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ORIGINAL RESEARCH

Study on the Therapeutic Effect and Mechanism of Gu Pi Sheng Ji Hua Zhuo Jie Du Decoction in Inducing Remission of Crohn's Disease

Jiaming He, Zhibin Huang, Jie Zheng, Xu Deng, Minghui Wu, Gang Liu, Shuilin Chen, Yan Chen

Department of Gastroenterology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, 510120, People's Republic of China

Correspondence: Yan Chen, Email YanChenTCM@outlook.com

Objective: To investigate the therapeutic effects of Gupishengji-Huazhuojiedu Decoction (GHD) in inducing remission in Crohn's disease (CD) and to explore its potential underlying mechanisms.

Methods: A two-stage exploratory study was conducted. Stage one included a retrospective analysis (17 GHD, 27 infliximab [IFX]) and a prospective single-arm trial (n=8), assessing clinical remission (CDAI <150), endoscopic response (≥50% SES-CD reduction), and inflammatory biomarkers (CRP, fecal calprotectin). Stage two applied network pharmacology, machine learning (random forest, LASSO, XGBoost), and immunohistochemistry to explore GHD mechanisms.

Results: In the retrospective analysis, the GHD group exhibited a higher clinical remission rate than the IFX group (88.2% vs 51.9%, p=0.01), with trends toward higher clinical response (88.2% vs 63.0%, p=0.07) and endoscopic remission rates (58.8% vs 37.0%, p=0.16). In the prospective study, 87.5% (7/8) of patients achieved both clinical remission and endoscopic response after 12 weeks of treatment. CDAI, SES-CD, CRP, and fecal calprotectin levels were all significantly reduced compared with baseline ($p<0.05$). Bioinformatics analysis identified 13 key functional components (KFCGs) from GHD, and intersection of their 369 targets with CD differentially expressed genes yielded 36 candidate genes. Machine learning further prioritized six feature genes (IDO1, PRKG2, TGM2, ALDH1A2, ACPP, CASP1). Immune infiltration analysis revealed differences in immune cell populations between CD patients and healthy controls. Immunofluorescence experiments confirmed that GHD treatment significantly reduced the expression of CASP1, IDO1, CD11c, CD83, KLRG1, and CD45RO in intestinal mucosal tissue ($p<0.05$).

Conclusion: This study suggests that GHD may induce remission in CD through a multi-component, multi-target mechanism, particularly by modulating pathways related to CASP1 and IDO1, thereby improving clinical symptoms and endoscopic findings. The underlying mechanism may involve regulation of the intestinal immune-inflammatory microenvironment. GHD holds promise as a potential traditional Chinese medicine strategy for treating CD, but further validation in larger randomized controlled trials is warranted.

Keywords: Crohn's disease, chronic inflammatory disease, gastrointestinal tract, pharmacological therapies, traditional Chinese medicine

Introduction

Crohn's disease (CD) is a chronic inflammatory condition that can affect the entire gastrointestinal tract.[1] It is a lifelong and currently incurable disease, characterized by a natural history of gradual progression of intestinal damage and potential disability, following repeated cycles of active and quiescent phases.[1] Notably, over 80% of individuals diagnosed with CD are under the age of 40.[2] The global incidence of CD has risen significantly over the past few decades, particularly in developing regions of Asia and South America, making it a worldwide health concern.[3,4]

The exact pathophysiological mechanisms underlying CD remain incompletely understood, but it is widely accepted that the disease results from a complex interplay of genetic susceptibility, environmental factors, immune dysregulation, and alterations in gut microbiota.[5] Currently, there is no definitive cure for CD. Pharmacological therapy remains the primary

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treatment approach for most patients, aimed at controlling symptoms, reducing intestinal inflammation, and maintaining a satisfactory quality of life. Biologics targeting tumor necrosis factor-α (TNF-α), such as infliximab (IFX), have revolutionized CD management. Infliximab represents the gold standard biological therapy for CD, with extensive clinical evidence supporting its efficacy in inducing and maintaining clinical remission.[6,7] It has demonstrated superior efficacy and safety compared to conventional therapies, particularly in cases of refractory disease, severe active episodes, and for patients with contraindications to other medications.[8]

Concurrently, Traditional Chinese Medicine (TCM) is playing an increasingly significant role in the management of inflammatory bowel disease (IBD). Numerous Chinese herbal formulations have been confirmed to alleviate intestinal inflammation, modulate immune responses, and regulate gut microbiota.[9] In TCM theory, the pathogenesis of CD is primarily attributed to spleen deficiency leading to impaired transport function, coupled with internal accumulation of dampness-heat and stasis-toxin. The combination of these pathogenic factors ultimately induces the disease. Through continuous inheritance and development by modern TCM practitioners, the current treatment principle for CD focuses on tonifying Qi, strengthening the spleen, removing dampness, resolving blood stasis, and detoxifying.

Gupishengji-Huazhuojiedu decoction (GHD) is a patented TCM formulation developed specifically for treating CD based on these principles. The formula comprises 15 herbs: Atractylodes macrocephala (Bai Zhu), Angelica sinensis (Dang Gui), Poria cocos (Fu Ling), Pueraria lobata (Ge Gen), Gallus gallus domesticus (Ji Nei Jin), Bombyx batryticatus (Jiang Can), Forsythia suspensa (Lian Qiao), Paeonia suffruticosa (Mu Dan Pi), Callerya speciosa (Niu Da Li), Zingiber officinale (Sheng Jiang), Agrimonia pilosa (Xian He Cao), Alpinia oxyphylla (Yi Zhi), Coix lacryma-jobi (Yi Yi Ren), Artemisia scoparia (Yin Chen), and Fritillaria thunbergii (Zhe Bei Mu). Based on their known phytochemical profiles, these herbs are rich in bioactive compounds such as flavonoids, saponins, and various glycosides, which are believed to contribute to anti-inflammatory, immunomodulatory, and intestinal barrier-protective effects.[10–12] The therapeutic strategy of GHD—"fortifying the spleen and boosting vitality, resolving dampness and detoxifying"—is specifically tailored to address the core pathogenesis patterns observed in CD patients. Network pharmacology analyses of analogous TCM formulations suggest multi-target mechanisms, potentially involving the modulation of the PI3K-AKT and MAPK signaling pathways, and the promotion of intestinal epithelial repair.[13] A distinguishing feature of GHD is its exclusive development for CD management, contrasting with the broader TCM approach of "treating different diseases with the same formula". Preliminary clinical observations are promising, indicating that GHD treatment can achieve endoscopic response rates of 75% in CD patients, with mucosal healing observed in 25% of cases after treatment—results comparable to those reported for biologics like infliximab.[14]

High-quality evidence increasingly supports the efficacy of TCM in IBD management. Recent clinical studies and meta-analyses have demonstrated that TCM formulations can induce and maintain remission in CD patients, with some showing comparable effectiveness to conventional biologics while exhibiting favorable safety profiles.[15,16] For instance, specific TCM protocols have achieved clinical remission rates of 63.6%-84.9% in patients with drug-refractory CD,[17] outcomes comparable to newer biologic agents.[18] The rationale for comparing GHD against infliximab in this study is robust. Infliximab, as the gold standard biologic therapy for CD with extensive clinical validation, provides a rigorous benchmark for evaluating GHD's therapeutic potential.[19] Furthermore, its well-characterized mechanism of action offers a solid foundation for comparative mechanistic investigations.[20]

Therefore, this study was designed to first examine the effectiveness of GHD in inducing remission in CD patients by assessing clinical symptoms, inflammatory biomarkers, and endoscopic findings, using IFX as an active comparator. Subsequently, the potential mechanism of action of GHD against CD was explored using an integrated strategy combining network pharmacology, machine learning, and experimental validation. The specific aims of this study were: (1) to compare the effectiveness and safety of GHD versus IFX in inducing clinical remission in patients with CD; (2) to evaluate the efficacy of GHD as a single-arm intervention in achieving mucosal healing and biomarker response; and (3) to generate novel mechanistic insights into GHD's mode of action through comprehensive network pharmacology analysis and subsequent experimental validation.

Materials and Methods

Study Design

This investigation employed a dual-phase exploratory framework. The initial phase comprised both retrospective observational analysis and a prospective single-arm intervention trial conducted at The Second Affiliated Hospital (Gastroenterology Division) of Guangzhou University of Chinese Medicine, spanning from January 2020 to May 2024. Following the acquisition of clinically significant outcomes from these human studies, the subsequent phase adopted computational biology approaches. Specifically, we integrated bioinformatics tools with advanced machine learning algorithms to systematically explore the mechanistic basis of Gu Pi Sheng Ji Hua Zhuo Jie Du Decoction (GHD) efficacy, as illustrated in Figure 1.

The research protocol underwent stringent ethical review and received formal approval from institutional review boards at all collaborating centers. Prior to enrollment, comprehensive written informed consent documents were voluntarily executed by all study participants following detailed protocol explanations.

Retrospective Study

Patients

We retrospectively analyzed patients diagnosed with mild-to-severe CD who had been receiving GHD or IFX between January 2020 to March 2023.

Exclusion criteria comprised: (1) insufficient longitudinal clinical documentation, and (2) concurrent gastrointestinal pathologies requiring surgical management or presenting clinical features incompatible with standardized CDAI-based therapeutic response assessment. (3) incomplete medical records for key covariates needed for propensity score matching, and (4) pregnancy or lactation at initiation of therapy.

This investigation received full ethical clearance (Approval ID: BF2022-146-01) from the Institutional Review Board of Guangzhou University of Chinese Medicine Second Affiliated Hospital. Prior to enrollment, written informed consent documentation was voluntarily executed by all participants following comprehensive protocol disclosure.

Treatment

General management was the same in both groups: correction of water and electrolyte imbalances; transfusion of blood components in cases of anemia or hypoproteinemia; provision of enteral nutritional support according to the patient's nutritional status; and avoidance of allergenic foods.

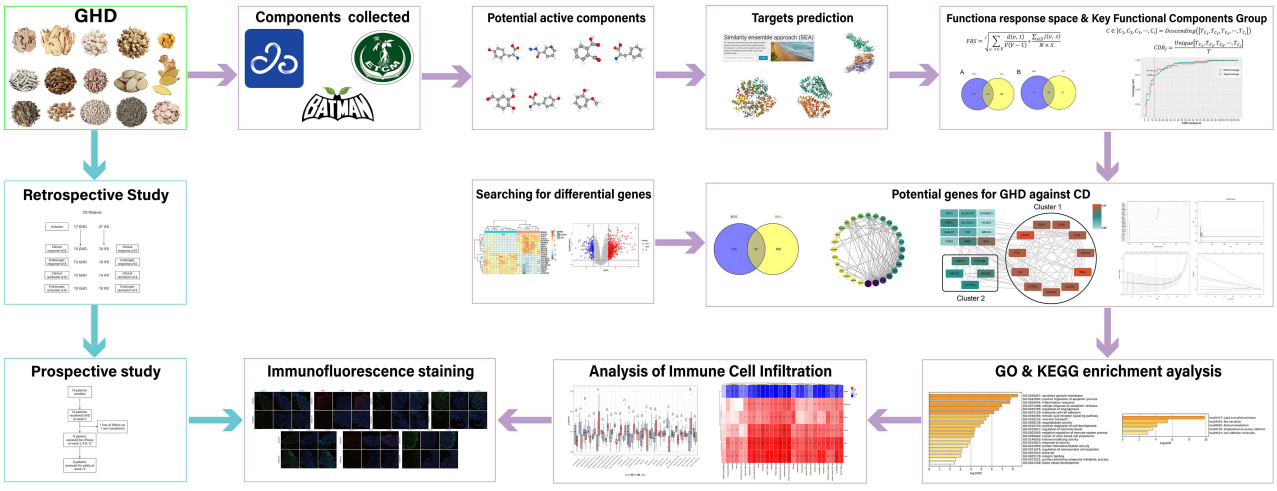

Figure 1 Study design and workflow.

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Gupishengji Huazhuojiedu decoction (GHD) Group: Patients received Gupishengji Huazhuojiedu decoction (GHD). The base prescription consisted of Atractylodes macrocephala 15 g, Angelica sinensis 5 g, Poria cocos 30 g, Pueraria lobata 30 g, Endothelium corneum Gigeriae galli 20 g, Bombyx batryticatus 5 g, Forsythia suspensa 5 g, Cortex moutan 5 g, Callerya speciosa 60 g, fresh ginger 10 g, Agrimonia pilosa 45 g, Alpinia oxyphylla 20 g, Coix lacryma-jobi 15 g, Artemisia capillaris 10 g, and Fritillaria thunbergii 30 g. Syndrome differentiation was independently conducted by two chief physicians of traditional Chinese medicine, and minor modifications to the prescription were permitted according to individual presentations, provided that the total number of herbs remained unchanged and single-herb dosage adjustments did not exceed 20%.

Decoction method: the herbs were soaked in 2000 mL water for 30 minutes, then decocted for 110 minutes using an automatic decoction machine. The extract was concentrated to 400 mL and packaged into two 200 mL sachets. Patients took one sachet twice daily, warmed before administration. The treatment course lasted continuously for 16 weeks, during which the use of other immunomodulators was prohibited.

Botanical authentication: All plant materials used in the decoction were authenticated by certified botanists. Voucher specimens for the herbs were deposited in public herbaria as follows: Angelica sinensis (GZTM0102624), Forsythia suspensa (GZTM0099062), Paeonia suffruticosa (0010330), Millettia speciosa (0019639), Pueraria lobata (0019744), Atractylodes macrocephala (GZTM0030894), and Poria cocos (GZTM0099795) were identified by botanists including Prof. Xun Zhang and deposited in the Herbarium of the School of Pharmacy, Guiyang University of Chinese Medicine (GZTM). Artemisia capillaris (SXTCM0002471) and Agrimonia pilosa (SXTCM0002996) were deposited in the Herbarium of Shanxi University of Chinese Medicine (SXTCM). Zingiber officinale (HEAC0012852) was deposited in the Herbarium of Henan Agricultural College (HEAC). Fritillaria thunbergii (KUN1481961) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

IFX group: Patients received infliximab (Cilag AG, Registration No. S20171001). The regimen was: induction therapy at weeks 0, 2, and 6 with intravenous infusion of 5 mg/kg, followed by maintenance therapy with the same dosage once every 8 weeks. The total treatment duration was 14 weeks (3 induction doses + 1 maintenance dose). During treatment, concomitant use of stable-dose 5-aminosalicylates was permitted, while other biologics were prohibited.

During the course of treatment, all other non-essential medications were discontinued. A light diet was advised, with avoidance of greasy foods.

Prospective Study

Patients Cohort

The prospective cohort enrolled adults (18–60 years) with confirmed Crohn's disease (CD) duration ≥6 months and baseline Crohn's Disease Activity Index (CDAI) scores 220–400.

Inclusion permitted stable-dose 5-aminosalicylate regimens maintained ≥4 weeks pre-screening. Exclusion criteria comprised: (1) immunomodulator/biologic exposure (cyclosporine, methotrexate, biologics, investigational agents) within 90 days pre-enrollment; (2) parenteral corticosteroid/corticotropin administration ≤4 weeks pre-baseline; (3) structural complications (symptomatic stenosis, ileal strictures); (4) surgical history (proctocolectomy, total colectomy, stoma creation); (5) active surgical gastrointestinal pathologies; (6) malignancy history; (7) pregnancy/lactation status; (8) neuropsychiatric comorbidities. The sample size for the prospective single-arm trial was determined based on historical response rates to biological therapies in CD patients. Assuming a clinical remission rate of 70% for standard biologics with a margin of non-inferiority of 20%, a sample size of 30 patients would provide 80% power at a one-sided alpha of 0.05. However, due to challenges in patient recruitment during the COVID-19 pandemic and the specific eligibility criteria, we ultimately enrolled 10 patients in the prospective cohort. We acknowledge that this small sample size represents a limitation of our study and may affect the generalizability of our findings.

This prospective study was not registered with the Chinese Clinical Trial Registry prior to participant enrollment.

Therapeutic Intervention

The Gupishengji Huazhuojiedu decoction (GHD) formulation and administration protocol mirrored the specifications detailed in Treatment of the retrospective investigation. The duration of the treatment programme was 12 weeks.

Study Protocol

Eligibility confirmation was completed 7 days prior to intervention initiation. All enrolled participants received standardized, once-daily GHD therapy over a 12-week observation period. Multiparametric assessments were conducted at protocol-defined intervals, including clinical visits at weeks 0 (baseline), 2, 4, and 12, during which adverse events were monitored through structured patient interviews, and disease activity was evaluated via CDAI scoring and routine laboratory profiling. Biomarker sampling included C-reactive protein (CRP) measurements at weeks 0, 4, and 12, and fecal calprotectin levels at weeks 0 and 12. Multidimensional evaluation encompassed endoscopic severity assessed by the Simple Endoscopic Score for Crohn's Disease (SES-CD), quality of life using the Inflammatory Bowel Disease Questionnaire (IBDQ), and anthropometric measurements such as body mass index (BMI). Safety analyses included all enrolled participants, with hepatic and renal function panels assessed at baseline and at the end of the study (week 12).

The primary outcome was defined as achievement of clinical remission, indicated by a CDAI score <150 at week 12. Secondary outcomes encompassed endoscopic activity, with endoscopy response defined as a decrease in SES-CD score of ≥50% from baseline and endoscopic remission defined as a SES-CD score ≤2; dynamic changes in C-reactive protein (CRP) levels between baseline and weeks 4 and 12; and terminal-phase improvements at week 12 compared to baseline in key biomarkers and clinical parameters, including fecal calprotectin levels, IBDQ scores, SES-CD scores, and BMI trajectory.

Bioinformatics Investigation

Phytochemical Profiling of GHD

Constituent identification was conducted through multi-database integration, leveraging: TCMSP: Traditional Chinese Medicine Systems Pharmacology Database (v2.3);[21] ETCM: Encyclopedia of TCM (2022 edition)[22] BATMAN: Bioinformatics Analysis Tool for Molecular Mechanisms of TCM (update 2021).[23] The integration of computational approaches (network pharmacology and machine learning) with experimental validation follows an established framework for studying complex herbal formulations. This methodology allows for systematic identification of bioactive compounds, prediction of potential targets, and prioritization of mechanisms for experimental verification, as detailed in subsequent methodology sections.

Bioactive Compound Screening

Pharmacokinetically favorable molecules were selected via SwissADME,[24] using multi-parameter filters: (1) Molecular descriptors: MW<500 Da; RotB≤10; (2) Solubility metrics: AliLogS>-2; Silicos-IT LogSw>-2; (3) Permeability criteria: H-bond donors<5; acceptors<10; (4) Absorption potential: High GI absorption classification.

Target Prediction Framework

Ligand-target interactions were modeled using the Similarity Ensemble Approach (SEA) platform (https://sea.bkslab.org), employing 3D structural similarity thresholds >0.7 for pharmacophore matching.

Network-Driven Component Optimization

A component-target bipartite network was constructed in Cytoscape (v3.9.1), with topological analysis executed via NetworkAnalyzer. Functional response space (FRS) modeling incorporated:

$$FRS = \sqrt[2]{\sum_{v,t \in V} \frac{d(v,t)}{V(V-1)} * \frac{\sum_{s \in S} j(v,s)}{N \times S}}$$

V represent the set of network G. Nodes v, t and s belong to set V. d(v,t) represent the shortest path between node v and node t. S represent the set of nodes s that share nodes with node v. j(v,s) represent the number of nodes shared between node v and node s. N represent the set of adjacent nodes of node v. If there is a direct connection between node v and node s, then j(v,s)+ 1.

To establish the Key Functional Component Group (KFCG) critical for elucidating GHD's pharmacological actions in Crohn's disease management, we implemented a knapsack optimization-driven computational strategy used to select KFCG:

$$C \in [C_1, C_2, C_3, \cdots, C_i] = Descending\left([T_{C_1}, T_{C_2}, T_{C_3}, \cdots, T_{C_i}]\right)$$

$$CDR_1 = \frac{Unique\left[T_{C1}, T_{C2}, T_{C3}, ..., T_{Cj}\right]}{T}$$

Tc represent the target of number *i* FRS component. Set C represent the set of FRS components. CDR is the cumulative contribution rate of component targets to the FRS network.

Transcriptomic Data Curation

Colonic mucosal transcriptomes were retrieved from GEO (GSE59071: 8 CD vs 11 controls) as discovery cohort, supplemented by GSE36807 (13 CD vs 7 controls) for external validation. Raw FASTQ files were processed through our in-house R pipeline (v4.2.1) implementing: (1) Read alignment (STAR v2.7.10b); (2) Count normalization (DESeq2 v1.38.3).

Differential Expression Analysis

Limma's empirical Bayes moderated *t*-test (FDR<0.05, |logFC|>1) identified disease-associated genes. Multivariate visualization employed: Volcano plots (EnhancedVolcano v1.16.0); Hierarchical clustering heatmaps (pheatmap v1.0.12).

Mechanistic Target Identification

Candidate genes were identified through the intersection of KFCG targets and Crohn's disease (CD) differentially expressed genes (DEGs), followed by comprehensive analysis. Protein–protein interaction (PPI) networks were constructed using STRING-DB with a confidence score >0.7, and clustered using the MCODE algorithm (k-core = 4). For target prioritization, machine learning approaches were employed, including LASSO regression (glmnet v4.1–4), Random Forest (randomForest v4.7–1.1), and validation via XGBoost (xgboost v1.7.3). Model performance was evaluated using area under the receiver operating characteristic curve (AUROC; pROC v1.18.0) and area under the precision-recall curve (AUPRC; PRROC v1.3.1).

Functional Enrichment Profiling

Functional enrichment of candidate genes was performed using Metascape. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted with the following parameters: minimum overlap ≥3, Benjamini-Hochberg adjusted p-value <0.01, and enrichment factor >1.5, with redundant terms removed. Interconnectivity among enriched pathways was visualized using Circos plots.

Immunological Landscape Characterization

ssGSEA (GSVA v1.46.0) quantified 28 immune cell infiltrates using LM22 gene signatures. Spearman correlation (Hmisc v4.8–0) linked core genes to immunocyte abundance.

Experimental Validation

Materials

In the prospective study, a total of 14 cases, 7 pre- and 7 post-treatment mucosal biopsies were taken from 7 patients, including 8 cases of terminal ileum, 2 cases of ileocecal valve, 2 cases of rectum and 2 cases of descending colon.

Immunofluorescence Staining

Paraffin-embedded colonic/ileal sections underwent antigen retrieval in EDTA buffer (pH 8.0, Powerfulbio B0035) at 95°C for 20min. Following three sequential 5-minute PBS rinses (Powerfulbio B0040), non-specific binding was blocked with 3% BSA (Solarbio A8010) under ambient conditions (25°C, 30min). Primary antibody incubation proceeded at 4°C for 16h using: Immune markers: CASP1 (PTG 16832-1-AP), IDO1 (PTG 13226-1-AP), CD45RO (PTG 60303-1-Ig), Activation markers: KLRG1 (PTG 14614-1-AP), CD11c (PTG 60185-1-Ig), Specialized antigens: CD83 (Immunoway YT5273), CD66b (Affinity DF13444). Alexa Fluor-conjugated secondary antibodies (Abcam ab150077/ab150113) were applied at 1:500 dilution (50min, RT), followed by nuclear counterstaining with DAPI (Powerfulbio B0025). Fluorescent signal acquisition utilized a Nikon 80i epifluorescence system with NIS-Elements AR 5.21 software. Quantitative intensity analysis was performed via Image-Pro Plus 6.0 using standardized thresholding protocols.

Statistical Analysis

Demographic characteristics were summarized as frequency distributions (%) with χ^2-tests evaluating categorical variables. Continuous parametric data (Shapiro–Wilk p>0.05) were expressed as mean±SD and analyzed via Student's two-tailed *t*-test. Non-parametric measures (median[IQR]) underwent Wilcoxon rank-sum testing. Significance thresholds maintained α=0.05 throughout. Analytical workflows were implemented in SPSS 25.0 (descriptive/inferential statistics) and R 4.1.0 (ggplot2 visualization, dplyr data wrangling).

Ethics Approval and Consent to Participate

This investigation received full ethical clearance (Approval ID: BF2022-146-01) from the Institutional Review Board of Guangzhou University of Chinese Medicine Second Affiliated Hospital. The study was conducted in accordance with the principles of the Declaration of Helsinki. Prior to enrollment, written informed consent documentation was voluntarily executed by all participants following comprehensive protocol disclosure.

Results

Retrospective Study

Clinical Characteristics

44 CD patients received GHD (n = 17) or IFX (n = 27). A flow chart of the study is shown in Figure 2. A comparison of the baseline characteristics (Table 1) revealed that the groups were comparable in all aspects except for gender. Nevertheless, current evidence suggests that gender does not affect the response to IFX in CD patients.[25]

Therapeutic Outcomes

The cohort demonstrated full protocol adherence, with all 44 participants (GHD: n=17; IFX: n=27) successfully completing their respective therapeutic courses. Safety monitoring revealed no treatment-related severe adverse events requiring intervention. Temporal analysis showed significant reductions in composite disease activity markers (P<0.05) at comparable timepoints (week 16 for GHD group and week 14 for IFX group) (Figure 3).

Comparative efficacy analysis revealed differential therapeutic profiles: GHD demonstrated statistically superior clinical remission rates vs IFX (88.2% vs 51.9%; p=0.01); Clinical response: 88.2% vs 63.0% (p=0.07); Endoscopic response: 76.5% vs 66.7% (p=0.49); Endoscopic remission: 58.8% vs 37.0% (p=0.16) (Figure 4).

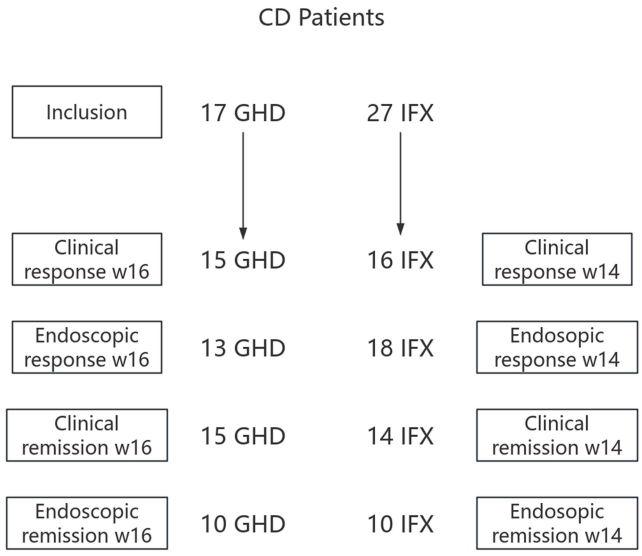

Figure 2 Flow chart of the retrospective study (CD: Crohn's disease; GHD: Gupishengji-Huazhuojiedu Decoction; IFX: infliximab).

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Table 1 Comparison of Baseline Clinical Characteristics Between the GHD and IFX Groups

	GHD Group n=17	IFX Group n=27	P Value
Male sex, n (%)	7 (41.2)	27 (100)	0
Age (years), mean±SD	32.41±12.04	29.93±11.81	0.42
Age at diagnosis, years, n (%)			
≤16 (A1)	2 (11.8)	1 (3.7)	0.15
17-40 (A2)	12 (70.6)	25 (92.6)	
>40 (A3)	3 (17.6)	1 (3.7)	
Involved site, n (%)			
Terminal ileum (L1)	1 (5.9)	4 (14.8)	0.11
Colonic (L2)	4 (23.5)	1 (3.7)	
Ileocolonic (L3)	12 (70.6)	22 (81.5)	
Disease type, n (%)			
Non-stricturing, non-penetrating (B1)	8 (47.1)	16 (59.3)	0.52
Stricturing (B2)	7 (41.2)	10 (37.00)	
Penetrating (B3)	2 (11.8)	1 (3.7)	
Disease activity, n (%)			
Mild	5 (29.4)	6 (22.2)	0.77
Moderate	11 (64.7)	18 (66.7)	
Severe	1 (5.9)	3 (11.1)	
CDAI, mean±SD	253.1±53.3	256.0±66.7	0.88
SES-CD, mean±SD	10.6±5.6	9.7±4.6	0.53
CRP (mg/liter), mean±SD	25.6±33.4	29.9±27.3	0.19

Prospective Study

Patients

Of 10 patients enrolled, 8 patients completed the study (Table 2).

The patients had moderate CD, a mean score on the CDAI was approximately 270. The mean of SES-CD, C-reactive protein (CRP) levels, Faecal Calprotectin (FC), Score on Inflammatory Bowel Disease Questionnaire (IBD-Q) were 16.38, 23.24mg/L, 1722.55μg/g, and 142.75, respectively.

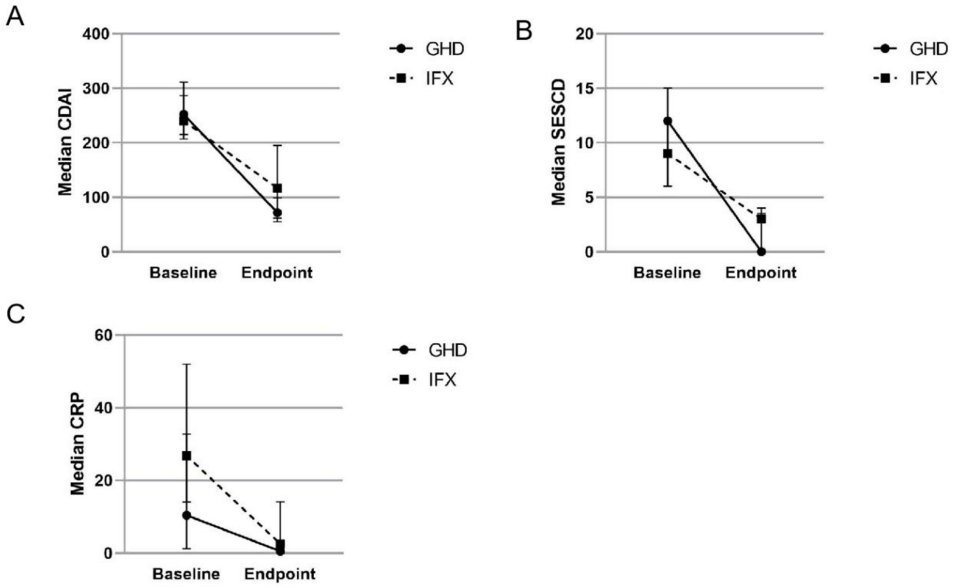

Figure 3 Longitudinal changes in key disease activity metrics. (**A**) Median CDAI; (**B**) Median SES-CD; (**C**) Median CRP; Bars=IQR. GHD (n=17), IFX (n=27).

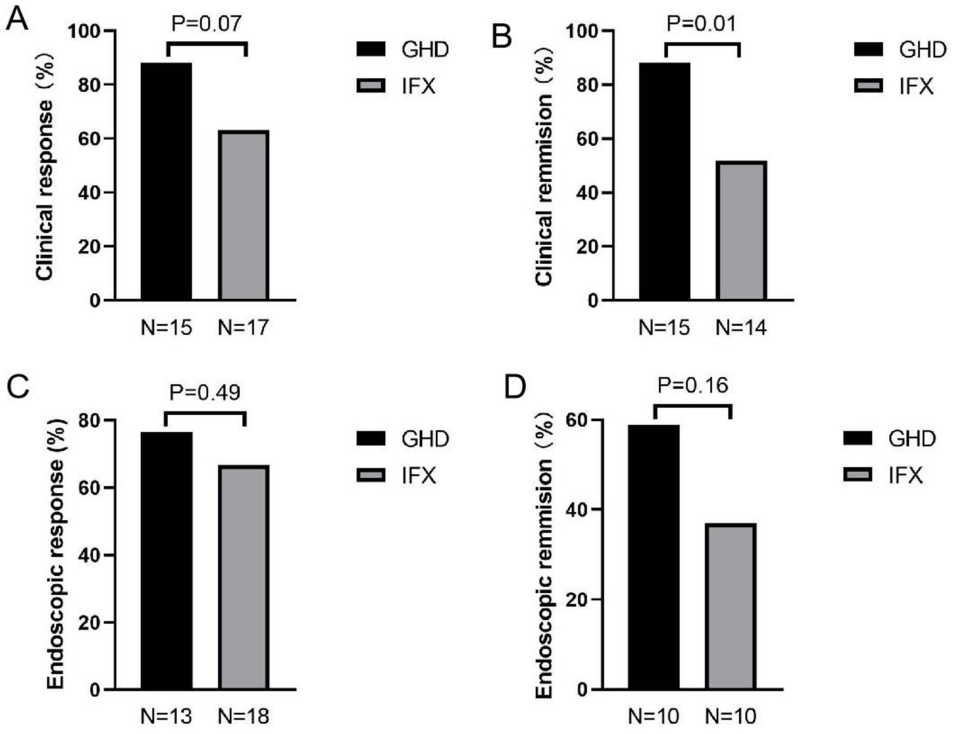

Figure 4 Comparative therapeutic achievement stratification. (**A**) shows the rates of clinical response; (**B**) shows the rates of clinical remission; (**C**) shows the rates of endoscopic response; (**D**) shows the rates of endoscopic remission.

Efficacy

Week 2, 4

At week 2, the reductions of CDAI were observed in all patients, and a continuously decreasing trend continued over the study period.

At week 4, all patients had a clinical response. The mean CDAI was recorded as 70, and CRP levels decreased in 6 patients, with the mean recorded as 5.35 mg/L, and the mean decrease was 17.89 mg/L.

Week 12

At week 12, 7 patients (87.5%) achieved both clinical remission and endoscopic response, among whom 1 patient (12.5%) achieved endoscopic remission, with a mean score on the CDAI and SES-CD was approximately 67, and 5.63,

Table 2 Baseline Demographic and Clinical Characteristics of Enrolled Patients (n = 8)

Number	1	2	3	4	5	6	7	8
Duration of disease-mon	168.00	8.00	91.00	24.00	60.00	72.00	4.00	8.00
Montreal classification	A2L3+L4B2p	A2L3+L4B1p	A2L3B2	A2L3B2	A2L3B2p	A2L2B2p	A2L3B2	A2L3+L4B2p
Sex	M	F	M	M	M	M	M	M
Age-yr	36	27	38	22	32	24	32	24
BMI-kg/m^2	17.60	16.60	19.90	17.90	15.00	16.00	20.80	19.60
CDAI	223.00	221.00	247.60	399.00	266.93	354.40	222.85	224.54
SES-CD	13	17	8	26	21	16	7	23
IBDQ	174.00	179.00	110.00	116.00	138.00	84.00	182.00	159.00
PG-SGA	6	1	11	15	16	14	6	8
CRP-mg/liter	22.90	0.10	9.80	8.30	52.61	60.20	18.30	13.70
FC-μg/g	2706.4	1089.15	1107.92	1230.56	1238.31	2753.89	54.17	3600

Table 3 Longitudinal Changes in Clinical, Inflammatory, and Nutritional Indices from Baseline to week 12

Variable	CDAI	IBDQ	SES-CD	CRP-mg/liter	FC-μg/g	PG-SGA
Base line, mean±SD	270±69	143±37	16±7	23±22	1723±1170	9.63±5.26
2 weeks, mean±SD	95±60					
4 weeks, mean±SD	70±45			5±5		
12 weeks, mean±SD	67±52	189±24	6±4	3±2	292±373	2.88±3.80

respectively. The FC and SES-CD levels of all patients decreased compared to baseline. The mean reduction in FC and SES-CD was 1430.72μg/g, and 10.75, respectively.

87.5% (7 of 8 patients) had improvement in IBDQ and BMI. Malnutrition (defined as PG-SGA>4) was observed in 4 patients, compared to 7 at baseline (Table 3).

In comparison with the baseline, Significant differences (P<0.05) were seen in CDAI, SES-CD, IBDQ and biomarkers of inflammation (CRP and FC).

Adverse Events and Safety

During the observation period, no SAE nor liver, kidney or cardiac (electrocardiograph) function injury were observed.

Bioinformatics Analysis

Phytochemical Profiling and Targetome Mapping

Through comprehensive screening of phytochemical constituents across established repositories (TCMSP/ETCM/BATMAN), we cataloged 2,811 unique molecules from GHD's 15 herbal components. SwissADME pharmacokinetic filtering (MW<500, H-bond donors<5) identified 227 bioactive candidates, subsequently expanded to 149 multi-target ligands via SEA prediction (structure similarity threshold >0.6). Network pharmacology modeling revealed an intricate component-target interactome (1,017 nodes, 3,707 edges), demonstrating polypharmacological characteristics with mean target multiplicity of 24.88 per compound (Figure 5).

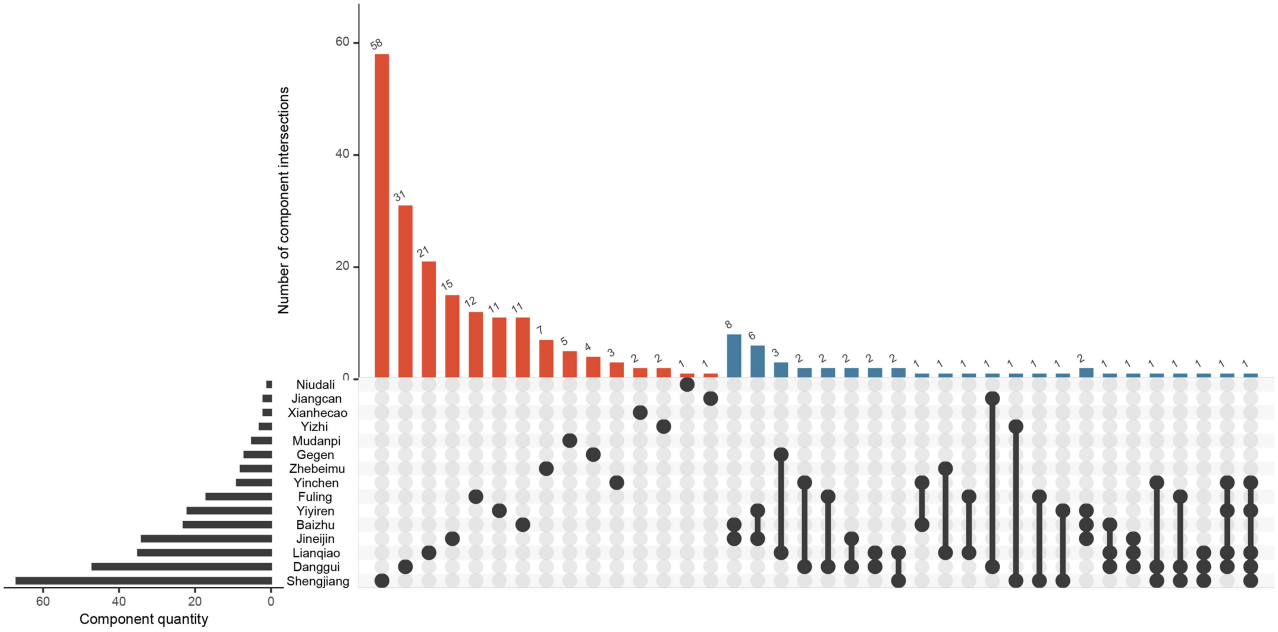

Figure 5 Phytochemical convergence among GHD constituents. Botanical nomenclature: Niudali (Millettia speciosa rhizome), Jiangcan (Bombyx mori larval preparation), Xianhecao (Agrimonia pilosa aerial parts), etc.

Key Functional Components Group Selection and Validation

To better understand the functional mechanisms of GHD, we first constructed a functional response space (FRS) based on the previously established C-T network. This FRS included 125 active components and 498 related targets. Enrichment analyses using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were then carried out. The results showed 115 enriched KEGG pathways and 1370 enriched GO terms. Notably, the FRS accounted for 67.72% of all enriched pathways and 67.48% of all enriched GO terms in the overall GHD dataset (Figure 6A–D), indicating that the FRS accurately reflects the biological functions of GHD.

Furthermore, a backpacking algorithm model was developed to optimize the selection of key functional components (KFCG). According to the cumulative contribution ranking, the top 13 compounds covered 74.2% of all targets and 81.7% of the KEGG pathways. Beyond this point, the rate of increase in coverage began to plateau. Therefore, these 13 compounds along with their 369 associated targets were defined as the KFCG (Figures 7 and 8).

Gene Differential Expression Analysis

To explore gene expression differences between healthy individuals and CD patients, differential gene expression analysis was performed using the Gene Expression Omnibus (GEO) dataset GSE59071 (adjusted $P < 0.05$). A total of 675 differentially expressed genes (DEGs) were identified (Figure 9A and B).

Screening of Potential Target Genes Combine with MCODE and Machine Learning Methods

By intersecting the 675 DEGs with the 369 KFCG-related targets, 36 overlapping genes were identified (Figure 10). These genes represent potential therapeutic targets of GHD in the treatment of CD.

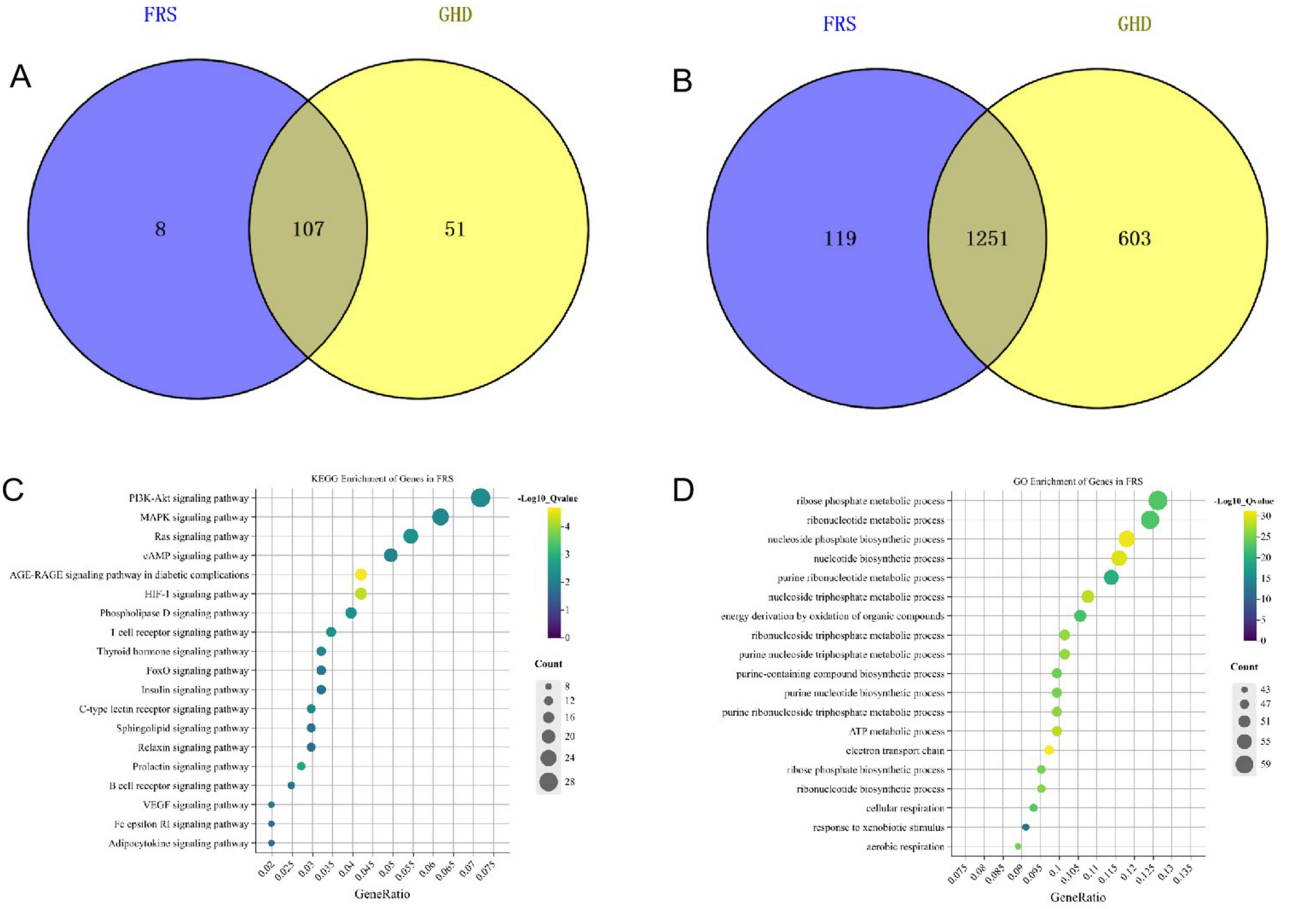

Figure 6 Construction and validation of the functional response space (FRS). (**A**) Comparison of signaling pathway counts between FRS and GHD; (**B**) Comparison of GO term counts between FRS and GHD; (**C**) KEGG enrichment analysis of genes within FRS; (**D**) GO enrichment analysis of genes within FRS.

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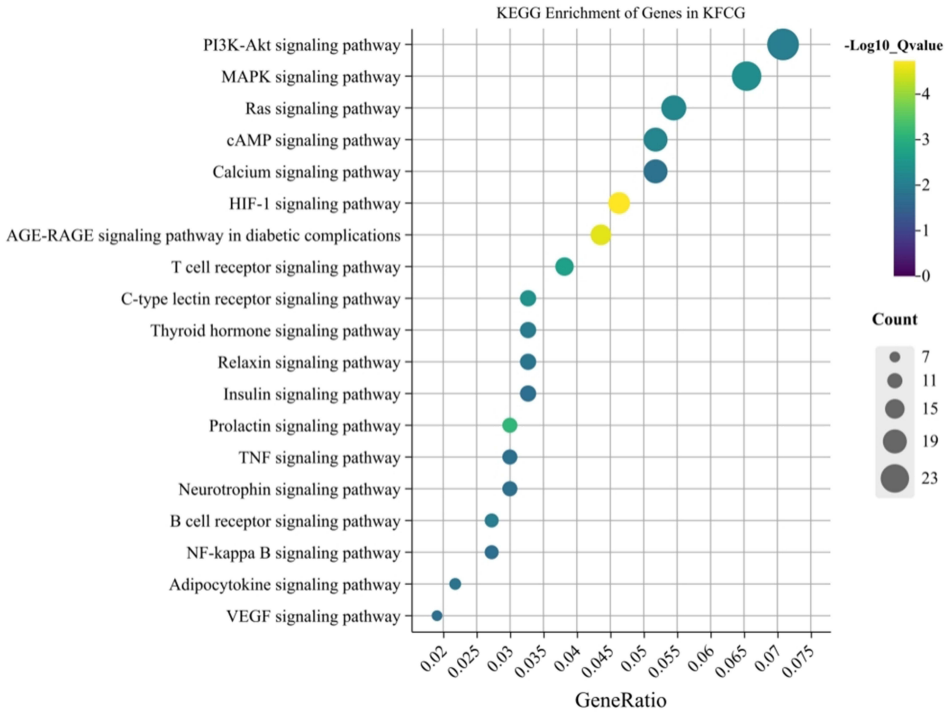

Figure 7 KEGG pathway enrichment analysis of genes in the KFCG.

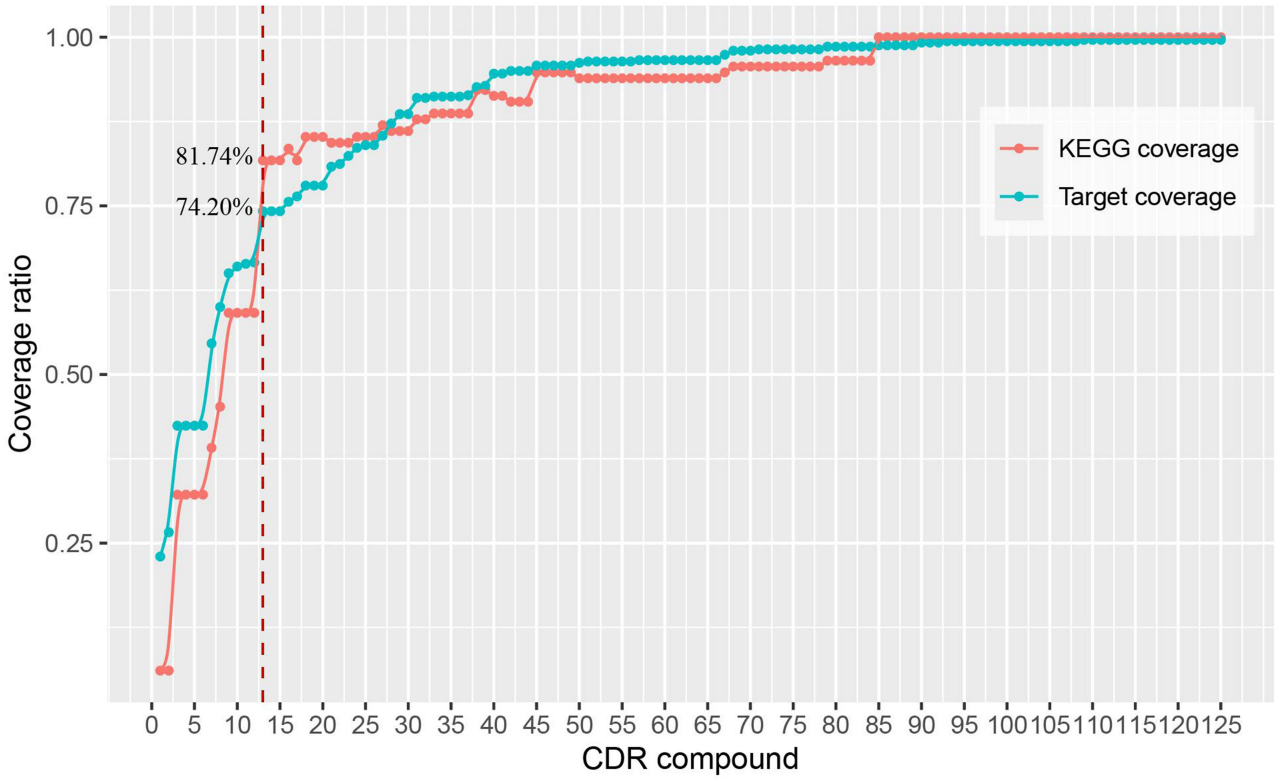

Figure 8 Dynamic plot showing the cumulative contribution rate (CDR) of compounds in the KFCG.

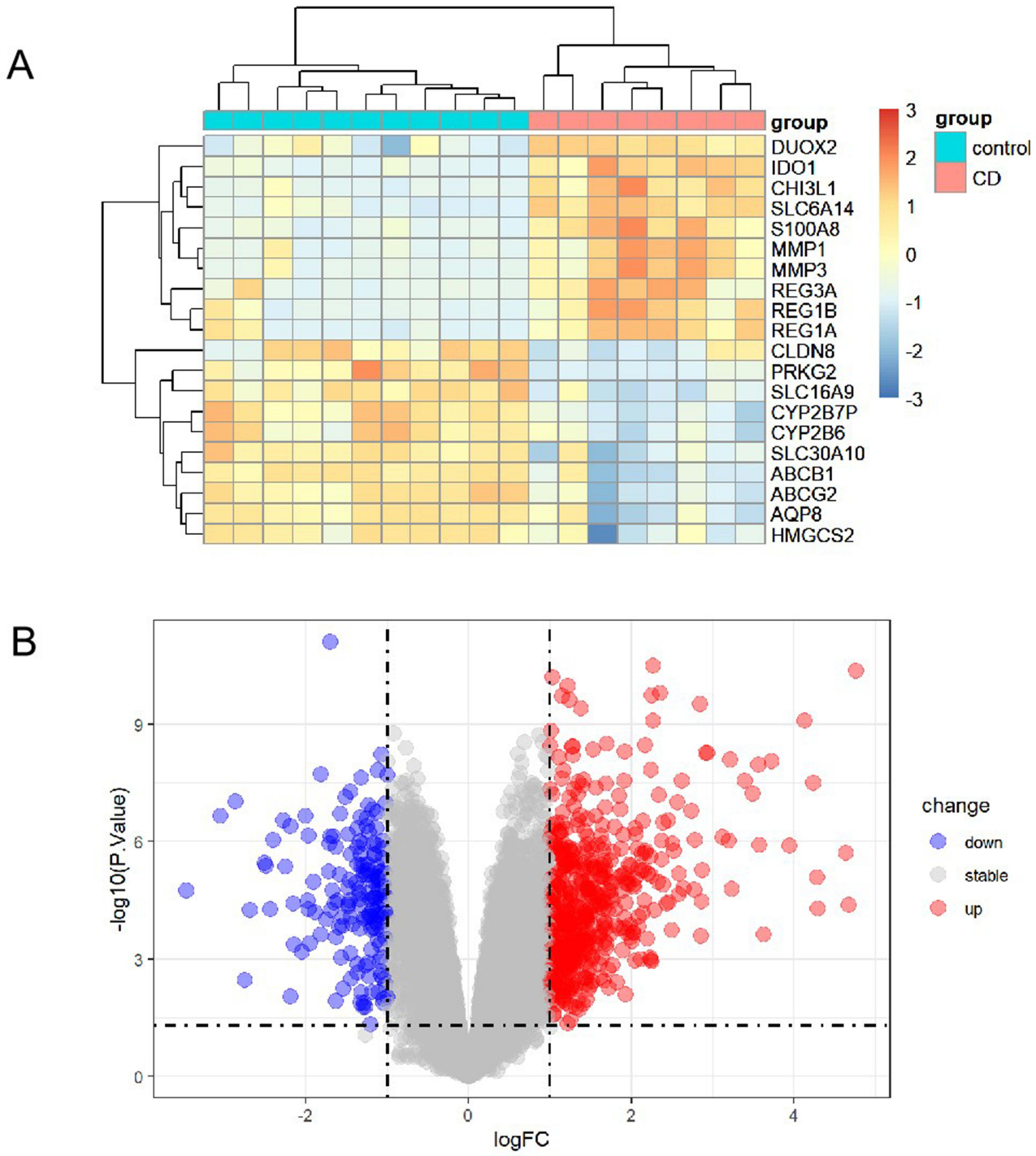

Figure 9 Analysis of gene expression differences between CD and normal tissues. (**A**) Heatmap showing the expression profiles of DEGs ($P < 0.05$); (**B**) Volcano plot displaying the distribution of upregulated and downregulated DEGs.
Abbreviation: FC: fold change.

To further investigate the functional importance of these 36 genes, a protein-protein interaction (PPI) network was constructed using the STRING database (https://cn.string-db.org) (Figure 11). The Molecular Complex Detection (MCODE) plugin in Cytoscape was applied to identify significant clusters within the PPI network, resulting in the identification of two major subnetworks (Figure 12).

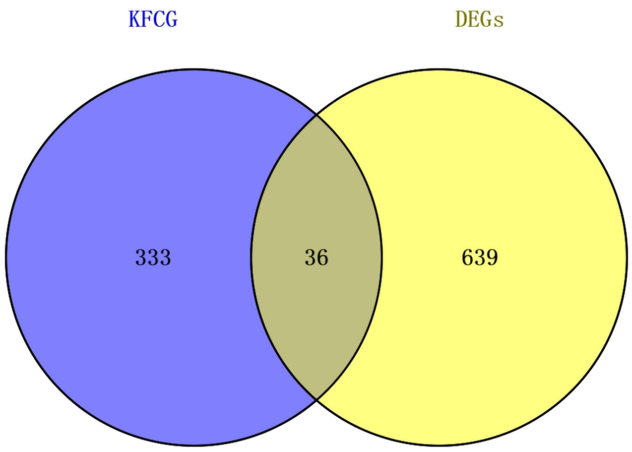

Figure 10 Venn diagram illustrating the overlap between KFCG targets and DEGs in CD.

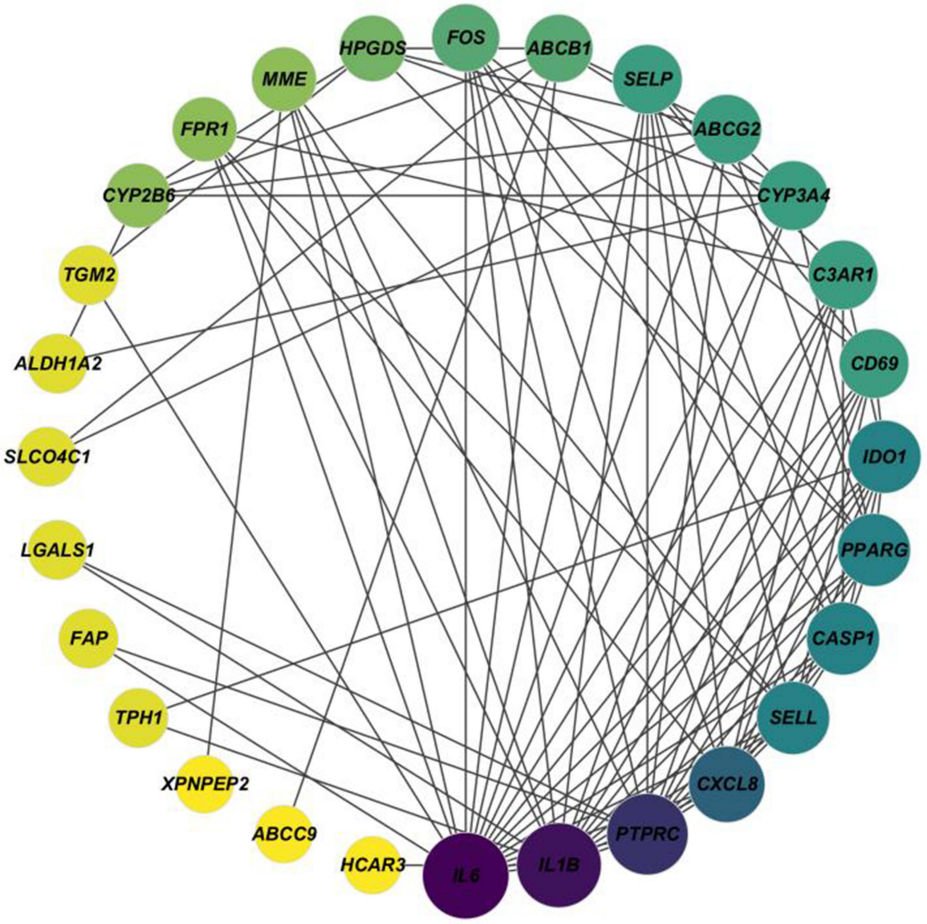

Figure 11 Visualization of the PPI network, including topological characteristics of the identified potential target genes.

In parallel, machine learning methods were employed to refine the list of candidate genes. First, a random forest algorithm was used, identifying 15 important genes. Then, a LASSO regression model narrowed this list down to six genes: IDO1, PRKG2, TGM2, ALDH1A2, ACPP, and CASP1. Receiver operating characteristic (ROC) curve analysis

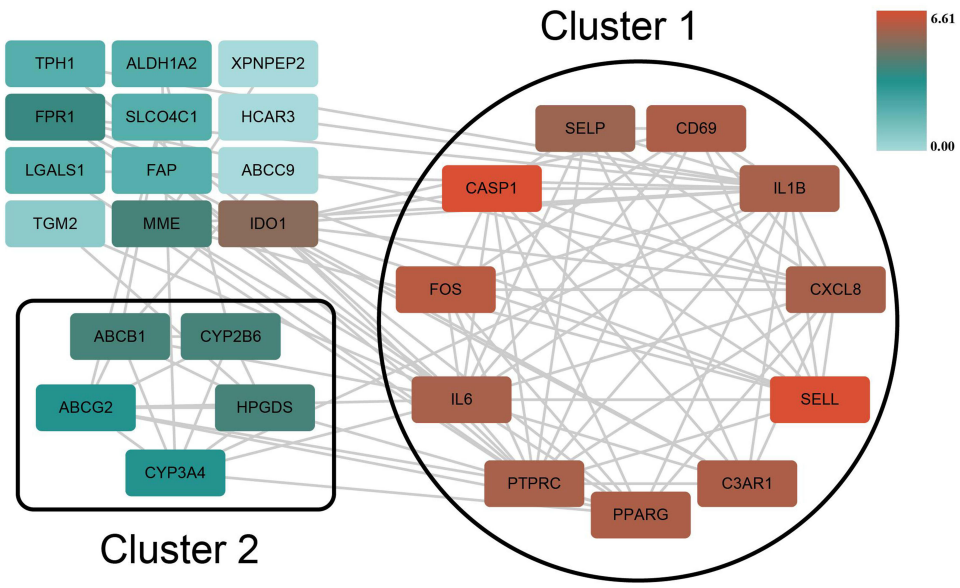

Figure 12 Clustering of genes using the MCODE plugin in Cytoscape.

demonstrated high predictive accuracy for these genes, with area under the curve (AUC) values ranging from 0.909 (ALDH1A2) to 1.0 (others) (Figure 13).

The six selected genes were subsequently used to train an XGBoost classification model. The trained model achieved an AUROC of 0.786 and an AUPRC of 0.899 when validated on an external dataset (Figure 14), confirming their robustness as feature genes.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Pathway Analysis

The 36 potential target genes were subjected to functional enrichment analysis using Metascape. GO analysis revealed that these genes are involved in biological processes such as apoptosis, inflammatory responses, xenobiotic stress response, angiogenesis, and leukocyte adhesion. Cellular component analysis indicated localization to secretory granule membranes and actin-based cell projections (Figure 15A). KEGG pathway enrichment highlighted retinol metabolism as a significantly enriched pathway (Figure 15B).

Immune Cell Infiltration Analysis

We analyzed immune cell composition in colon mucosal biopsies from CD patients and healthy controls. The results showed higher levels of central memory CD8+ T cells, immature dendritic cells, gamma delta T cells, and CD56dim natural killer cells in the control group. Conversely, effector memory CD8+ T cells, neutrophils, activated dendritic cells, myeloid-derived suppressor cells (MDSC), and activated CD4+ T cells were more abundant in CD samples (Figure 16).

Correlation analysis between the six feature genes and immune cell infiltration revealed that CASP1 and IDO1 were positively correlated with several immune cell types, including activated CD4+ T cells, plasmacytoid dendritic cells, MDSCs, macrophages, type 1 helper T cells, neutrophils, follicular helper T cells, central memory CD4+ T cells, activated dendritic cells, effector memory CD8+ T cells, monocytes, activated B cells, immature B cells, activated CD8+ T cells, gamma delta T cells, memory B cells, natural killer T cells, regulatory T cells, CD56bright natural killer cells, effector memory CD4+ T cells, natural killer cells, and type 2 helper T cells. In contrast, PRKG2 showed negative correlations with these same immune cell populations, suggesting that changes in gene expression may influence immune cell infiltration patterns in CD.

Immunofluorescence (IF) Staining

Given that the core genes CASP1 and IDO1 showed strong positive correlation with neutrophil, activated dendritic cell and effector memory CD8+ T cell, we performed the IF staining of CASP1, IDO1, marker of neutrophil (CD66b),[26] markers of matured dendritic cell (CD11c and CD83),[27,28] and markers of Effector memory $CD8^+$ T cell (KLRG1 and CD45RO).[29,30]

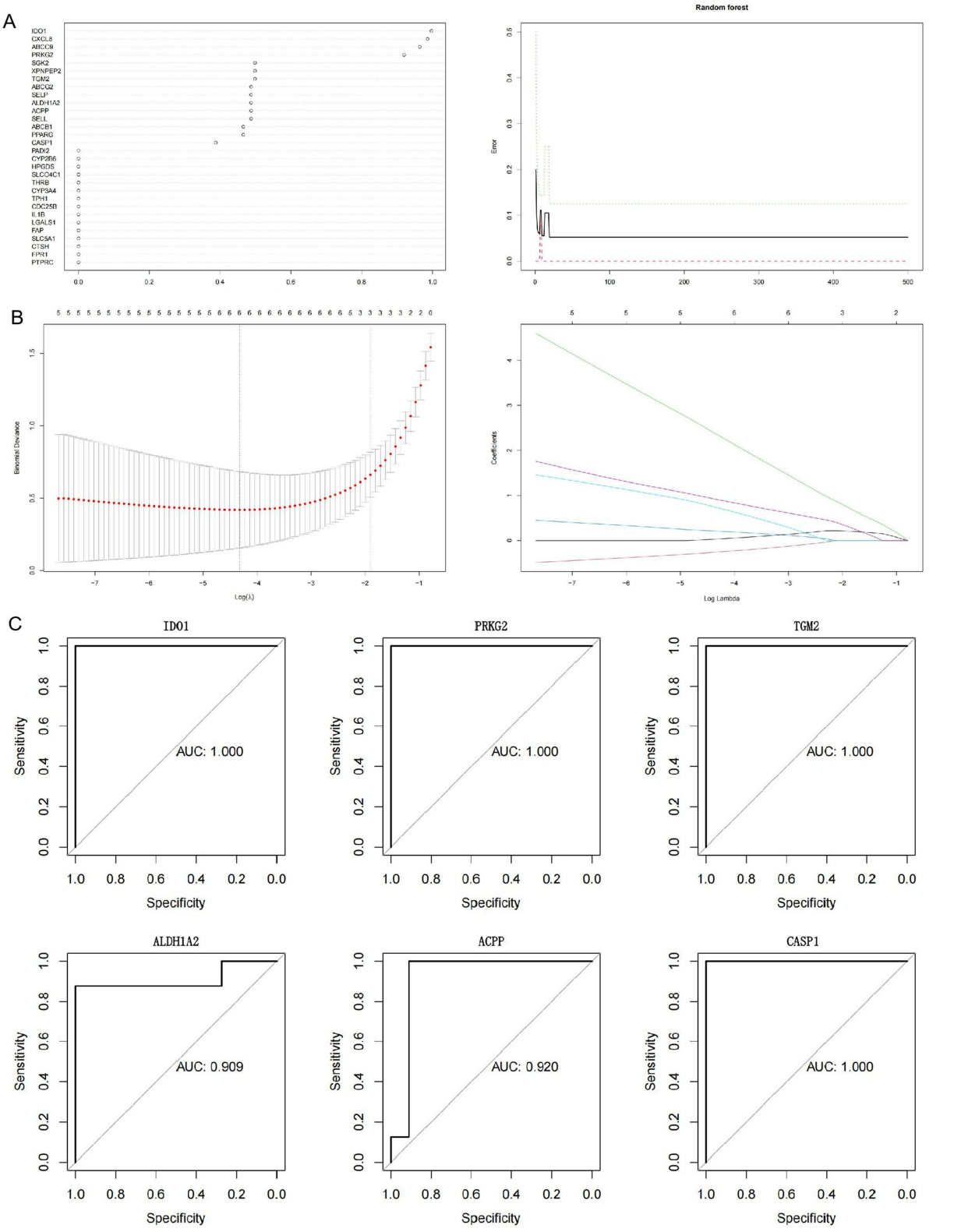

Figure 13 Application of machine learning algorithms for gene screening. (**A**) Random forest algorithm; (**B**) Least Absolute Shrinkage and Selection Operator (LASSO) regression; (**C**) ROC curves of the six selected feature genes (IDO1, PRKG2, TGM2, ALDH1A2, ACPP, and CASP1) in the training set.

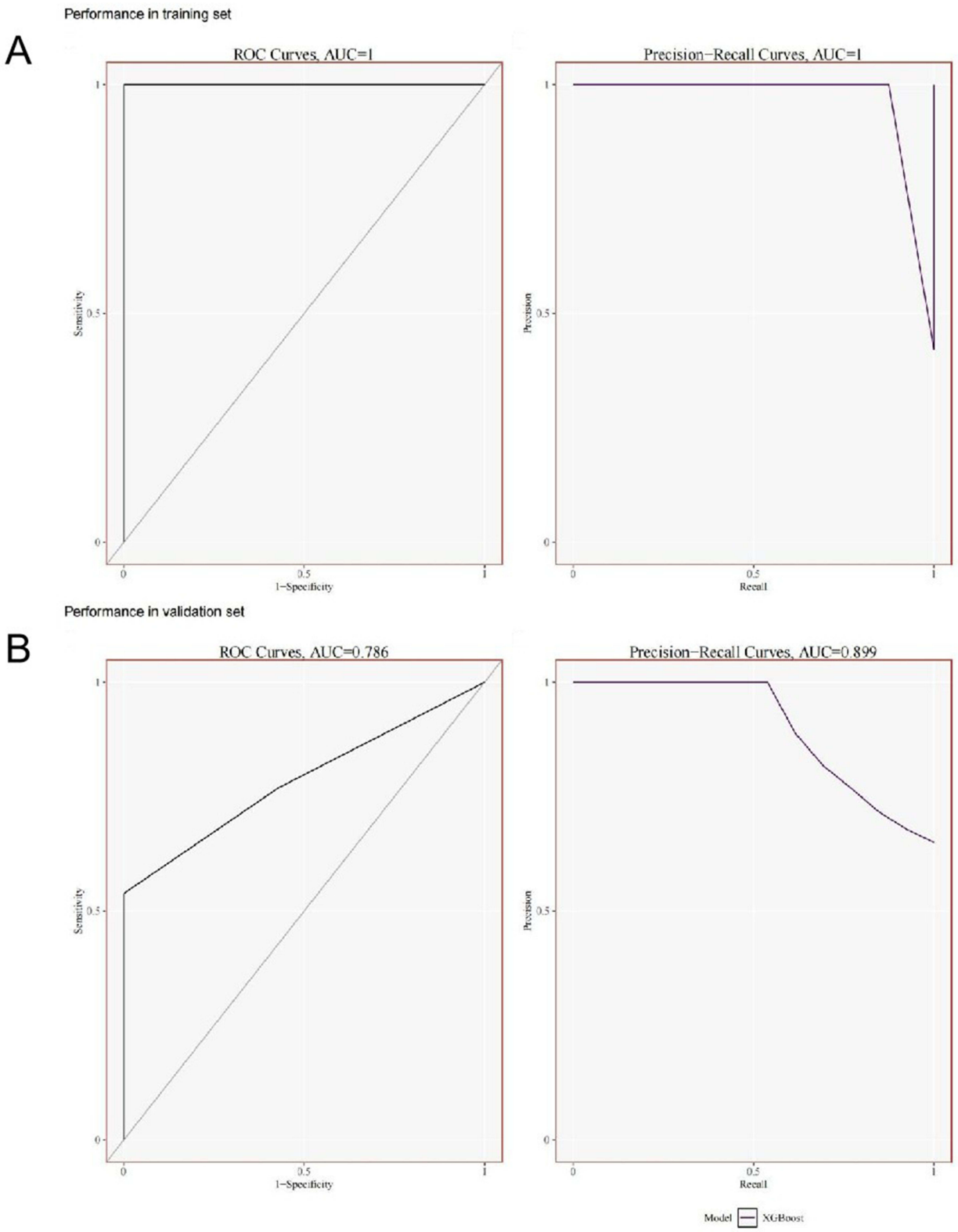

Figure 14 Performance evaluation of the XGBoost model. (**A**) Results in the training set; (**B**) Results in the external validation set.

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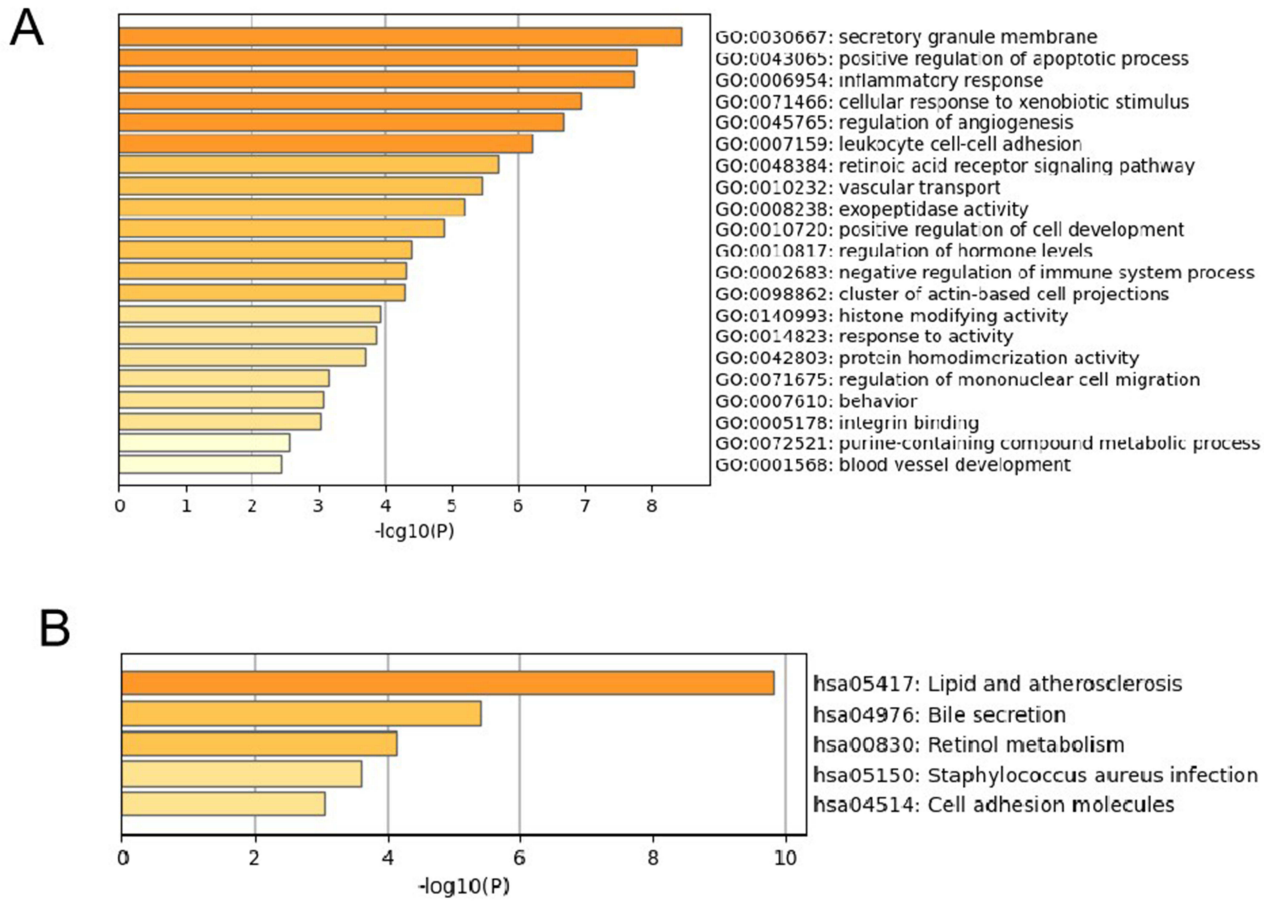

Figure 15 Functional enrichment analysis of the 36 potential target genes. (**A**) GO enrichment results generated by Metascape; (**B**) KEGG pathway enrichment results generated by Metascape.

At the end of the study, immunofluorescence staining revealed a significant reduction in CASP1, IDO1, CD11c, CD83, KLRG1 and CD45RO fluorescence intensity, while the difference in CD66b intensity was not statistically significant (Figure 17).

This finding suggest that the expression of CASP1 and IDO1 decreased after GHD treatment, and might lead to the improvement of matured dendritic cell and Effector memory CD8+ T cell infiltration in the intestinal mucosal tissue.

Discussion

The multifactorial etiology of Crohn's disease necessitates therapeutic approaches capable of addressing its complex pathophysiology.[19] While current pharmacological strategies target specific inflammatory pathways, our findings suggest that Gupishengji-Huazhuojiedu decoction (GHD) may offer a multi-targeted approach that aligns with the systems biology perspective of traditional Chinese medicine. This study provides clinical evidence supporting GHD's potential in CD management while offering mechanistic insights through integrated computational and experimental approaches.

Our clinical observations indicate that GHD treatment was associated with significant improvement in both clinical symptoms and endoscopic findings. In the retrospective analysis, GHD demonstrated comparable efficacy to infliximab in achieving clinical response (88.2% vs 63.0%, p=0.07) and remission (88.2% vs 51.9%, p=0.01), though these findings should be interpreted cautiously given the observational nature of the comparison. The prospective single-arm study further supported these observations, with 87.5% of patients achieving both clinical remission and endoscopic response after 12 weeks of treatment. These results are consistent with previous reports on TCM formulations in IBD management. Guo et al [31] demonstrated in their meta-analysis that Chinese herbal medicine combined with conventional therapy

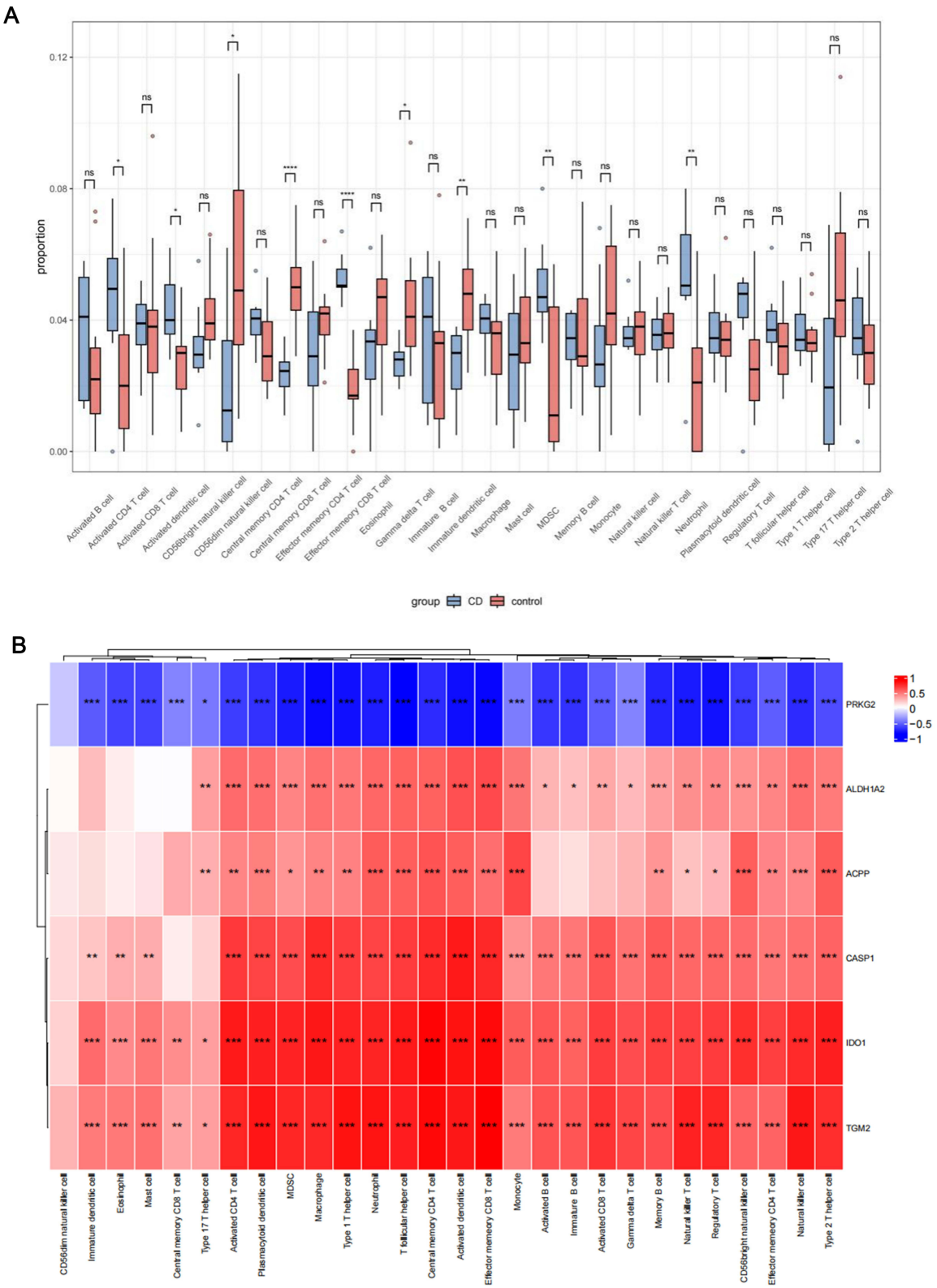

Figure 16 Immune cell infiltration analysis. (**A**) Box plots comparing the abundance of 28 immune cell types in CD (blue) and normal (red) colon tissues. ns, not significant ($P > 0.05$); *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$. (**B**) Correlation matrix depicting the relationship between the six feature genes (PRKG2, ALDH1A2, ACPP, CASP1, IDO1, and TGM2) and various infiltrating immune cell populations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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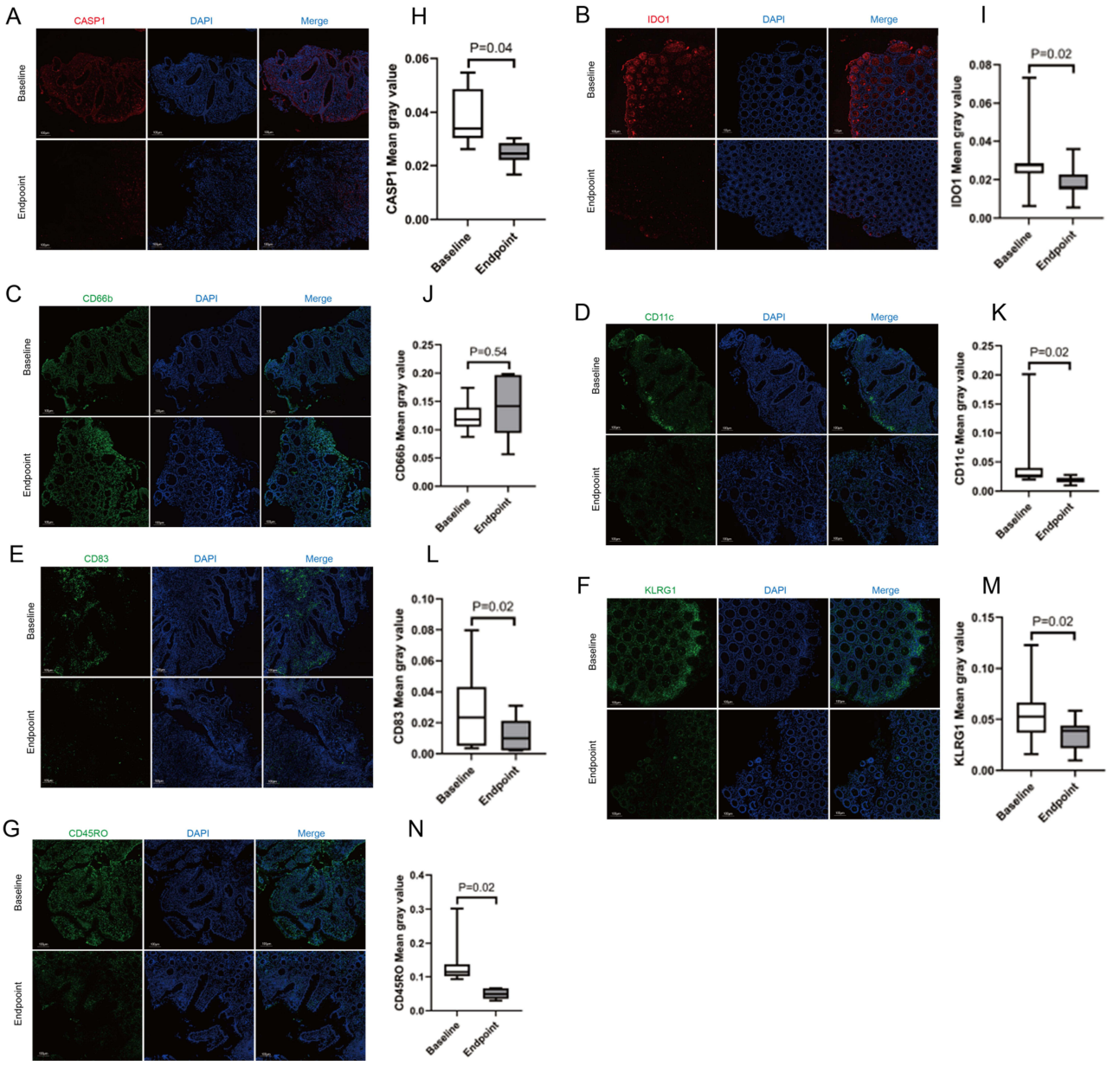

Figure 17 Comparison of CASP1, IDO1, CD66b, CD11c, CD83, KLRG1 and CD45RO expression in paired mucosal biopsies taken from the same anatomical site before and after GHD treatment. (**A**–**G**) Immunofluorescence staining. (**H**–**N**) Quantitative fluorescence analyses. Scale bars = 100 μm.

showed superior clinical efficacy compared to conventional therapy alone, particularly in achieving endoscopic improvement.

The rapid clinical response observed as early as week 2, accompanied by parallel reductions in inflammatory biomarkers (CRP and fecal calprotectin), suggests that GHD may exert both symptomatic and anti-inflammatory effects. The particularly pronounced reduction in fecal calprotectin, which correlates strongly with histological inflammation,[32] indicates potential effects on mucosal healing. This pattern of response is consistent with the known kinetics of biological therapies in CD,[33] though the precise mechanisms may differ.

Through our integrated network pharmacology and bioinformatics approach, we identified multiple potential mechanisms through which GHD may exert its therapeutic effects. The identification of 36 candidate target genes through the intersection of KFCG-related targets and CD differentially expressed genes provides a systems-level view of GHD's potential actions. We specifically prioritized CASP1 and IDO1 for experimental validation based on several

considerations: (1) their well-established roles in intestinal inflammation and immune regulation;[34,35] (2) their central positions in the protein-protein interaction network; (3) their strong performance in machine learning feature selection; and (4) the availability of reliable antibodies for experimental validation. While the other four feature genes (PRKG2, TGM2, ALDH1A2, and ACPP) also represent interesting candidates, their characterization in CD pathophysiology is less established, making them suitable for future investigations.

The machine learning approaches employed in this study provided a rigorous framework for target prioritization. Random forest algorithm, known for its robustness in handling high-dimensional data,[36] identified 15 important genes from the initial 36 candidates. Subsequent LASSO regression, which performs feature selection through L1 regularization,[37] further refined this list to six genes. The external validation using XGBoost[38] and performance evaluation on independent datasets helped mitigate overfitting concerns, though the perfect AUC values for some genes should be interpreted cautiously given the limited sample size.

Our experimental findings demonstrated significantly reduced expression of both CASP1 and IDO1 following GHD treatment, accompanied by improved immune cell infiltration profiles. Caspase-1 (CASP1) plays a crucial role in inflammasome activation and pyroptosis, processes increasingly recognized as important in CD pathogenesis.[39] The observed reduction in CASP1 expression suggests that GHD may modulate these pathways, potentially contributing to reduced epithelial damage and inflammation. This finding aligns with previous studies showing increased caspase-1 expression in CD tissues[34] and the protective effects of caspase-1 inhibition in experimental colitis.[40]

The case of IDO1 presents a more complex narrative. While traditionally viewed as an immunosuppressive enzyme through its role in tryptophan metabolism, recent evidence suggests context-dependent functions in inflammation.[35,41] Our observation of reduced IDO1 expression following successful treatment may reflect resolution of inflammation rather than indicating a primary therapeutic target. This interpretation is supported by studies showing that IDO1 expression often increases during active inflammation as a compensatory mechanism.[42] Based on current evidence, we hypothesize rather than conclude that CASP1 may represent a more direct therapeutic target of GHD, while IDO1 reduction may be a downstream consequence of inflammation resolution.

The immunofluorescence findings further support the potential immunomodulatory effects of GHD. The reduction in mature dendritic cells (CD11c+, CD83+) and effector memory CD8+ T cells (KLRG1+, CD45RO+) suggests that GHD may modulate antigen presentation and T cell activation in the intestinal mucosa. These cell populations have been implicated in the pathogenesis of CD,[43,44] and their reduction following treatment may contribute to decreased inflammation and tissue damage.

Limitations

This study has several limitations that should be considered when interpreting the results. First, the clinical components were limited by small sample sizes—particularly in the prospective single-arm trial (n=8)—and the non-randomized design of the retrospective comparison. These methodological constraints limit the generalizability of our efficacy findings and introduce potential for selection bias. Second, the chemical profiling of GHD relied exclusively on published databases rather than experimental quantification of bioactive components through methods such as HPLC or mass spectrometry. This approach may not fully capture the complex phytochemical composition or pharmacokinetic properties of the decoction.

Third, while our integrated bioinformatics approach identified promising mechanistic leads, the absence of direct functional validation experiments means that causal relationships between target modulation (particularly of CASP1 and IDO1) and therapeutic effects remain speculative. Without knock-down experiments, specific inhibitor studies, or in vitro validation, we cannot definitively establish whether these targets are central to GHD's mechanism of action. Fourth, the machine learning models, while rigorously validated, showed perfect AUC values for some genes, which may reflect overfitting despite our cross-validation efforts; independent validation in larger datasets is necessary.

Fifth, the relatively short treatment duration (12–16 weeks) prevents assessment of long-term efficacy, safety, and durability of response. Sixth, the single-arm design of the prospective study lacks a control group, making it difficult to distinguish drug-specific effects from natural history or placebo responses. Finally, although several genes showed near-perfect AUC values, this finding should be interpreted cautiously due to the limited sample size and the risk of model overfitting. Independent external validation is warranted to confirm these preliminary findings. Future studies should

address these limitations through randomized controlled trials, comprehensive chemical characterization, functional validation of targets, and longer follow-up periods.

Conclusion

This exploratory study provides preliminary evidence that Gupishengji-Huazhuojiedu decoction (GHD) may induce clinical remission in Crohn's disease, as evidenced by improvements in symptoms, inflammatory biomarkers, and endoscopic findings. Our integrated network pharmacology and machine learning approach suggests that this effect is likely mediated through a multi-target mechanism, primarily involving the modulation of immune-inflammatory pathways such as those related to CASP1 and IDO1.

However, these findings are constrained by the study's methodological limitations, including its small sample size and non-randomized design. Therefore, the results should be interpreted as exploratory and associative rather than definitive. Further validation through adequately powered randomized controlled trials and functional experimental studies is essential to confirm GHD's efficacy and elucidate its precise mechanisms of action. Despite these limitations, GHD presents a promising candidate for future development as a multi-target therapeutic strategy for Crohn's disease.

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Disclosure

The authors declare that they have no competing interests in this work.

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