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Identification of potential therapeutic targets of fatty acids for inflammatory bowel diseases: An *in silico* approach

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Inflammatory bowel diseases (IBD) are chronic disorders of the gastrointestinal tract, often associated with long-term adverse effects from conventional therapies. Here we investigated the potential of a collection of odd- and even-chain fatty acids (FAs) as alternative therapeutic agents for IBD. IBD-associated genes were retrieved from DisGeNET and the Therapeutic Target Database, while the molecular structures of 14 odd-chain and 58 even-chain FAs were obtained from PubChem. ADME profiling was conducted using SwissADME, and target prediction was performed via SwissTargetPrediction and STITCH, followed by interaction network construction using Cytoscape. Gene ontology and KEGG pathway enrichment analyses were carried out using the DAVID bioinformatics tool. The analysis identified pristanic acid, stearic acid, oleic acid and its isomers, and eicosapentaenoic acid as potential candidates for IBD therapy. GO analysis revealed involvement in inflammatory responses and extracellular matrix disassembly, while KEGG pathway analysis indicated significant associations with the TNF, IL-17, HIF-1, and PI3K-Akt signaling pathways, all of which play crucial roles in IBD. These findings were further supported by molecular docking studies that showed strong interactions between the identified FAs and key proteins in these pathways. Overall, the results suggest that fatty acids may modulate signaling pathways relevant to IBD, though further *in vitro* and *in vivo* validation is required.

Keywords: Fatty acids, Gene ontology, Inflammatory bowel disease, Signal transduction and extracellular matrix

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and Ulcerative colitis (UC) are chronic inflammatory disorders of the digestive tract[1-3].They typically begin before the age of 40, posing significant health challenges relatively in the young population[4]. CD patients have a slightly higher risk of mortality due to complications, whereas UC patients do not[1–3]. Both CD and UC have an increased risk of developing colorectal cancer (CRC) due to persistent inflammation, necessitating keen observation and preventative measures[5]. The highest incidence and prevalence rates of both CD and UC are found in Northern Europe and North America, while rates continue to rise in Southern Europe, Asia, and most developing countries[6].

Since the mechanism of action of IBD is not well understood, therapies generally involve nonspecific anti-inflammatory and immune-suppressive medications or surgery when medical therapy fails[7]. Oral delivery of such compounds is successful against intestinal inflammation; nonetheless, it has several limitations, as most of the administered drug is delivered to nonspecific cells in the body, resulting in either infusion reactions or undesirable side effects[1]. While a cure for IBD remains elusive, it is clear that several pharmaceutical compounds can reduce intestinal inflammation. Small molecules, such as FAs, are among the new and developing treatment choices for IBD[8,9]. Omega-3 polyunsaturated fatty acids (PUFAs) have been found to reduce inflammatory responses in IBD[10].

FAs are diverse molecules with varying hydrocarbon chain lengths and degrees of saturation. Long-chain (LCFAs) and very-long-chain fatty acids (VLCFAs) are mainly obtained through diets, while short-chain fatty acids (SCFAs) are reported to be produced by gut bacteria fermenting dietary fibers[11]. In addition, FAs are readily available naturally[12]. Currently, small molecules that have the ability to regulate the inflammatory signaling pathways have been identified as effective therapeutic options for treating IBD[13].

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Suppl. Data available on respective page of NOPR

The anti-inflammatory effects of omega-3 FAs are helpful in the treatment of UC and CD by reducing the production of precursors of pro-inflammatory cytokines such as leukotrienes and prostaglandins[10]. The omega-6 PUFAs also make significant contributions to inflammation by being the precursors to a number of potent pro-inflammatory mediators including leukotrienes and prostaglandins[14]. In addition, SCFAs play crucial roles in regulating metabolism and inflammation through interactions with gut microbiota[11].

Considering the previous reports that address the anti-inflammatory effects of FAs, the present study was planned to investigate potential drug targets of IBD through computational analysis using a collection of odd- and even-chain FAs (14 odd-chain and 58 even-chain fatty acids).

Materials and Methods

Classification of FAs

The structures of fatty acids (FAs) were obtained from the PubChem database. The FAs were classified based on the number of carbon atoms, distinguishing between odd- and even-chain fatty acids. Additionally, they were categorized according to their degree of saturation, identifying them as either saturated or unsaturated. For detailed characterization, the chemical names, molecular formulas, and SMILES (Simplified Molecular Input Line Entry System) notations were also retrieved.

Target prediction

Target prediction of FAs

Identifying the biological targets of FAs reveals how these molecules interact with various molecular and cellular components within the body. These targets include enzymes, receptors, and signaling pathways that FAs modulate, influencing a wide array of physiological processes. Based on the SMILES, targeted genes of FAs were extracted through SwissTargetPrediction[15] and STITCH[16] tools. The search process was restricted to *Homo sapiens* in both databases, and the SMILES notations were uploaded to SwissTargetPrediction.

Target prediction of IBD

Prediction of target genes associated with IBD was performed using DisGeNET[17] and TTD: Therapeutic Target Databases[18]. These databases offer brief genomic data and functional annotations for well-studied human genes. The ultimate shared genes among both the FA targets and disease targets, both across and within them, were identified and visually represented using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/).

Network construction

Network construction integrates diverse data to unravel the complexity of biological systems. By constructing and analyzing biological networks, it is possible to gain deeper insights into the underlying mechanisms driving cellular processes, disease pathogenesis, and therapeutic interventions. For this purpose, Cytoscape 3.10.1[19] was used.

Gene ontology and KEGG pathway enrichment analysis

Gene ontology categorizes gene functions and attributes across three main domains: biological process (BP), molecular function (MF), and cellular component (CC). The ontology allows for comprehensive annotation and analysis of gene sets across diverse biological contexts. DAVID Bioinformatics Database[20] was used to predict the role of overlapped genes for each classified FA. Gene ontology enrichment analysis was visualized using Shiny GO 0.80[21] and SR Plots[22].

SWISS ADME profiling

ADME refers to the systematic evaluation of a compound's Absorption, Distribution, Metabolism, and Excretion properties. It plays a crucial role in drug discovery and development by assessing the pharmacokinetic and pharmacodynamic characteristics of potential drug candidates. To carry out this task, SWISS ADME[23] was employed to distinguish the physicochemical properties essential for the analysis. The SWISS ADME profile provides information on molecular weight (MW), oral bioavailability, gastrointestinal (GI) absorption, and blood-brain barrier (BBB) permeability. To be effective in treating IBD, a drug should have a molecular weight of no more than 500, a minimum oral bioavailability of 30%, excellent GI absorption, and should not penetrate the BBB (typically, drugs intended for use in the central nervous system (CNS) must cross the blood-brain barrier (BBB).

BOILED-Egg (Brain Or IntestinaL EstimateD permeation predictive model) graph in SWISS ADME helps to visualize two properties, gastro-intestinal absorption and blood-brain barrier (BBB) permeability[24]. The graph itself is divided into two main regions: The white region is the physicochemical space of molecules with the highest probability of being absorbed by the gastrointestinal tract, and the yellow region (yolk) is the physicochemical space of molecules with the highest

probability of permeating to the brain. Yolk and white areas are not mutually exclusive. Molecules plotted outside the ellipses, in the grey area, are predicted to have low GI absorption and poor BBB penetration[24]. Ideally, a drug candidate might be in one of the regions based on the desired effect.

Molecular docking

The pro-inflammatory cytokines Interleukin-6 (IL-6; PDB ID: 8D82), Interleukin-17 (IL-17; PDB ID: 7AMA), and Tumor necrosis factor-α (TNF-α; PDB ID: 2AZ5) along with a key signaling component in the PI3K signaling pathway, Phosphoinositide 3-kinase-α (PI3K-α; PDB ID: 8EXU), Protein Kinase B (AKT; PDB ID: 3CQW) and Hypoxia-inducible factor 1 (HIF-1; PDB ID: 8II0) were considered for docking studies based on KEGG Pathway analysis. In order to accomplish site-specific interactions, PDB structures with bound ligands were selected for further docking analysis[25-29]. ForIL-6, the receptor binding site was considered as the target site[30]. Discovery Studio was used to clean the protein structures. Moreover, the coordinates (Suppl. Table 1) for site-specific docking were generated by constructing a sphere around the existing small molecules, and the configuration files were created. AutoDock Tools 1.5.6 was used to generate pdbqt files for the proteins.

Pristanic acid, stearic acid, oleic acid, and eicosapentaenoic acid were considered for molecular docking studies. AutoDock Tools 1.5.6 was used to generate the PDBQT files required for docking. AutoDockVina was employed to perform molecular docking of the target proteins IL-6, IL-17, TNF-α, PI3K-α, AKT, and HIF-1 with the potential ligands mentioned above[31]. The vina.exe command was used to generate the docking poses, and the poses were split using the vina_split.exe command. BIOVIA Discovery Studio was utilized to visualize and analyze the docking poses for interactions. The docking with the highest number of hydrogen bonds was considered, and representative structures highlighting these interactions were generated using PyMOL and BIOVIA Discovery studio.

Results

Classification of FAs

The FAs were classified based on the number of carbon atoms as odd-chain and even-chain FAs. In total, 14-odd chain fatty acids and 58 even-chain FAs were employed. Furthermore, the even-chain FAs were classified based on their saturation as even-chain saturated (14) and unsaturated (44) FAs.

Identification of gene targets of FAs and genes associated with IBD

Gene targets of FAs

Gene targets for odd- and even-chain FAs were retrieved from the SwissTargetPrediction and STITCH databases. The collected targets from both databases were analyzed for overlaps, and any duplicates were removed. The final count of gene targets for each fatty acid (FA) in the classified odd- and even-chain categories was determined. The distribution of gene targets across the FAs within each category was relatively uniform. Notably, gene targets associated with odd- and even-chain saturated FAs comprised approximately 7–8% of the total targets for most FAs (Table 1), while those associated with even-chain unsaturated FAs ranged between 2-3% (Table 1).

Genes associated with IBD and the identification of overlapping gene targets between FAs and IBD

A total of 1,599 gene targets were identified to be associated with IBD (from DisGeNET and TTD databases). The exclusion of the overlapping gene targets of FAs determined the final count of gene targets as 325 for odd-chain FAs and 578 for even-chain FAs (Fig. 1A). These FA targets were then utilized to identify overlapping genes associated with IBD. The results revealed 98 overlapping gene targets for odd-chain FAs (Fig. 1B) and 166 for even-chain FAs (Fig. 1C).

Identification of biological properties associated with FAs targeting IBD-associated genes

For odd-chain and even-chain FAs, the majority of gene targets were enzymes, including proteases, kinases, phosphatases, *etc*. The rest consists of receptors, proteins, HDAC erasers and others as indicated in (Fig. 2A & B).

Identification of potential FAs that target IBD-associated genes

Potential fatty acids (FAs) from various FA classes that could target a large number of genes associated with IBD were identified. Within the odd-chain FA category, pentadecanoic acid, heneicosylic acid, tricosylic acid, and pristanic acid demonstrated a significant number of targets related to IBD (Table 2). Similarly, among even-chain saturated FAs, lignoceric acid, stearic acid, cerotic acid, and lauric acid exhibit a high number of targets associated with IBD (Table 2).

Table 1 — Number of identified gene targets (%) of odd-chain and even-chain FAs

(A) Gene targets of odd-chain FAs		(B) Gene targets of even-chain FAs (Saturated)		(C) Gene targets of even-chain FA (Unsaturated)					
Odd-chain FA	Targets (%)	Even-chain FA	Targets (%)	Even-chain FA	Targets (%)	Even-chain FA	Targets (%)	Even-chain FA	Targets (%)
Propionic acid	35 (2%)	Butyric acid	91 (6%)	Crotonic acid	25 (1%)	Linoleic acid	119 (3%)	Eicosatrienoic acid	113 (2%)
Valeric acid	112 (8%)	Caproic acid	113 (7%)	Trans-2-Octenoic acid	103 (2%)	Linoelaidic acid	110 (2%)	Arachidonic acid	122 (3%)
Oenanthic acid	106 (7%)	Caprylic acid	116 (7%)	2-Decenoic acid	107 (2%)	Rumenic acid	107 (2%)	Eicosatetraenoic acid	108 (2%)
Pelargonic acid	113 (8%)	Capric acid	116 (7%)	(2E,4E)-2,4-Decadienoic acid	106 (2%)	α-Linolenic acid	116 (2%)	Eicosapentaenoic acid	113 (2%)
Undecylic acid	112 (8%)	Lauric acid	118 (8%)	Lauroleic acid	107 (2%)	γ-linolenic acid	116 (2%)	Bosseopentaenoic acid	109 (2%)
Tridecylic acid	113 (8%)	Myristic acid	118 (8%)	Myristoleic acid	101 (2%)	Pinolenic acid	109 (2%)	Erucic acid	103 (2%)
Pentadecanoic acid	118 (8%)	Palmitic acid	119 (8%)	8Z-tetradecenoic acid	101 (2%)	Punicic acid	112 (2%)	Docosadienoic acid	115 (2%)
Margaric acid	105 (7%)	Stearic acid	117 (7%)	Palmitoleic acid	115 (2%)	Calendic acid	110 (2%)	Adrenic acid	113 (2%)
Pristanic acid	121 (8%)	Arachidic acid	114 (7%)	4-Hexadecenoic acid	109 (2%)	Stearidonic acid	110 (2%)	Clupanodonic acid	110 (2%)
Heneicosylic acid	108 (7%)	Behenic acid	109 (7%)	Sapienic acid	108 (2%)	Gadoleic acid	108 (2%)	Osbond acid	111 (2%)
Tricosylic acid	110 (7%)	Lignoceric acid	111 (7%)	Oleic acid	114 (2%)	Paullinic acid	103 (2%)	Docosahexaenoic acid	120 (3%)
Hyenic acid	108 (7%)	Cerotic acid	117 (7%)	Elaidic acid	107 (2%)	Eicosenoic acid	108 (2%)	Nervonic acid	103 (2%)
Carboceric acid	109 (7%)	Montanic acid	109 (7%)	Petroselinic acid	109 (2%)	Eicosadienoic acid	108 (2%)	9Z,12Z,15Z,18Z,21Z – tetracosapentaenoic acid	110 (2%)
Nonacosanoic acid	103 (7%)	Melissic acid	103 (7%)	Vaccenic acid	104 (2%)	Mead acid	106 (2%)	Nisinic acid	114 (2%)
				12-Octadecenoic acid	106 (2%)	Dihomo-γ-linolenic acid	118 (2%)		

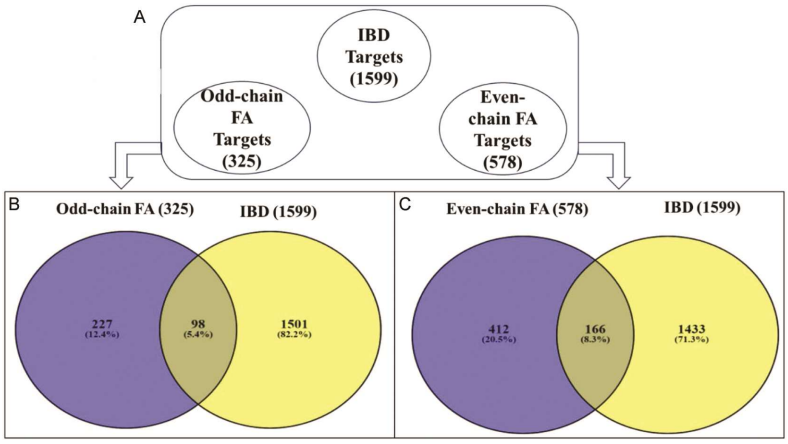

Fig. 1 — Genes linked to inflammatory bowel disease (IBD) and the discovery of common gene targets shared between FAs and IBD. (A). IBD gene targets were obtained from DisGeNET and TTD databases. After excluding the overlapping gene targets, the final number of gene targets obtained for IBD was 1,599. The SwissTargetPrediction and STITCH databases were utilized to obtain gene targets of odd- and even-chain FAs. Following the removal of overlapping gene targets within databases and FAs, the final total number of gene targets was determined to be 325 for odd-chain FAs and 578 for even-chain FAs. These odd- and even-chain FA targets were then utilized to identify overlapping gene targets with IBD; (B) and (C). The analysis using Venny 2.1.0 showed 98 overlapping gene targets for odd-chain FAs and 166 for even-chain FAs with IBD-associated genes

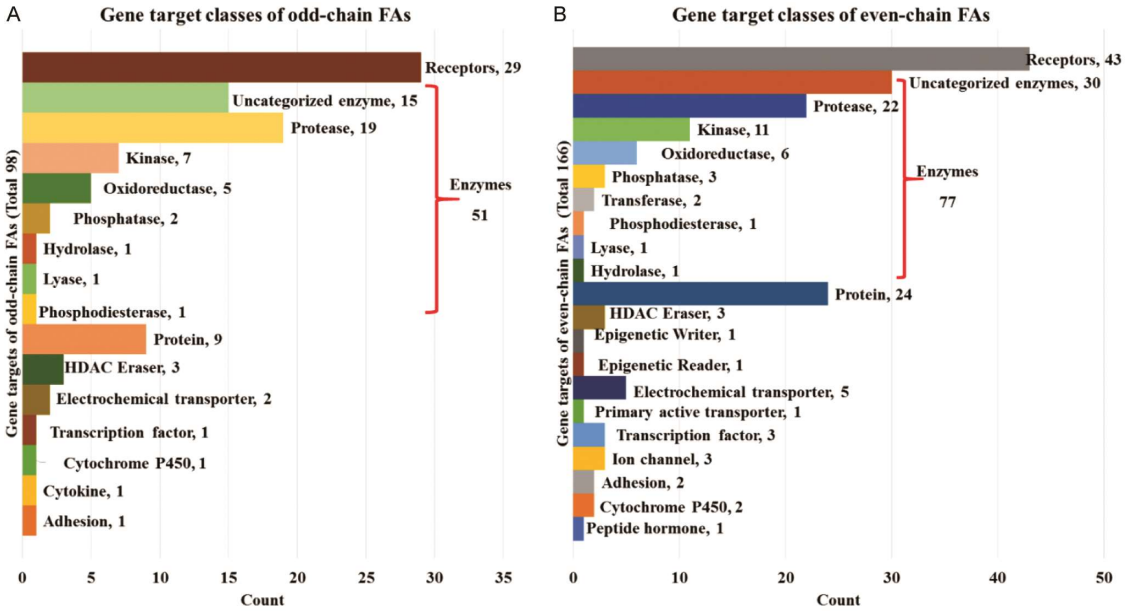

Fig. 2 — The biological properties associated with overlapping gene targets of odd-chain and even-chain fatty acids (FAs) reveal distinct classifications. For odd-chain FAs, the overlapping gene targets include 29 receptors, such as G protein-coupled receptors—Family A (9) and Family B (2)—a receptor for short-chain FAs (1), nuclear receptors (9), a proton-sensing receptor (1), a low-affinity receptor for leukotrienes (1), membrane receptors (5), and other receptors (1). There are also 51 enzymes, including uncategorized enzymes (15), proteases (19), kinases (7), oxidoreductases (5), phosphatases (2), a hydrolase (1), a lyase (1), and a phosphodiesterase (1). The protein group consists of 9 proteins, including secreted proteins (3) and unclassified proteins (6). Additional categories include HDAC erasers (3), electrochemical transporters (2), a transcription factor (1), cytochrome P450 (1), a cytokine (1), and an adhesion molecule (1). For even-chain FAs, the overlapping gene targets include 43 receptors, comprising G protein-coupled receptors—Family A (18) and Family B (2)—a receptor for short-chain FAs (1), nuclear receptors (11), a proton-sensing receptor (1), a low-affinity receptor for leukotrienes (1), membrane receptors (8), and other receptors (1). The 77 enzymes identified include uncategorized enzymes (30), proteases (22), kinases (11), oxidoreductases (6), phosphatases (3), transferases (2), a phosphodiesterase (1), a lyase (1), and a hydrolase (1). Among the 24 proteins are secreted proteins (6), unclassified proteins (2), other cytosolic proteins (4), and proteins (12). Ion channels (3) include a voltage-gated ion channel (1), a ligand-gated ion channel (1), and another ion channel (1). Epigenetic regulators (5) include an epigenetic writer (1), an epigenetic reader (1), and HDAC erasers (3). Transporters (6) include electrochemical transporters (5) and a primary active transporter (1). Additional classifications include transcription factors (3), adhesion molecules (2), cytochrome P450 enzymes (2), and a peptide hormone (1).

Table 2 — Overlapping gene targets of odd- and even-chain FAs with IBDs

A. Odd-chain FAs		B. Even-chain FAs (Saturated)				C. Even-chain FAs (Unsaturated)			
Odd-chain FAs	Overlapping gene targets	Even-chain FAs	Overlapping gene targets	Even-chain FAs	Overlapping gene targets	Even-chain FAs	Overlapping gene targets	Even-chain FAs	Overlapping gene targets
Pentadecanoic acid	39	Lignoceric acid	37	Arachidonic acid	48	Eicosapentaenoic acid	41	γ-linolenic acid	38
Heneicosylic acid	36	Stearic acid	37	α-Linolenic acid	47	Bosseopentaenoic acid	41	Gadoleic acid	38
Tricosylic acid	35	Cerotic acid	36	Linoleic acid	46	9Z,12Z,15Z,18Z,21Z-tetracosapentaenoic acid	41	Erucic acid	38
Pristanic acid	35	Lauric acid	36	Pinolenic acid	46	Elaidic acid	40	Nervonic acid	38
Nonacosanoic acid	33	Behenic acid	35	Oleic acid	44	Stearidonic acid	40	Lauroleic acid	37
Tridecylic acid	33	Palmitic acid	35	Punicic acid	44	Paullinic acid	40	Eicosenoic acid	37
Pelargonic acid	33	Caproic acid	35	Linoelaidic acid	43	Eicosatrienoic acid	40	Clupanodonic acid	37
Carboceric acid	32	Myristic acid	34	Dihomo-γ-linolenic acid	43	Myristoleic acid	39	Nisinic acid	37

(*Contd.*)

Table 2 — Overlapping gene targets of odd- and even-chain FAs with IBDs (*Contd*.)

(A) Odd-chain FA		(B) Even-chain FAs (Saturated)				(C) Even-chain FAs (Unsaturated)			
Margaric acid	32	Melissic acid	33	Osbond acid	43	Sapienic acid	39	Petroselinic acid	36
Hyenic acid	30	Caprylic acid	33	4-Hexadecenoic acid	42	Mead acid	39	12-Octadecenoic acid	36
Oenanthic acid	30	Montanic acid	32	Calendic acid	42	Docosadienoic acid	39	Eicosatetraenoic acid	36
Valeric acid	30	Capric acid	32	Vaccenic acid	41	Adrenic acid	39	Docosahexaenoic acid	36
Undecylic acid	28	Arachidic acid	29	Rumenic acid	41	8Z-tetra decenoic acid	38	Trans-2-Octenoic acid	31
Propionic acid	6	Butyric acid	21	Eicosadienoic acid	41	Palmitoleic acid	38	(2E,4E)-2,4-Decadienoic acid	31
								2-Decenoic acid	27
								Crotonic acid	5

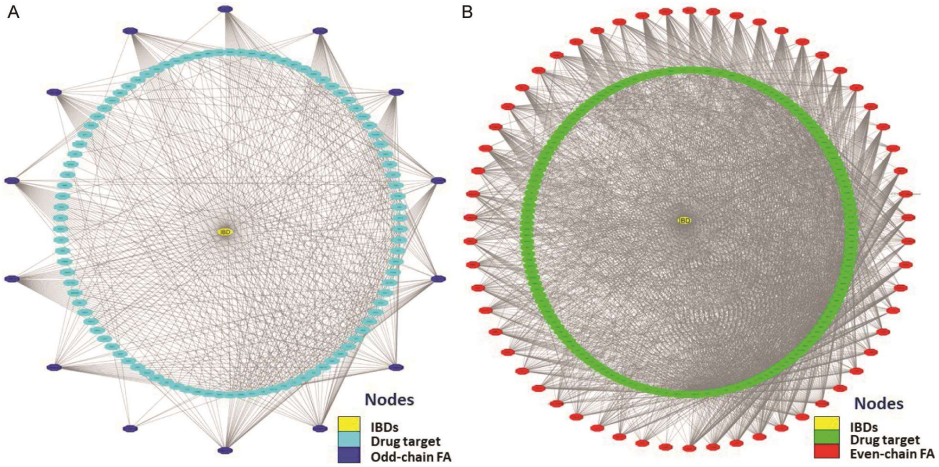

Fig. 3 — Construction of networks comprising FAs and IBD-associated genes. (A). Network showing odd-chain FA and IBD targets. Yellow node: IBD, light blue nodes: gene targets; blue nodes: odd-chain FAs; solid lines describe the interaction between the nodes; (B). Network showing even-chain FAs and IBD targets. Yellow node: IBD, light green nodes: gene targets; red nodes: odd-chain FAs; solid lines describe interaction between the nodes

In contrast, even-chain unsaturated FAs show a markedly higher number of gene targets in IBDs, by exceeding over 40 genes. Specifically, arachidonic, α-linolenic, linoleic, pinolenic, and oleic acids were identified as having a substantial number of targets in IBD (Table 2).

Upon comparing these different categories of FAs, it becomes evident that even-chain unsaturated FAs exhibit a higher potential than both odd-chain and even-chain saturated FAs, as indicated by the number of genes they target. The targeting range of even-chain unsaturated FAs in IBD spans from 5 to 48 genes, whereas odd-chain FAs target between 6 to 39 genes, and even-chain saturated FAs target between 21 to 37 genes. This comparison features the superior targeting efficacy of even-chain unsaturated FAs in the context of IBD.

Network construction

Through network analysis, a potential relationship between genes (including proteins, enzymes, receptors, transcription factors, *etc*.) and FAs relevant to IBD can be observed (Fig. 3A & B). In this network, IBD is centrally positioned, with the first immediate layer comprising potential drug targets that can be influenced by various FAs. This initial layer, consisting of disease-associated genes, represents promising targets for therapeutic intervention.

The network layout reveals functionally related genes that may contribute to the pathogenesis of IBD. Moreover, the interactions between nodes,

particularly between genes and FAs, suggest potential therapeutic mechanisms, highlighting the intricate connections that could be addressed for drug development.

GO ontology and KEGG pathway enrichment analysis

According to Gene Ontology (GO) enrichment analysis, the biological functions (BF), cellular components (CC), and molecular functions (MF) of the common gene targets of odd-chain and even-chain FAs (Fig. 4A & B) were identified.

GO analysis of odd-chain FAs revealed that the target genes were primarily involved in biological functions/properties such as extracellular matrix disassembly, inflammatory response, and collagen catabolic processes (Fig. 4A). The cellular components significantly associated with these genes included the cell surface, plasma membrane, and cytoplasm (Fig. 4A). In terms of molecular functions, peptidase activity and endopeptidase activity (Fig. 4A) were notably involved. For even-chain FAs, the target genes were primarily involved in biological functions/properties such as inflammatory response, response to lipopolysaccharide, and extracellular matrix disassembly (Fig. 4B). The cellular components most significantly associated with these genes included the plasma membrane, cell surface, and external side of the plasma membrane (Fig. 4B). In terms of molecular functions, RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, peptidase activity, and endopeptidase activity were notably involved (Fig. 4B).

KEGG pathway analysis of odd-chain FAs indicated significant enrichment of genes in the TNF and IL-17 signaling pathways (Fig. 5A). In the TNF-signaling pathway, the notable proteins enriched included p38, ERK1/2, IKKβ, Akt, and CASP3, highlighting their potential roles in mediating the effects of odd-chain FAs on inflammatory processes (Suppl. Fig. 1A). For even-chain FAs, the targets were significantly enriched in the PI3K-Akt, HIF-1, TNF and, IL-17 signaling pathways (Fig. 5B). In the HIF-1 signaling pathway, significant enrichment of proteins such as IL-6, TLR4, STAT3, ERK, and AKT were observed, underscoring their potential roles in the molecular mechanisms mediated by even-chain FAs (Suppl. Fig. 1B).

SWISS-ADME analysis of FAs

The Swiss -ADME analysis of both odd-chain and even-chain FAs reveals that almost all the FAs possess a molecular weight of ≤500 Daltons, and exhibit an oral bioavailability (OB) of ≥30%. Despite these common characteristics, notable differences were observed in terms of gastrointestinal (GI) absorption and blood-brain barrier (BBB) permeability

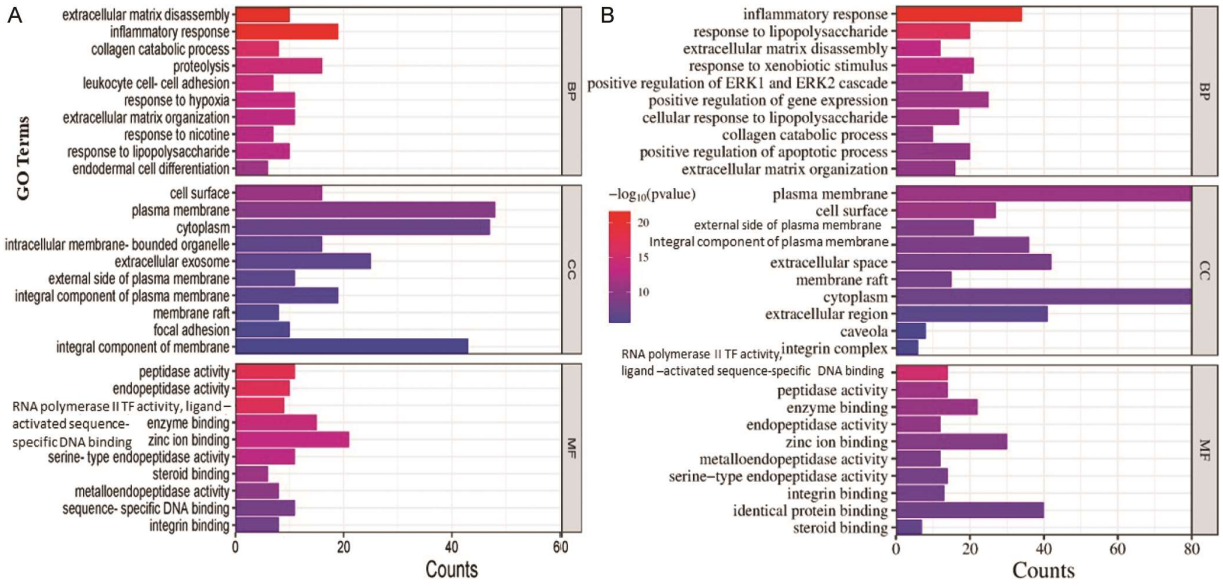

Fig. 4 — Gene ontology (GO) ontology pathway enrichment analysis of odd-and even-chain FAs. (A) Gene ontology enrichment analysis of odd-chain FAs gene targets; and (B) Gene ontology enrichment analysis of even-chain FAs gene targets. GO encompasses three main categories: Biological Processes (BP), Cellular Components (CC), and Molecular Functions (MF). The significance of the results is inversely related to the p-value, represented as $-\log_{10}$ (p-value). As the $-\log_{10}$ (p-value) increases, the p-value decreases, indicating a more significant result. In the visual representation of the data, highly significant results are denoted by a red color, while poorly significant results are indicated by a blue color, highlighting the varying levels of statistical significance across different GO terms

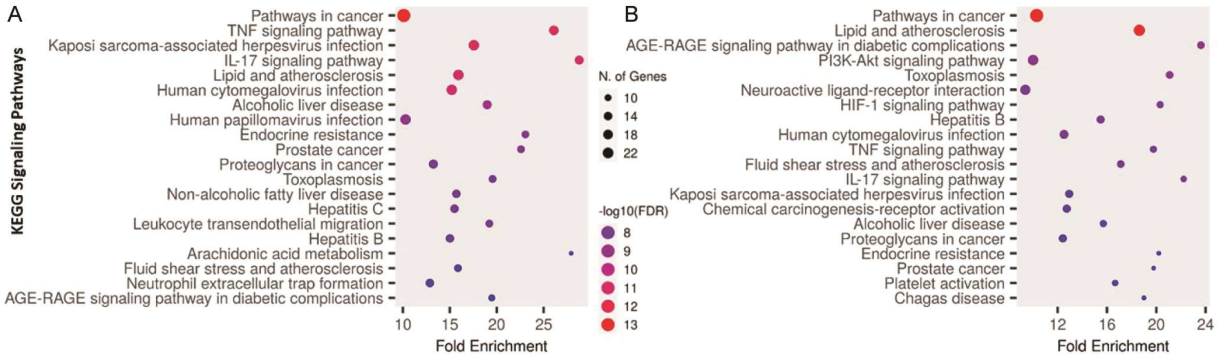

Fig. 5 — KEGG pathway analysis of odd-and even-chain FAs. (A) KEGG pathway analysis odd-chain FA targets; and (B) KEGG pathway analysis even-chain FA targets. This provides critical insights into the underlying biological mechanisms. Here, the size of each dot represents the number of genes associated with a particular pathway. The -$\log_{10}$ (FDR) value is used to denote the significance of the results; as the –$\log_{10}$ (FDR) value increases, the FDR value

among the FAs. These variations highlight the distinct pharmacokinetic profiles that may influence their therapeutic potential and applications.

Among the odd-chain FAs, pristanic acid exhibits high GI absorption and non-permeability through the BBB, distinguishing it from other odd-chain FAs that also demonstrate high GI absorption but are capable of crossing the BBB (Suppl. Table 2). This distinction is further explained by the BOILED-egg plot, where pristanic acid is exclusively positioned within the white ellipse (representing compounds with high GI absorption and no BBB permeability), and while all other FAs are located in the yellow yolk, indicating their ability to traverse the BBB (Suppl. Fig. 2A).

Among the even-chain saturated FAs, stearic acid is distinguished by its high GI absorption coupled with an inability to permeate the BBB (Suppl. Table 2). Similar to odd-chain FAs, this distinction is clearly illustrated in the boiled egg plot, where stearic acid is distinctively positioned within the white ellipse, while all other FAs are found in the yellow yolk, highlighting their potential to cross the BBB (Suppl. Fig. 2B).

In the analysis of even-chain unsaturated FAs, although arachidonic acid and α-linolenic acid target a high number of genes in inflammatory bowel diseases (IBD), they exhibit differing pharmacokinetic profiles. Arachidonic acid demonstrates low GI absorption, whereas α-linolenic acid is capable of crossing the BBB. Consequently, linoleic, oleic, vaccenic, elaidic, petroselinic, 12-octadecenoic, eicosapentaenoic, bosseopentaenoic, eicosatrienoic and eicosatetraenoic acids emerge as potential FAs with desirable features for therapeutic applications (Suppl. Table 2). The BOILED-egg plot (Suppl. Fig. 2C) further supports this by indicating that other even-chain unsaturated FAs are also positioned within the white ellipse, suggesting their suitability for drug development compared to other odd-chain and even-chain saturated FAs.

Molecular docking

To further validate the interactions between the identified enriched proteins and the potential drug candidates, molecular docking studies were performed. The target proteins were categorized into pro-inflammatory cytokines (IL-6, IL-17, and TNF-α) and regulatory housekeeping proteins (PI3K, AKT, and HIF-1). The binding affinity and detailed set of interacting residues of these proteins with their corresponding ligands are provided in (Table 3). The pro-inflammatory cytokines IL-6, IL-17, and TNF-α exhibited binding affinities in the range of -2.9 to -7.1 kcal/mol. Among these, IL-6 exhibited the least binding affinity toward the fatty acids, in the range of -2.9 to -4 kcal/mol, with the common interacting residues Arg52, Leu112, Phe153, Lys156, and Lys157 (Suppl. Fig. 3 and Table 3).

For IL-17, the binding affinities were relatively stronger, ranging from -6.7 to -7.1 kcal/mol, with key interacting residues Tyr62, Pro63, Gln94, Ile96, Leu97, Leu99, and Leu112 (Suppl. Fig. 4 and Table 3). In the case of TNF-α, binding affinities ranged from -4.5 to -5.9 kcal/mol, with common interacting residues Leu57, Tyr59, Tyr119, and Tyr151 (Suppl. Fig. 5 and Table 3). Overall, IL-17 demonstrated the best binding affinity compared to IL-6 and TNF-α.

In the case of PI3K-α, AKT, and HIF-1, these proteins have exhibited binding affinities in the range of -4.7 to -5.7 kcal/mol. Among these, PI3K-α showed better binding affinities, ranging from -5.1 to -5.7 kcal/mol, with common interacting residues

Table 3 — Binding affinities and interacting residues of fatty acids with selected proteins

Name of proteins and their PDB IDs	Fatty acids	Affinity values (kcal/mol)	Interacting amino acid residues
Interleukin-6 (8D82)	Pristanic acid	-3.4	Ser50, Asp54, and Arg210
	Stearic acid	-2.9	Arg52, Leu112, Phe153, Lys156, and Lys157
	Oleic acid	-4.0	Arg52, Leu112, Lys156, Lys157, and Met212
	Eicosapentaenoic acid	-4.0	Arg52, Phe153, Lys156, and Lys157
Interleukin-17 (7AMA)	Pristanic acid	-6.7	Tyr62, Pro63, Gln94, Ile96, Leu97, and Leu99
	Stearic acid	-6.1	Pro63, Gln94, Ile96, Leu97, Leu99, and Leu112
	Oleic acid	-6.1	Pro63, Gln94, Ile96, Leu97, Leu99, and Leu112
	Eicosapentaenoic acid	-7.1	Tyr62, Pro63, Ile66, Ile96, Leu97, Val98, Leu99, and Val117
Tumor necrosis factor-α (2AZ5)	Pristanic acid	-5.6	Leu57, Tyr59, Tyr119, and Tyr151
	Stearic acid	-4.5	Leu57, Tyr59, Tyr119, and Tyr151
	Oleic acid	-5.1	Leu57, Tyr59, Tyr119, Tyr151, and Ile155
	Eicosapentaenoic acid	-5.9	Tyr59, Tyr119, and Tyr151
Phosphoinositide 3-kinase-α (8EXU)	Pristanic acid	-5.7	Ser774, Ile848, Tyr836, Val850, Met922, and Ile932
	Stearic acid	-5.1	Trp780, Ile800, Lys802, Ile848, Val850, Val851, Met922, and Ile932
	Oleic acid	-5.1	Trp780, Ile800, Lys802, Tyr836, Ile848, Val850, and Met922
	Eicosapentaenoic acid	-5.6	Pro778, Trp780, Ile800, Lys802, Tyr836, Ile848, Val850, Val851, Thr856, and Met922
Protein Kinase B (AKT-1) (3CQW)	Pristanic acid	-5.6	Leu156, Val164, Ala177, Met227, Tyr229, Ala230, Glu234, Glu278, Met281, and Phe438
	Stearic acid	-4.9	Leu156, Val164, Ala177, Met227, Ala230, Met281, Thr291, Asp292, and Phe438
	Oleic acid	-4.9	Leu156, Val164, Met227, Ala230, Met281, and Phe438
	Eicosapentaenoic acid	-5.3	Leu156, Val164, Ala177, Ala230, Met281, Asp292, and Phe438
Hypoxia-inducible factor 1 (8II0)	Pristanic acid	-5.5	Tyr102, Leu186, Thr196, and Trp296
	Stearic acid	-4.9	Tyr102, Ser184, Leu186, Leu188, His199, and Ile281
	Oleic acid	-4.7	Tyr93, Tyr102, Leu186, Leu188, His199, Phe207, Ile281, and Trp296.
	Eicosapentaenoic acid	-5.7	Tyr93, Tyr102, Gln147, Leu186, Leu188, His199, His279, and Trp296.

Trp780, Ile800, Lys802, Ile848, Val850, Val851, Met922, and Ile932 (Suppl. Fig. 6 and Table 3). For AKT, the binding affinities were in the range of -4.9 to -5.6 kcal/mol, with key interacting residues Leu156, Val164, Ala177, Met227, Ala230, Met281, Asp292, and Phe438 (Suppl. Fig. 7 and Table 3). In the case of HIF-1, the binding affinities also ranged from -4.7 to -5.7 kcal/mol, with key interacting residues Tyr93, Tyr102, Leu186, Leu188, His199, Ile281, and Trp296 (Suppl. Fig. 8 and Table 3). Overall, PI3K-α, AKT, and HIF-1 exhibit average binding affinities of approximately -5 kcal/mol.

Discussion

Inflammatory bowel diseases (IBD), including CD and UC, are chronic inflammatory conditions of the gastrointestinal tract that cause significant health challenges[32]. Conventional pharmacotherapy for IBD often lacks effectiveness, leading to discontinuation due to intolerance or side effects. This highlights the need for the identification of new drug candidates such as small molecules using computational drug discovery approaches[33].

This study was designed to screen potent drug targets of odd- and even-chain FAs against IBD. Notably, this study identified 98 odd-chain and 166 even-chain targets against IBD. The majority of these gene targets encompass enzymes such as proteases, kinases, phosphatases, *etc*. Additional targets include transporters (electrochemical and primary active transporters), epigenetic regulators (HDAC erasers, epigenetic readers and writers), proteins, transcription

factors, *etc*. The comparison among different FA categories revealed that even-chain unsaturated FAs show greater potential in targeting a higher number of genes associated with IBD. Especially, even-chain unsaturated FAs such as arachidonic acid, alpha-linolenic acid, linoleic acid, pinolenic acid, oleic acid, *etc.,* target more IBD-associated genes. The construction of a network linking fatty acids, gene targets, and disease unveiled intricate relationships that could be leveraged for novel drug development.

SWISS-ADME analysis identified the most promising FAs for IBD treatment, distinguished by high GI absorption and limited ability to cross the BBB. Among the findings, pristanic acid was identified as the most favourable odd-chain FA, while stearic acid was recognized as the best even-chain saturated FA. Among even-chain unsaturated fatty acids, linoleic, oleic, vaccenic, elaidic, petroselinic, 12-octadecenoic, eicosapentaenoic, bosseopentaenoic, eicosatrienoic, and eicosatetraenoic acids were identified as the most suitable candidates.

Previous studies have shown that omega-3 and omega-9 FAs possess anti-inflammatory potential and can inhibit pro-inflammatory pathways[34] while omega-6 FAs have been found to promote inflammation[11]. Based on this, there is some controversy regarding the most suitable drug candidates in even-chain unsaturated FAs. According to the analysis, linoleic and eicosatetraenoic acids demonstrated desirable pharmacokinetic properties (ADME), suggesting their potential for treating inflammatory bowel disease (IBD). However, since they are omega-6 fatty acids, their use as drug candidates for IBD may be less desirable due to the pro-inflammatory potential often associated with omega-6 FAs. In contrast, omega-3 fatty acids—such as eicosapentaenoic acid, bosseopentaenoic acid, and eicosatrienoic acid—as well as omega-9 fatty acids, including oleic acid and its isomers (vaccenic acid, elaidic acid, petroselinic acid, and 12-octadecenoic acid), may be considered more promising for the treatment of IBD.

GO functional enrichment analysis was performed to enhance the understanding of biological processes, cellular components, and molecular functions associated with gene targets. This analysis highlighted the key pathways commonly involved in treating IBD are TNF, IL-17, PI3K-Akt, and HIF-1 signaling pathways.The development of IBD is associated with the production of pro-inflammatory cytokines, such as IL-6, IL-17, and Tumor necrosis factor-α (TNF-α)[35]. TNF-α plays a crucial role in mediating intestinal inflammatory processes and is one of the primary cytokines involved in the pathogenesis of IBD[36]. Hypoxia-inducing factor (HIF) plays a crucial role in intestinal hypoxia, with HIF-1 and HIF-2 pathways significantly affecting inflammatory disease progression. Numerous studies suggest that HIF-1 holds therapeutic potential and can be targeted to influence signaling pathways related to hypoxic intestinal disorders[37]. Targeting glycolysis, driven by PI3K signaling to AKT, shows promising therapeutic approach for treating chronic IBD[38]. High levels of IL-6, IL-17, TNF-α, and IL-8 are common in inflammatory conditions like IBD[8,35]. It is interesting to note that alpha-linolenic acid (ALA)-rich diets have been reported to affect toll-like receptor signaling (TLR) and decrease IL-6 and TNF-α, while increasing the anti-inflammatory IL-10[8]. Furthermore, the identified targets were validated by docking with potential FAs.

Computational methods play a crucial role in IBD genetic research, offering numerous gene enrichment and pathway analysis tools. While both experimental and computational approaches are utilized to identify drug targets, experimental methods often incur high costs and require extensive scientific labour to explore chemical compound spaces fully. Consequently, researchers and pharmaceutical companies increasingly rely on computational methods for initial investigations before resorting to experimental validation[33]. Thus, computational methods hold a promising approach for the recognition of drug targets of IBD where in the future this could be implied in drug development. The results of this preliminary study provide rationale to investigate the *in vitro* and *in vivo* efficacy of even-chain unsaturated FAs such as arachidonic acid, alpha-linolenic acid, linoleic acid, pinolenic acid, oleic acid alone or in combination for IBD treatment.

Limitations of the investigation

In silico models often predict drug targets based on computational algorithms, but experimental validation (*in vitro* or *in vivo* testing) may be limited, resulting in uncertainty about the functional relevance of predicted targets[39] . While computational approaches can identify potential targets, they often lack detailed mechanistic explanations about how odd- and even-chain FAs interact with these targets to regulate inflammation. It is also possible that both odd- and even-chain FAs might not cover the full range of IBD-related metabolic interactions.

Conclusion

FAs are small molecules that can be promising drugs for the identified targets of IBD. Our *in silico* findings indicate that pristanic acid, stearic acid, eicosapentaenoic acid, eicosatrienoic acid, oleic acid, and its isomers play crucial roles in modulating drug targets within signaling pathways associated with IBD.

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Conflict of interest

All authors declare no conflict of interest.

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