitor of TGF-b1 signaling

4. Reducing factor XI to prevent venous thrombosis - antisense oligo to prevent post-operative venous thrombosis
Transthyretin amyloidosis (TTA) – familial amyloid polyneuropathy

Figure 1. Schematic illustration of FAP amyloidosis. Wild-type and mutant TTR (open and closed circles and solid lines) are synthesized in the liver. A fraction of TTR tetramers dissociates into monomers and/ or misfolded TTR tetramers. Both monomers and misfolded TTR tetramers enter the bloodstream, where they deposit as fibrils in affected organs (e.g., peripheral nerves, heart, kidney, skin, and thyroid gland). These fibrils aggregate and cause tissue damage, leading to clinical manifestations of the disease.
Mongersen, an oral SMAD7 antisense oligonucleotide

• Inhibition of gene expression by means of nucleic acids,
• Gene silencing effect
Mongersen, an oral SMAD7 antisense oligonucleotide which increase transforming growth factor-b levels in the mucosa

- This first-generation antisense technology-based (21 base single strand phosphorothioate oligonucleotide) oral drug
- Was shown to be effective in inducing clinical remission and response at week 2 (primary efficacy endpoint) and week 4 in Crohn’s Disease.
- The drug was well-tolerated.
- Needs further confirmatory evidence in the short and the long term and further validation of its mechanism of action.
Mongersen

• Produced clinical remission rates as high as 65.1% in a Phase II trial in 166 patients with moderate to severe Crohn’s disease, according to an abstract published in advance of the United European Gastroenterology’s meeting in Vienna.

• In the trial, 55% of patients receiving 40 mg/day of mongersen and 65.1% of those receiving 160 mg/day achieved clinical remission compared with 9.5% of placebo patients (p<0.0001 for both).

• A cohort receiving 10 mg/day achieved a clinical remission rate of 12.2%, which was not significantly better than placebo.
Mongersen

- Mongersen is an oligonucleotide that inhibits ileal and colonic SMAD7, a protein that prevents transforming growth factor beta1-mediated suppression of inflammatory genes.

- This perfect drug for IBD would be something that’s effective, safe, and well tolerated; orally administered; shows long-term efficacy; and can be dosed as needed.

- If the data in the current phase III trials are half as good, mongersen may be what we’re looking for.
Antisense oligonucleotides
A new strategy of treatment of IBD

• If Safety and efficacy of Mongersen is confirmed, it will introduce a different strategy of treatment of IBD by providing a synthetic compound that enhances the anti-inflammatory regulatory immune processes.

• Whether such a strategy enhances stable long-term mucosal healing will be interesting to observe in the future
oral synthetic targeted therapies offer new strategies for IBD Therapy

• without being too concerned about immunogenicity and loss of response.
• These will include:
  ➢ stop–start strategies,
  ➢ Intercalated short-term additional therapy to background maintenance therapy,
  ➢ combining therapies with complementary mechanism of action
  ➢ Novel topical therapies
A cure for Crohn’s disease by 2032

Filgotinib, a selective janus kinase (JAK1) inhibitor, in Crohn’s disease
FITZROY Trial
Lancet Jan 2017
Non-selective JAK inhibitor

- Previous phase 2 studies of the non-selective JAK inhibitor, tofacitinib, suggested efficacy in ulcerative colitis but only a modest biochemical response and no statistically significant improvement in clinical remission or response rates in Crohn’s disease.
Phase 2 multicentre randomised controlled trial examining the efficacy of Filgotinib.

• In the present trial, 8,174 patients with moderate-to-severe Crohn’s disease confirmed by centrally read endoscopy were randomised across 52 centres in Europe to placebo or Filgotinib 200 mg daily for 10 weeks.

• An exploratory arm of the study randomly assigned patients to placebo, Filgotinib 100 mg daily, or Filgotinib 200 mg daily for a further 10 weeks.
Primary & secondary outcome

• The primary outcome was clinical remission at 10 weeks.
• Secondary outcomes including endoscopic and histological response, and the impact on biomarkers of inflammation and patient reported outcomes were examined.
• In an intention to-treat analysis, 60 (47%) of 128 patients randomly assigned to active treatment achieved clinical remission compared with ten (23%) of 44 patients in the placebo.
Research is not keeping up with need to manage CD

• Since 1932, when Burrill Crohn, Leon Ginzburg, and Gordon Oppenheimer codified regional ileitis as a distinct clinical and pathological entity, successive approaches to care have been unable to achieve universal success against the relapsing nature of the illness.

• Research is not keeping up with need.

• Each year Crohn’s disease snatches the quality out of life for an increasing number of young adults, in more and more countries
JAK1 inhibitor filgotinib for CD

• In this issue Lancet, Séverine Vermeire and colleagues report the results of FITZROY, a phase 2 randomised controlled trial that compared the JAK1 inhibitor filgotinib with placebo for clinical remission in patients with moderate-to-severe Crohn’s disease.
Promise for several reasons: Although modestly sized, short-term, and preliminary, the study offers promise for several reasons:

- The different approach to cytokine blockade,
- Oral dosing,
- Stratification by prior treatment with anti-TNF compounds,
- Use of meaningful patient-reported outcomes.

When considered alongside a recent study of stem cells for perianal fistula, it shows a shift to broader thinking in ways to improve the treatment of Crohn’s disease.
Findings from FITZROY

• Findings from FITZROY add to the rapidly accumulating knowledge of genetic, environmental, microbiota, molecular, and immune factors involved in inflammatory bowel disease.

• They should serve to bolster and accelerate recent momentum in research about Crohn’s disease and the translation of new insights into opportunities for prevention and earlier intervention.
offering new hope to the next generation of people with the disease

• What better way to mark the centenary of Crohn’s description in 15 years’ time than by offering new hope to the next generation of people with the disease?

• Doing so requires more reliable biomarkers that correlate better with endoscopic findings and prognosis, improved understanding of individual responsiveness, bolder attitudes about treatments, their goals and timing, and more emphasis on care in resource-limited settings.
Alternative mechanisms, such as repair of disordered signalling pathways and the microbiome, should also be considered.

In the countdown to 2032, real progress can be made with concerted, collaborative effort; particularly if researchers and funders and funders aspire beyond the target of deep remission to the possibility of cure.
The Main present Challenge

• Searching for biomarkers which could predict efficacy or failure of new biologics and small molecules in the hope of personalized therapy
Proposed management strategies and options for personalized treatment of IBD.

**Diagnosis**
- Assessment of disease severity
- Prediction of disease course

**Selection of therapy**
- New predictive tools: Omics, serologic markers, serum and fecal biomarkers
- High risk patients: Early combination therapy
- Low risk patients: Rapid step up therapy
- Predicting response to therapy
- Determining who needs early surgery

**Treat to target:** No symptoms and mucosal healing
- Tight control: frequent re-assessment / monitoring pharmacokinetics / objective disease monitoring

Legend:
- Currently proposed management strategies
- Potential future personalized management strategies
# Personalization-Assessing Disease severity

Eight Factors That Define the Severity of Crohn’s Disease, Proposed by the International Organization for the Study of Inflammatory Bowel Diseases

<table>
<thead>
<tr>
<th>Items</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effects of disease</strong></td>
<td></td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td>Frequency of loose stool (&gt;10/wk) and/or daily abdominal pain (pain scale &gt;1)</td>
</tr>
<tr>
<td></td>
<td>Anorectal symptoms (pain, urgency, incontinence, discharge, tenesmus, active fistula)</td>
</tr>
<tr>
<td></td>
<td>Disease having significant impact on daily activities</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Impact on daily activities</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory burden</td>
<td></td>
</tr>
<tr>
<td>Serum biomarkers</td>
<td>Anemia (WHO criteria; abnormal) and/or elevated CRP (cutoff 5) and/or low albumin</td>
</tr>
<tr>
<td></td>
<td>Presence of large and/or deep ulcers</td>
</tr>
<tr>
<td>Mucosal lesions (MRI, endoscopy)</td>
<td></td>
</tr>
<tr>
<td>Disease course</td>
<td>Fistula at time of clinic visit and/or abscess at time of clinic visit and/or stricture at time of clinic visit and/or stoma at time of clinic visit and/or &gt;1 intestinal resection or 1 intestinal resection &gt;40 cm</td>
</tr>
<tr>
<td>Complicated disease</td>
<td>Steroid use within past year and/or failure of biologics and/or immunomodulators</td>
</tr>
<tr>
<td></td>
<td>Extensive disease (&gt;40 cm ileal involvement and/or pancolitis)</td>
</tr>
<tr>
<td>Response to medication</td>
<td></td>
</tr>
<tr>
<td>Disease extent</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. From Peyrin-Biroulet,\textsuperscript{91} adapted with permission. MRI, magnetic resonance imaging; WHO, World Health Organization.
Recent progress in stem cell therapy in IBD

• Autologous hematopoietic stem cell transplantation will likely remain a last resort rescue therapy in specialized centers for patients failing all available drugs.

• Local administration of mesenchymal stem cells for fistula healing in crohn disease may soon be ready for clinical practice.
No quality data support the use of any of the following therapies:

• Diet (including gluten-free, specific carbohydrate, etc.).
• Complementary and alternative or traditional therapies (CAM)
• Physicians should understand the evidence (or lack thereof) behind the various Traditional or CAM modalities so that they can offer rational advice to patients
Importance of psychological, family and social support

• There is increasing evidence, from experimental models and clinical studies, that the brain-gut axis plays an important role in IBD.

• We should not overlook chronic pain, anxiety, depression, and sleep disorders which can strongly impact the quality of life of our patients and increase healthcare utilization.

• We should talk with IBD patients reassure them of cure and normal life span, accommodate specialized personnel who apply behavioral interventions and/or prescribed psychotropic medications when needed.
IBD & IBS

• The prevalence of IBS-like symptoms in UC patients in remission is about three times higher than in controls, and these patients have impaired HRQOL comparable with that of UC patients in the active phase.
### Distribution of IBS subgroups in controls and patients

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=100)</th>
<th>UC patients in remission</th>
<th>P value (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS</td>
<td>13 (13%)</td>
<td>23 (46%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Functional diarrhea</td>
<td>10 (10%)</td>
<td>22 (44%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Functional abdominal bloating</td>
<td>20 (20%)</td>
<td>18 (36%)</td>
<td>&lt; 0.004</td>
</tr>
<tr>
<td>Incomplete defecation</td>
<td>11 (11%)</td>
<td>14 (28%)</td>
<td>&lt; 0.035</td>
</tr>
<tr>
<td>Functional constipation</td>
<td>7 (7%)</td>
<td>1 (2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Relief of abdominal pain after defecation</td>
<td>6 (6%)</td>
<td>22 (44%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

NS, not significant. Figures are reported in number (%).

Ansari R Malekzadeh R et al Eur J Gast & Hep 2008
IBD: Cumulative Incidence of Pregnancy Within 5 Years

ULCERATIVE COLITIS IN PREGNANCY

INCIDENCE OF RELAPSE

Control (25%)

Lower frequency but higher severity

1st  2nd  3rd  POST-PARTUM
TRIMESTER
Disease activity in pregnancy

- **Education** and planning required
- **Folic acid** advised before conception
- Maintenance treatment may be continued
- Flare-up should be actively treated
- **Disease activity is more dangerous than most drugs**
- Nutritional assessment
## Safety of IBD medications during pregnancy

<table>
<thead>
<tr>
<th>Safe</th>
<th>Limited data</th>
<th>Contraindicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral mesalamine</td>
<td>Azathioprine</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Topical mesalamine</td>
<td>6-Mercaptopurine</td>
<td>Thalidomide</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>Cyclosporine</td>
<td>Diphenoxylate</td>
</tr>
<tr>
<td>Ciprofloxacin, metronidazole (after first trimester)</td>
<td>Infliximab</td>
<td>Doxycycline</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td></td>
<td>Tetracycline</td>
</tr>
</tbody>
</table>
Safety of 6-Mercaptopyruvate

- Outcomes analyzed comparing pregnancies from men and women to control population
- No statistical difference
  - Live births
  - Spontaneous abortion
  - Abortion secondary to birth defect
  - Major congenital malformations
  - Infections
  - Neoplasia
Safety of 6-Mercaptopurine

- Severe exacerbation may result in significant harm to both fetus and mother
- Indication to restrict 6-MP
  - Homozygous TPMT deficient
  - Noncompliance with follow up care
  - ABNORMAL PAP SMEARS
    - ASSOCIATED WITH SMOKING, HPV INFECTION
IBD Drugs in Pregnancy: Evidence From Clinical Experience (cont’d)

Cyclosporine
- In severe steroid refractory disease, safer than colectomy in pregnancy
- FDA Category C
- Breastfeeding: safety unknown

Tacrolimus
- FDA Category C
- Breastfeeding: safety unknown
INFLIXIMAB AND CHILDBEARING

- Classified as Pregnancy Category B
- Animal reproduction studies have not been conducted
- Toxicity study conducted in mice using an analogous antibody
- No evidence of maternal and embryo toxicity or teratogenicity
Infliximab and Pregnancy

• Intentional exposure during pregnancy
  – No congenital malformations
  – 3 preterm, 1 LBW
• Infliximab levels in infants
  – Detectable up to 6 mos from birth
  – Consider dosing interval adjustment in 3rd trimester
  – Implications: vaccination status, immune system development
Screening before Steroid or Biologic therapy:

• TB skin test
• Chest PA
• HBSAg
• HCV RNA
Vaccination

- All adult patients with IBD, regardless of immunosuppression status, should receive non-live vaccines in accordance with national guidelines, including:
  - Trivalent inactivated influenza vaccine
  - Pneumococcal vaccination (PCV13 and PPSV23)
  - Hepatitis A
  - hepatitis B
  - Haemophilus influenza B
  - Human papilloma virus (HPV)
  - Tetanus
  - Pertussis
Statin use is associated with reduced risk of colorectal cancer in inflammatory bowel diseases

Results – II
Statin use and incident CRC
- New diagnosis of CRC
  - Non-users: 287 / 9,625 (3%)
  - Statin users: 30 / 1,376 (2%)
    - age-adjusted OR: 0.35, 0.24 – 0.53

Ashwin N Ananthakrishnan, MD, MPH
Massachusetts General Hospital
Boston, MA
IBD Research Group
DDRI/TUMS
Figure 2. Increasing incidence of Crohn’s disease
Age distribution of Crohn’s disease in Iran.
Crohn’s Disease and Early Exposure to Domestic Refrigeration

Fatemeh Malekzadeh¹, Corinne Alberti²,³, Mehdi Nouraei¹, Homayoon Vahedi¹, Isabelle Zaccaria²,³, Ulrich Meinzer⁴,⁵,⁶, Siavosh Nasseris-Moghaddam¹, Rasoul Sotoudehmanesh¹, Sara Momenzadeh¹, Reza Khaleghe Nejad¹, Shahrooz Rashtak¹, Golrokh Olfati¹, Reza Malekzadeh¹, Jean-Pierre Hugot⁴,⁵,⁶,⁷

¹ Digestive Disease Research Center, Medical Sciences, University of Tehran, Tehran, Iran, ² APHP, Unité d’épidémiologie clinique, Hôpital Robert Debré, Paris, France, ³ INSERM U1015, Paris, France, ⁴ AP-HP, service de gastroentérologie pédiatrique, Hôpital Robert Debré, Paris, France, ⁵ Université Paris Diderot, Paris, France, ⁶ INSERM UB43, Paris, France

Abstract

Background: Environmental risk factors playing a causative role in Crohn’s Disease (CD) remain largely unknown. Recently, it has been suggested that refrigerated food could be involved in disease development. We thus conducted a pilot case control study to explore the association of CD with the exposure to domestic refrigeration in childhood.

Methodology/Principal Findings: Using a standard questionnaire we interviewed 199 CD cases and 207 age-matched patients with irritable bowel syndrome (IBS) as controls. Cases and controls were followed by the same gastroenterologists of tertiary referral clinics in Tehran, Iran. The questionnaire focused on the date of the first acquisition of home refrigerator and freezer. Data were analysed by a multivariate logistic model. The current age was in average 34 years in CD cases and the percentage of females in the case and control groups were respectively 48.3% and 63.7%. Patients were exposed earlier than controls to the refrigerator (X² = 9.9, df = 3, P = 0.04) and refrigerator exposure at birth was found to be a risk factor for CD (OR = 2.08 (95% CI: 1.01 – 4.29), P = 0.05). Comparable results were obtained looking for the exposure to freezer at home. Finally, among the other recorded items reflecting the hygiene and comfort at home, we also found personal television, car and washing machine associated with CD.

Conclusion: This study supports the opinion that CD is associated with exposure to domestic refrigeration, among other household factors, during childhood.
Emerging Epidemic of Inflammatory Bowel Disease in a Middle Income Country: A Nation-wide Study from Iran

Masoud M. Malekzadeh MD1, Homayoon Vahedi MD1, Kimiya Gohari BSc2,3, Parinaz Mehdipour MSc2,4, Sadaf G. Sepanlou PhD1, Nasser Ebrahim E Daryani MD5, Mohammad Reza Zali MD6, Fariborz Mansour-Ghanaci MD7, Alireza Safaripour MD9, Rahim Aghazadeh MD8, Hassan Vossoughinia MD9, Hafez Fakheri MD10, Mohammad H. Somi MD11, Iraj Maleki MD10, Vahid Hoseini MD10, Mohammad Reza Ghadir MD12, Hamed Daghaghzadeh MD13, Payman Adibi MD13, Hamid Tavakoli MD13, Alireza Taghavi MD8, Mohammad Javad Zahedi MD14, Taghi Amirian MD15, Masoud Tabib MD16, Zainab Alipour MD16, Hossein Nobakht MD17, Abbas Yazdanbod MD18, Masoud Sadreddini MD19, Alireza Bakhshipour MD20, Ahmad Khosravi MD9, Pejman Khosravi MD3, Siavosh Nasser-Moghadam MD1, Shahin Merat MD1, Rasoul Sotoudehmanesh MD1, Farhad Barazandeh MD22, Peyman Arab MD23, Nadieh Baniassadi MD24, Seyyed Javad Pournaghi MD21, Mahboubeh Parsaefan PhD1,23, Farshad Farzadfar MD MPH DSc1,4, Reza Malekzadeh MD*1

Abstract

Background: The burden of inflammatory bowel disease (IBD) hasn’t been reported in Iran. We aimed to estimate the prevalence and incidence of IBD and its trend in Iran at national and subnational level from 1990 to 2012.

Methods: We conducted a systematic review of English and Persian databases about the epidemiology of IBD. We also collected outpatient data from 17 provinces of Iran using almost all public and private referral gastroenterology clinics. Prevalence and incidence rate was calculated at national and subnational levels. The Kriging method was used to extrapolate provinces with missing data and GPR model to calculate time trends of rates at subnational level.

Results: We found 16 case series, two population-based studies, and two review articles. We collected 11,000 IBD cases from outpatient databases. Among them, 9,269 (84.26%) had ulcerative colitis (UC), 1,646 (14.96%) had Crohn's disease (CD), and 85 had intermediate colitis (IC). A total of 5,452 (49.56%) patients were male. Mean age at diagnosis was 32.80 years (CI: 13 – 61) for UC and 29.98 years (CI: 11 – 58) for CD. Annual incidences of IBD, UC, and CD in 2012 were 3.11, 2.70, and 0.41 per 100,000 subjects respectively. Prevalence of
Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations


Ulcerative colitis and Crohn’s disease are the two main forms of inflammatory bowel disease (IBD). Here we report the first trans-ancestry association study of IBD, with genome-wide or Immunochip genotype data from an extended cohort of 86,640 European individuals and Immunochip data from 9,846 individuals of East Asian, Indian or Iranian descent. We implicate 38 loci in IBD risk for the first time. For the majority of the IBD risk loci, the direction and magnitude of effect are consistent in European and non-European cohorts. Nevertheless, we observe genetic heterogeneity between divergent populations at several established risk loci driven by differences in allele frequency (NOD2) or effect size (TNFSF15 and ATG16L1) or a combination of these factors (IL23R and IRGm). Our results provide biological insights into the pathogenesis of IBD and demonstrate the usefulness of trans-ancestry association studies for mapping loci associated with complex diseases and understanding genetic architecture across diverse populations.
Nonadherence to Medication in Inflammatory Bowel Disease: Rate and Reasons

Mohammad Reza Ghadir, Mohammad Bagheri, Homayoon Vahedi, Nasser Ebrahim Daryani, Reza Malekzadeh, Ahmad Hormati, Shadi Kolahdoozan, Meghdi Chaharmahali

ABSTRACT

BACKGROUND

This study is the first study to evaluate the nonadherence rate and reasons of same patient with inflammatory bowel disease (IBD) in Iran.

METHODS

During 9 months, 500 patients with IBD were enrolled in the study. Patients were interviewed about their nonadherence behaviors. Factor analysis was used to analyze the collected answers.

RESULTS

The overall rate of nonadherence was 33.3% (27.6% intentional nonadherence and 5.7% unintentional nonadherence). 33.6% of the patients had at least one relapse after discontinuing treatment. The most frequent reason for intentional nonadherence was discontinuing the treatment after recovering from symptoms (42.7%). The most frequent reason for unintentional nonadherence was forgetfulness (5.2%). 19.8% of the patients did not visit their gastroenterologist on time and they purchased drugs from the drugstore. These patients reported that their clinics were too far and difficult to access. There was no significant relationship between nonadherence and demographic variables.

CONCLUSION

Multiple reasons are suggested as factors of medication nonadherence and they seem to be different among different populations. Determining these possible reasons, could lead to finding suitable strategies to overcome or reduce them.
Three Common CARD15 Mutations are not Responsible for the Pathogenesis of Crohn’s Disease in Iranians

Ladan Teimoori-Toolabi1, Homayoun Vahedi1, Hamid Mollahajian1, Esmaf Kamali2, Shohreh Hajizadeh-Sikaroodi3, Sirous Zeinali4, Tahmineh Tabrizian1, Golrokh Olfati1, Shahrooz Rashak1, Fatemeh Malekzadeh1, Alireza Ghodossi, Reza Malekzadeh1

1Digestive Disease Research Center (DDRC), Shariati Hospital, Tehran University; Medical Sciences North Kargar Street, Tehran, Iran, Postal code: 14117-13135
2Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran 69th Pasteur Avenue, Kargar Street, Postal code: 13169-43551

Corresponding Author: Ladan Teimoori-Toolabi
Digestive Disease Research Center (DDRC), Shariati Hospital, Tehran University; Medical Sciences North Kargar Street, Tehran, Iran, Postal code: 14117-13135
Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran 69th Pasteur Avenue, Kargar Street, Postal code: 13169-43551
Tel: +982166953311 Ext 2473, Fax: +982166480780, E-mail: liosha1380@yahoo.com

ABSTRACT

Background/Aims: Crohn’s disease frequency has increased in recent years in Iran. Genetic and environmental factors predispose people to this disease. Mutation in Caspase Recruitment Domain 15 (CARD15) gene is the most well known genetic predisposing factor to this disease. Prevalence of three common CARD15 mutations has been studied in different ethnic groups. We aimed to study the frequency of these mutations in Iranian patients affected with Crohn’s Disease.

Methodology: One hundred fifteen proved cases of Crohn Disease and 115 age and sex matched normal controls were recruited in this study. Lf1007fs, R702W and G908R mutations were studied by Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP) followed by direct sequencing.

Results: Lf1007fs and G908R mutations were not found in either patients or age-sex matched controls. Just in two patients, R702W mutation was proved by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and sequencing. None of these patients had ileal or fibrostenotic type of disease while 14.7% of total patients had stricturing type of disease. No complication was seen in these two patients while 50.4% of patients had acquired complications during the course of disease.

Conclusion: The three mutations described are not responsible for the pathogenesis of Crohn’s Disease in Iranians. The results are in accordance with other Asian nations’ studies on IBD Patients.
Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations


Ulcerative colitis and Crohn's disease are the two major forms of inflammatory bowel disease (IBD). Here we report the first trans-ancestry association study of IBD, with genome-wide or Immunochip genotype data from an extended cohort of 86,640 European individuals and Immunochip data from 9,846 individuals of East Asian, Indian or Iranian descent. We implicate 38 loci in IBD risk for the first time. For the majority of the IBD risk loci, the direction and magnitude of effect are consistent in European and non-European cohorts. Nevertheless, we observe genetic heterogeneity between divergent populations at several established risk loci driven by differences in allele frequency (NOD2) or effect size (TNFSF15 and ATG16L1) or a combination of these factors (IL23R and IRGM). Our results provide biological insights into the pathogenesis of IBD and demonstrate the usefulness of trans-ancestry association studies for mapping loci associated with complex diseases and understanding genetic architecture across diverse populations.
Genetics of IBD
163 Confirmed Loci

CD genes

30 CD-specific loci
NOD2
PTPN22

UC genes

23 UC-specific loci

110 IBD loci
Common pathways:
- Leprosy
- Mycobacterial susceptibility
- Other immune-mediated disease

Genes in common

Trans-Ethnic Working Group

- **Iran (DDRI)**
  - 169/428 CD/UC
  - 663 controls

- **India**
  - 184/1,239 CD/UC
  - 990 controls

- **China**
  - 172/155 CD/UC
  - 264 controls

- **Korea**
  - 210/243 CD/UC
  - 144 controls

- **Japan**
  - 1,318/736 CD/UC
  - 3,311 controls

**Total non Caucasian 10,216**

- 2,043 Crohn’s Disease
- 2,801 Ulcerative colitis
- 5,372 Healthy controls
Cross-ethnic meta-analysis: 6 New UC genes

<table>
<thead>
<tr>
<th>Chr</th>
<th>ID</th>
<th>TRAIT</th>
<th>EUR_OR</th>
<th>IRA_OR</th>
<th>IND_OR</th>
<th>EAS_OR</th>
<th>IRA_P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs2</td>
<td>UC</td>
<td>0.93</td>
<td>0.69</td>
<td>0.78</td>
<td>0.85</td>
<td>0.0100802</td>
</tr>
<tr>
<td>2</td>
<td>rs8</td>
<td>UC</td>
<td>1.08</td>
<td>1.19</td>
<td>1.21</td>
<td>1.17</td>
<td>0.152725</td>
</tr>
<tr>
<td>3</td>
<td>rs11</td>
<td>UC</td>
<td>1.09</td>
<td>0.95</td>
<td>1.16</td>
<td>0.96</td>
<td>0.628002</td>
</tr>
<tr>
<td>3</td>
<td>rs12</td>
<td>UC</td>
<td>0.93</td>
<td>0.86</td>
<td>0.97</td>
<td>0.99</td>
<td>0.172844</td>
</tr>
<tr>
<td>3</td>
<td>rs13</td>
<td>UC</td>
<td>1.08</td>
<td>1.27</td>
<td>1.19</td>
<td>1.18</td>
<td>0.0441663</td>
</tr>
<tr>
<td>21</td>
<td>rs31</td>
<td>UC</td>
<td>0.92</td>
<td>0.85</td>
<td>0.87</td>
<td>0.89</td>
<td>0.265811</td>
</tr>
</tbody>
</table>

6 novel UC variants: 4 same effect as EU, 5 as Indians, 6 as Asians
Cross-ethnic meta-analysis: 11 New CD Variants

<table>
<thead>
<tr>
<th>Chr</th>
<th>ID</th>
<th>TRAIT</th>
<th>EUR_OR</th>
<th>IRA_OR</th>
<th>IND_OR</th>
<th>EAS_OR</th>
<th>IRA_P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>rs7</td>
<td>CD</td>
<td>1.17</td>
<td>1.45</td>
<td>0.67</td>
<td>NA</td>
<td>0.222275</td>
</tr>
<tr>
<td>2</td>
<td>rs10</td>
<td>CD</td>
<td>1.09</td>
<td>1.03</td>
<td>1.07</td>
<td>1.04</td>
<td>0.810311</td>
</tr>
<tr>
<td>6</td>
<td>rs18</td>
<td>CD</td>
<td>0.93</td>
<td>0.97</td>
<td>0.60</td>
<td>0.94</td>
<td>0.845333</td>
</tr>
<tr>
<td>6</td>
<td>rs20</td>
<td>CD</td>
<td>1.09</td>
<td>0.93</td>
<td>1.06</td>
<td>1.11</td>
<td>0.627883</td>
</tr>
<tr>
<td>7</td>
<td>rs21</td>
<td>CD</td>
<td>0.93</td>
<td>1.17</td>
<td>0.97</td>
<td>0.91</td>
<td>0.342518</td>
</tr>
<tr>
<td>12</td>
<td>rs23</td>
<td>CD</td>
<td>0.93</td>
<td>1.05</td>
<td>0.86</td>
<td>NA</td>
<td>0.697382</td>
</tr>
<tr>
<td>17</td>
<td>rs26</td>
<td>CD</td>
<td>1.09</td>
<td>0.76</td>
<td>1.13</td>
<td>1.05</td>
<td>0.0660535</td>
</tr>
<tr>
<td>18</td>
<td>rs27</td>
<td>CD</td>
<td>1.12</td>
<td>1.19</td>
<td>0.88</td>
<td>0.69</td>
<td>0.635792</td>
</tr>
<tr>
<td>19</td>
<td>rs28</td>
<td>CD</td>
<td>0.93</td>
<td>0.96</td>
<td>0.93</td>
<td>NA</td>
<td>0.765237</td>
</tr>
<tr>
<td>20</td>
<td>rs30</td>
<td>CD</td>
<td>0.93</td>
<td>0.88</td>
<td>1.13</td>
<td>0.92</td>
<td>0.355424</td>
</tr>
<tr>
<td>22</td>
<td>rs32</td>
<td>CD</td>
<td>1.10</td>
<td>0.93</td>
<td>0.93</td>
<td>0.95</td>
<td>0.608975</td>
</tr>
</tbody>
</table>

11 CD_SNPs: 5 on the same direction, 4 with indians, 5 with Asians,
## Cross-ethnic meta-analysis: 16 New IBD genes

<table>
<thead>
<tr>
<th>Chr</th>
<th>ID</th>
<th>TRAIT</th>
<th>EUR_OR</th>
<th>IRA_OR</th>
<th>IND_OR</th>
<th>EAS_OR</th>
<th>IRA_P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs1</td>
<td>IBD</td>
<td>1,24</td>
<td>0,74</td>
<td>0,68</td>
<td>9,48</td>
<td>0,691872</td>
</tr>
<tr>
<td>1</td>
<td>rs3</td>
<td>IBD</td>
<td>1,07</td>
<td>1,33</td>
<td>1,05</td>
<td>1,06</td>
<td>0,0157786</td>
</tr>
<tr>
<td>1</td>
<td>rs4</td>
<td>IBD</td>
<td>1,06</td>
<td>1,23</td>
<td>1,04</td>
<td>1,18</td>
<td>0,0289052</td>
</tr>
<tr>
<td>1</td>
<td>rs5</td>
<td>IBD</td>
<td>0,95</td>
<td>0,87</td>
<td>0,93</td>
<td>1,61</td>
<td>0,229956</td>
</tr>
<tr>
<td>1</td>
<td>rs6</td>
<td>IBD</td>
<td>1,07</td>
<td>1,11</td>
<td>0,96</td>
<td>0,88</td>
<td>0,273083</td>
</tr>
<tr>
<td>2</td>
<td>rs9</td>
<td>IBD</td>
<td>0,94</td>
<td>0,98</td>
<td>0,88</td>
<td>0,91</td>
<td>0,840088</td>
</tr>
<tr>
<td>4</td>
<td>rs14</td>
<td>IBD</td>
<td>1,10</td>
<td>1,18</td>
<td>1,07</td>
<td>1,24</td>
<td>0,308381</td>
</tr>
<tr>
<td>4</td>
<td>rs15</td>
<td>IBD</td>
<td>1,06</td>
<td>1,02</td>
<td>0,97</td>
<td>1,09</td>
<td>0,802702</td>
</tr>
<tr>
<td>5</td>
<td>rs16</td>
<td>IBD</td>
<td>0,93</td>
<td>1,15</td>
<td>1,04</td>
<td>1,00</td>
<td>0,176056</td>
</tr>
<tr>
<td>6</td>
<td>rs17</td>
<td>IBD</td>
<td>1,07</td>
<td>1,08</td>
<td>1,00</td>
<td>1,05</td>
<td>0,402933</td>
</tr>
<tr>
<td>6</td>
<td>rs19</td>
<td>IBD</td>
<td>0,94</td>
<td>0,95</td>
<td>0,88</td>
<td>1,05</td>
<td>0,65069</td>
</tr>
<tr>
<td>7</td>
<td>rs22</td>
<td>IBD</td>
<td>0,93</td>
<td>0,82</td>
<td>1,03</td>
<td>0,87</td>
<td>0,0586876</td>
</tr>
<tr>
<td>12</td>
<td>rs24</td>
<td>IBD</td>
<td>0,90</td>
<td>0,71</td>
<td>0,82</td>
<td>83,00</td>
<td>0,0296335</td>
</tr>
<tr>
<td>13</td>
<td>rs25</td>
<td>IBD</td>
<td>0,91</td>
<td>0,93</td>
<td>0,97</td>
<td>1,04</td>
<td>0,545358</td>
</tr>
<tr>
<td>19</td>
<td>rs29</td>
<td>IBD</td>
<td>0,84</td>
<td>0,84</td>
<td>0,69</td>
<td>1,11</td>
<td>0,47788</td>
</tr>
</tbody>
</table>

16 novel IBD SNPs: 13 same effect as EU, 13 as Indians, 6 as Asians
Genetic overlap among 163 loci – European vs Iranian
Estimating shared additive genetic variation explained by all Ichip SNPs
Pervasive sharing of genetic risk factors and similarity of phenotypes in Iranian too,

- Largest Genetic Study in non-European IBD patients,
- >20 novel IBD genes identified,
- Pervasive sharing of genetic risk factors in Iranian too,
- Relative contribution of genes can vary between Iranian and others, specially genes associated with autophagy & innate immunity in CrD.
- Clinical relatively similar phenotypes,
Reasons for worldwide differences in IBD incidence

• Differences in the distribution of susceptibility genes (e.g. NOD2) between populations living in the Western world and newly industrialized countries influence incidence

• Environmental exposures and the consequences of these exposures differ between populations living in the Western world and newly industrialized countries

• Advances in health-care infrastructure, greater adoption of medical technology (e.g. colonoscopy), and improved access to health care affect the incidence of IBD

• Methodological differences in surveillance of IBD artificially widen the gap between the reported incidence of IBD in the Western world and in newly industrialized countries
Recommendations to mitigate the burden of IBD over the next 10 years

• Utilize forecasting models that predict the regional health-care utilization and resource allocation needed to care for patients with IBD

• Implement efficient, cost-effective and innovative models of health-care delivery that adapt to the rising number of cases of IBD

• Reduce the use of biologic agents through the discovery of biomarkers that optimize patient selection, dosing and discontinuation

• Minimize the cost of biologic agents through the adoption of biosimilar agents and market-place negotiations to bundle costs
Recommendations 2

• Fund basic science and clinical research that explores the origin of IBD (e.g. microbiome research) to foster novel treatment strategies and to prevent disease development

• Conduct interventional studies that alter exposure to environmental risk factors that are shown to exacerbate IBD

• Introduce population-level interventions to modify exposures and activities that can increase the risk of developing IBD (e.g. smoking cessation, encouraging breast-feeding and avoiding unnecessary antibiotics)

• Target high-risk populations (e.g. first-degree relatives of patients with Crohn’s disease) for prevention through recommendations on environmental modification
Statins Associated With Decreased Risk of New Onset Inflammatory Bowel Disease

• Any statin exposure was associated with a significantly decreased risk of IBD (OR 0.68, 95% CI 0.64–0.72), CD (0.64, 95% CI 0.59–0.71), and UC (OR 0.70, 95% CI 0.65–0.76). This effect was similar for most specific statins and regardless of intensity of therapy.

• Statins may have a protective effect against new onset IBD, CD, and UC. This decreased risk is similar across most statins and appears to be stronger among older patients, particularly in CD.
Genetic Data Suggest Dividing IBD Into Three Forms

Data from a genetic association study

• Suggest that IBD should be divided into a three-group continuum, rather than the current division between Crohn's disease and ulcerative colitis.

• "The current clinical classifications of IBD, while important and useful, are a simplification of the true biological variation of this disease

• "Ultimately, if we improve this classification system, we'll hopefully have more successful trials for new medicines and better ability to give the right drug to the right patient."
Methods
Genetic risk scores from nearly 30,000 IBD patients to study genetic heterogeneity underlying the natural history of IBD.

- Three genetic loci achieved genome-wide significance: 3p21 (MST1), NOD2, and the major histocompatibility complex (MHC).
Ileal and colonic Crohn's disease are distinct entities.

- At least 163 susceptibility loci related to IBD have been identified so far, with most conferring risk of both ulcerative colitis and Crohn's disease.
- Molecular studies have suggested that ileal and colonic Crohn's disease are distinct entities because of specific variants in NOD2 (in small bowel disease) and HLA alleles (in colonic disease).
- The genetic risk score strongly favored a model in which colonic Crohn's disease is intermediate between ileal Crohn's disease and ulcerative colitis over the model that grouped both Crohn's disease subphenotypes as a single category.
New Classification Of IBD

• Ulcerative colitis
• Crohn’s Disease of Colon
• Ileal crohn’s Disease
HLA alleles correlated with IBD

• Several human leukocyte antigen (HLA) alleles correlated with IBD susceptibility, location, and extent: HLA-DRB1*07:01 was the strongest signal for colonic disease and the strongest shared risk allele for Crohn's disease and ulcerative colitis; rs77005575 was associated with Crohn's disease behavior; HLA-B*08 was the top signal for the extent of ulcerative colitis; and HLA-DRB1*13:01 was the top signal for age at diagnosis of ulcerative colitis.
200 regions of the genome have been shown to be associated with IBD risk three of them were individually strongly associated with clinical phenotype.

- "Despite the fact that more than 200 regions of the genome have been shown to be associated with IBD risk, only found three of them were individually strongly associated with clinical phenotype.

- The way in which we clinically stratify patients is imperfectly capturing the true underlying biology of their disease.

- In future with further genetic study the results will help to improve that stratification in the future."
Personalized treatments

• For personalized treatments we need to understand which cells are affected in particular patients, and how they've gone wrong in maintaining the balance between healthy and exaggerated inflammation.

• The quest to identify biomarkers that can allow clinicians to predict disease course and disease complications is gaining increasing importance.

• It is also increasingly obvious that no single biomarker will be able to perform such a task in a complex disease as IBD."
Precision medicine will become real in IBD

• in the future, algorithms that incorporate specific genetic risk alleles with other markers (e.g., antimicrobial markers, specific proteomic signature, microRNAs, etc.) will be used to predict complications, response to specific therapies, risk for surgery, etc., allowing true precision medicine to become real in IBD," Dr. Torres said.

• "Genetics alone are not useful in predicting disease course, disease complications, or behavior," she concluded. "Other avenues of research that integrate genetic with non-genetic factors (such as the microbiome, early life environmental exposures, etc.) need to be developed if any light is to be shed onto the complex pathophysiology of inflammatory bowel disease."