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Original Research Article - Translational

OCTN1 variants shape innate immunity and predict individual response to anti-TNFα in ulcerative colitis patients: An exploratory study

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Abstract

Background: **Ulcerative colitis (UC) exhibits substantial heterogeneity in inflammatory pathways and therapeutic response. The organic cation transporter OCTN1 (SLC22A4) and its rs1050152 missense variant (L503F) have been implicated in innate immune activation and disease susceptibility. We investigated whether OCTN1 variants modulate monocyte inflammatory responses and predict clinical outcomes in UC patients treated with anti-tumor necrosis factor α (TNFα) agents.**

Methods: **We combined preclinical and clinical approaches. In THP-1 cells engineered for OCTN1 knockdown or variant-specific overexpression, and in primary monocytes isolated from genotyped donors and UC patients, we assessed by enzyme-linked immunosorbent assay and Western blot interleukin (IL)-1β production following bacterial stimulation (peptidoglycan, *Fusobacterium nucleatum*, *Escherichia coli*, *Salmonella*). In the clinical study, UC patients initiating anti-TNFα therapy were genotyped and evaluated for 1-year therapy persistence, steroid-free clinical remission, and endoscopic response. Logistic regression models were used to identify predictors of outcomes. An exploratory machine learning analysis (regularized logistic regression and neural networks) was performed to rank the variables contributing to each endpoint.**

Results: **OCTN1-deficient THP-1 cells showed markedly reduced interleukin-1β secretion, whereas the same cell line or primary monocytes carrying the 503F variant exhibited significantly enhanced cytokine release upon microbial stimulation. On the clinical side, 105 patients with UC were enrolled. The homozygous 503F genotype (TT) was strongly associated with higher rates of steroid-free remission, endoscopic response, and therapy persistence. Heterozygous carriers displayed intermediate outcomes. Machine learning models demonstrated moderate predictive performance (area under the curve 0.60-0.72), consistently ranking OCTN1 among relevant, though not dominant, determinants of therapeutic success.**

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Conclusions: **The OCTN1 503F variant enhances interleukin-1β–mediated innate immune activation and is associated with improved long-term response to anti-TNFα therapy in UC. These findings support OCTN1 genotyping as a promising component of future precision medicine strategies in inflammatory bowel disease and warrant confirmation in larger prospective multicenter cohorts.**

Key words: ulcerative colitis, anti-TNFα therapy, pharmacogenetics, precision medicine, OCTN1

Lay Summary

The inflammatory bowel disease–associated 503F variant of the organic cation transporter OCTN1 promotes interleukin-1β production from leukocytes and predicts a better response to anti-tumor necrosis factor α therapy in patients with ulcerative colitis. Machine learning confirms this finding.

Key Messages

What is already known?

Response to anti-tumor necrosis factor α therapy in ulcerative colitis is highly variable and reliable predictive biomarkers are lacking.

What is new here?

The OCTN1 rs1050152 (L503F) variant modulates innate immune responses and is associated with improved clinical and endoscopic outcomes under anti-tumor necrosis factor α therapy; exploratory machine learning analyses support its relevance among predictive variables.

How can this study help patient care?

OCTN1 genotyping may contribute to patient stratification and support more personalized selection of biologic therapies in ulcerative colitis.

Introduction

Ulcerative colitis (UC) is a chronic, invalidating disease, with an estimated prevalence of 1 per 1000 individuals in Europe, usually diagnosed at a young age.[1] It is a multifactorial disorder, in which genetic predisposition, microbiota, and environmental factors play a major role.[2]

Current medications include aminosalicylates, steroids, and immune modulators, including small molecules and biologic drugs. For patients requiring second level medications, anti-tumor necrosis factor α (TNFα) drugs (infliximab, adalimumab, golimumab) still represent the first-line therapy in clinical practice.[3] Other medications, such as vedolizumab, have also proven effectiveness as first-line therapy in UC.[4]

Choosing the correct first-line therapy is a key determinant of long-term patients' prognosis and quality of life.[5] However, little evidence is available so far to predict therapeutic response to biologic medications. Throughout the years, many elements have been proposed as predictors of response to therapy, including disease phenotypes, genetics, cytokines, proteomics and tissue markers, with uncertain and inconclusive findings.[6]

Regarding genetics, Telesco et al[7] identified a gene expression signature in colon biopsies that could predict long-term clinical response to golimumab. However, this panel of genes, including sequences coding complement receptors, cytokines, fibroblast growth factors, synuclein alpha, superoxide dismutase, and others, did not reach a strong statistical significance in foretelling the achievement of mucosal healing.[7] Burke et al[8] also proposed an interesting model of genetic markers to predict individual response to anti-TNFα, analyzing almost 200 000 single nucleotide polymorphisms (SNPs).

The organic cation transporter Slc22A4/OCTN1 (OCTN1) (Figure 1), with its variants 503 L (wild-type), and 503F (inflammatory bowel disease [IBD] associated), represents one of the key genetic molecules associated with the individual susceptibility and disease severity gravity in UC. The OCTN1 rs1050152 (C1672T) polymorphism results in a leucine-to-phenylalanine substitution at position 503 (L503F). Genotypes were classified as CC (wild-type homozygous), CT (heterozygous), and TT (homozygous variant, 503F).[9,10] The molecular tertiary structure, as well as the functional role of OCTN1, are still unclarified. Nonetheless, the importance of OCTN1 in cell protection from oxidative and inflammatory damage is largely acknowledged, as is the role of OCTN1 in the trafficking of drugs, metabolites, antioxidants, and immunosuppressive agents.[11]

Current data suggest a direct involvement of OCTN1 in shaping the innate immune response to microbes and microbial products. In particular, the OCTN1/1672T variant has been associated with increased levels of interleukin (IL)-8 messenger RNA and predisposition to UC, and sporadic colorectal cancer in young individuals.[12] Furthermore, our group has previously demonstrated that OCTN1−/− mice show a better course of colitis and better microbial profiles in comparison to wild-type mice, together with attenuated response to infliximab.[13] With the present study, we aimed to (1) further elucidate the role of OCTN1 polymorphisms in modulating inflammation and (2) investigate its clinical relevance as a predictor of response to therapy in UC patients.

Methods

Cell lines and THP-1–based OCTN1 models

The human THP-1 monocytic cell line (ATCC TIB-202) was cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Differentiation into macrophage-like cells was induced with PMA (500 ng/mL for 3 hours), followed by an overnight resting phase to allow maturation before stimulation.

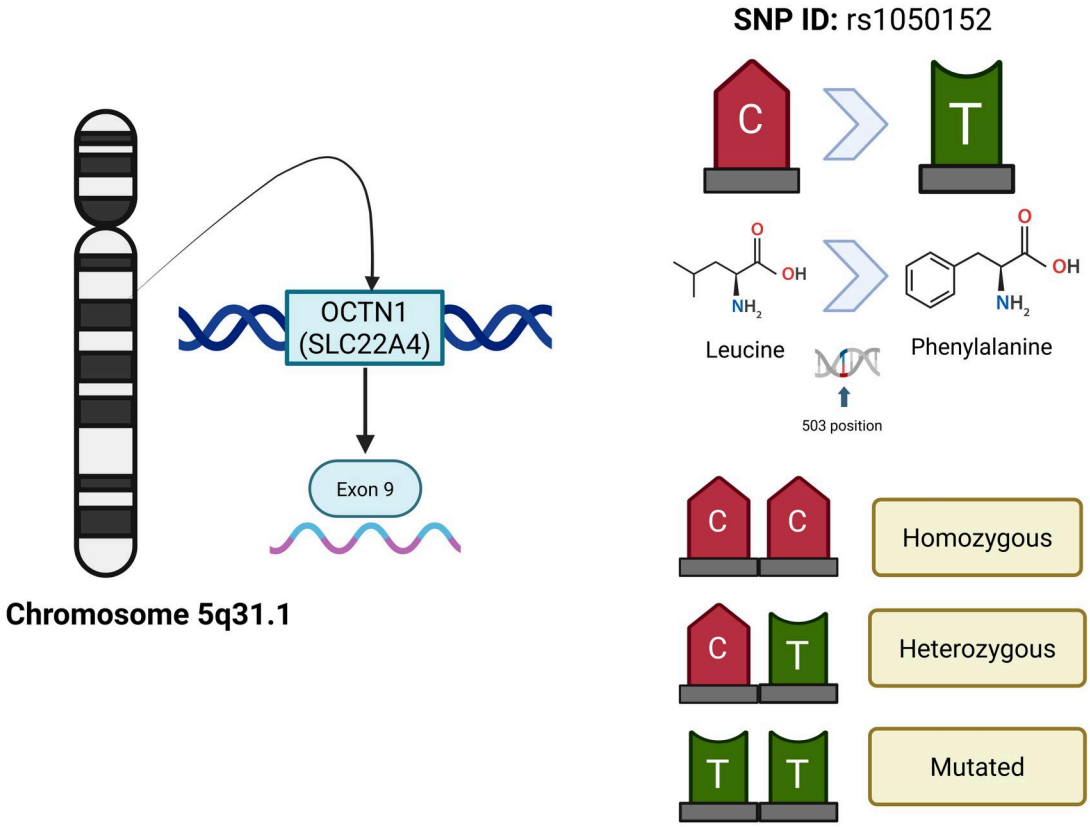

Figure 1 The OCTN1 gene (SLC22A4), located on chromosome 5q31.1, encodes an organic cation transporter involved in the trafficking of drugs and endogenous metabolites. The rs1050152 single nucleotide polymorphism (SNP) (C1672T), a missense variant located in exon 9, results in a leucine-to-phenylalanine substitution at amino acid position 503 (L503F).

To investigate the functional role of OCTN1, we generated THP-1 derivatives using lentiviral vectors based on the pLKO.1 system. Two short hairpin RNA sequences targeting human OCTN1 (#14 and #15) were used to obtain stable knockdown lines, while the empty vector served as control. In parallel, complementary DNAs encoding the OCTN1 503 L (wild-type) and 503F (risk variant) isoforms were cloned into the same backbone to obtain stable overexpression models. Viral particles were produced in HEK293T cells and used for transduction following standard procedures.

Primary monocyte isolation and culture

Primary monocytes were isolated from peripheral blood mononuclear cells (PBMCs) obtained from the buffy coats of 22 healthy donors and from fresh peripheral blood of 20 UC patients. PBMCs were separated by Ficoll density gradient centrifugation. Monocytes were enriched by short-term adhesion to plastic, a procedure that yields a highly enriched (>85%) monocyte population. Nonadherent cells were collected for genotyping, while adherent monocytes were maintained in serum-free medium for functional assays.

UC patient samples were processed using the same methodology, with scaled-down volumes proportional to available material.

OCTN1 genotyping

Genomic DNA was isolated from PBMCs, and the OCTN1 C1672T SNP (rs1050152), corresponding to the 503 L (C allele) and 503F (T allele) variants, was genotyped using a TaqMan SNP Genotyping Assay (Thermo Fisher Scientific), according to the manufacturer's instructions.

Cellular stimulation and cytokine quantification

Differentiated THP-1 cells and primary monocytes were stimulated with either purified peptidoglycan (PGN) or live bacteria, depending on the experiment (*Fusobacterium nucleatum* for THP-1; *Escherichia coli* and *Salmonella* for primary monocytes). Cells were incubated for 8 to 16 hours based on stimulus type.

Supernatants were collected, clarified, and stored at −80 °C. IL-1β concentrations were measured using a commercial enzyme-linked immunosorbent assay (ELISA) (Thermo Scientific). In selected experiments, protein expression in cell lysates or precipitated supernatants was evaluated by Western blot using standard protocols.

Clinical study design and patient enrollment

This observational ambispective study consecutively enrolled adult patients (>18 years of age) with confirmed UC who were candidates to start anti-TNFα therapy. Baseline clinical

characteristics, Mayo clinical and endoscopic scores, and prior/concomitant treatments were collected. Patients were reassessed after 1 year to determine therapy persistence, steroid-free clinical remission, and endoscopic response.

Exclusion criteria included prior allergic reactions to anti-TNFα, documented antidrug antibody development, or discontinuation of therapy due to drug-related adverse events or unrelated major complications (eg, cancer).

The study followed the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the institutional ethics committee. All patients provided written informed consent.

Clinical endpoints

Clinical endpoints were 1-year persistence (continued anti-TNF treatment at 12 months), steroid-free clinical remission (modified Mayo clinical score of 0 or 1 and no systemic corticosteroids for ≥12 weeks), and endoscopic response (≥1-point reduction in Mayo endoscopic score). Genotyping for the OCTN1 (503 L/503F) variants was performed in all enrolled patients.

Statistical analysis

As a pilot study with limited prior data, no formal sample size calculation was performed; Recommendations for pilot clinical investigations were followed. Quantitative variables were summarized as mean ± SD or median (interquartile range) and categorical variables as frequency. Normality was assessed using the Shapiro-Wilk test.

Between-group comparisons used chi-square or Fisher's exact test (categorical variables) and analysis of variance or Kruskal-Wallis test (continuous variables), with false discovery rate correction for multiple testing. Predictors of clinical outcomes were analyzed using univariate and multivariate logistic regression (backward stepwise).

Statistical significance was set at $P < .05$. Analyses were performed with Stata 12 (StatCorp) and R v4.2.2 (R Foundation for Statistical Computing).

Machine learning analysis

An exploratory machine learning analysis was performed using the Orange Data Mining platform. The objective was to rank the relative importance of clinical, demographic, and genetic variables in determining each outcome. Two models were applied: a regularized logistic regression and a simple neural network classifier.

The machine learning logistic regression differs from the traditional biostatistical model, as it is prediction-oriented, uses automatic regularization (L1/LASSO) to shrink or remove less informative variables, and does not rely on predefined linearity or interaction assumptions. Neural networks were included to explore potential nonlinear patterns in the data.

To optimize performance, we conducted parameter tuning (training/test proportion, regularization strength, and model settings) and evaluated models primarily through area under the curve (AUC). The resulting rankings were used to identify the variables most strongly associated with each endpoint.

Results

Reduced IL-1β secretion in response to PGN and *F nucleatum* in OCTN1-deficient THP-1 cells

We initially measured the release of the mature IL-1β (p17) protein in the supernatant of control THP-1 cells (pLKO.1) and the two different OCTN1-deprived lines (short hairpin RNA #14 and #15), before and after stimulation with PGN or *F nucleatum*. As shown in Figure 2A (bottom), supernatant immunoblotting revealed an abundant release of IL-1β (p17) in response to PGN, while the cytokine signal was undetectable in the supernatant of unstimulated cells. Importantly, such induction appears remarkably attenuated in the two OCTN1-silenced sublines as compared with the control pLKO.1 line (Figure 2A [bottom] and 2B). PGN-induced upregulation of the 40 kD pro-IL-1β precursor in whole lysates appeared unaffected by OCTN1 expression level (Figure 2A, top), suggesting that an impaired post-translational processing (signal 2, likely by inflammasomes) conceivably accounts for the defective IL-1β secretion observed in cells expressing reduced levels of OCTN1.

Similar to PGN-stimulated cells, pLKO.1 cells released significant amounts of IL-1β in response to both low (1 multiplicity of infection) and high (10 multiplicity of infection) loads of *F nucleatum*, while only the response to the highest bacterial load was detectable in OCTN1-silenced lines, indicating a reduced cell reactivity to the pathogen (Figure 2C). Moreover, when responses to the highest microbial load were considered, OCTN1 deficient lines consistently released about half the amount of IL-1β compared with control THP-1 cells (Figure 2D). Taken together, these observations reveal the need of OCTN1 for an optimal cytokine response to bacterial challenge in human monocytes.

Increased production of IL-1β in response to PGN and live bacteria in monocytes bearing the OCTN1 503F variant

To initially elucidate the functional significance of the OCTN1 503F variant in monocyte responses to bacteria, THP-1 cells were engineered to overexpress either OCTN1 503 L (wild-type) or OCTN1 503F (Crohn's disease associated) by lentiviral transduction of the respective complementary DNAs. When challenged with bacterial wall components muramyl dipeptide (MDP) or PGN, 503F-expressing cells displayed a much stronger IL-1β (p17) secretory response as compared with 503 L–transduced control cells (Figure 3A), consistent with an enhanced proinflammatory activity of the IBD-associated variant.

To confirm this relevant observation in a more physiological setting, primary monocytes isolated from buffy coats of genotyped healthy donors were stimulated in vitro with the same mixture of PGN from *Bacillus subtilis* and *Streptomyces*, and IL-1β measured in the supernatants by ELISA. As shown in Figure 3B, the release of IL-1β was significantly higher in subjects homozygous for the 503F variant (TT, n = 4) than in individuals (CC+CT, n = 18) carrying at least 1 "normal" C allele. Likewise (Figure 3C), PBMCs from UC patients bearing the 503F variant released more IL-1β in response to live bacteria (*E coli*

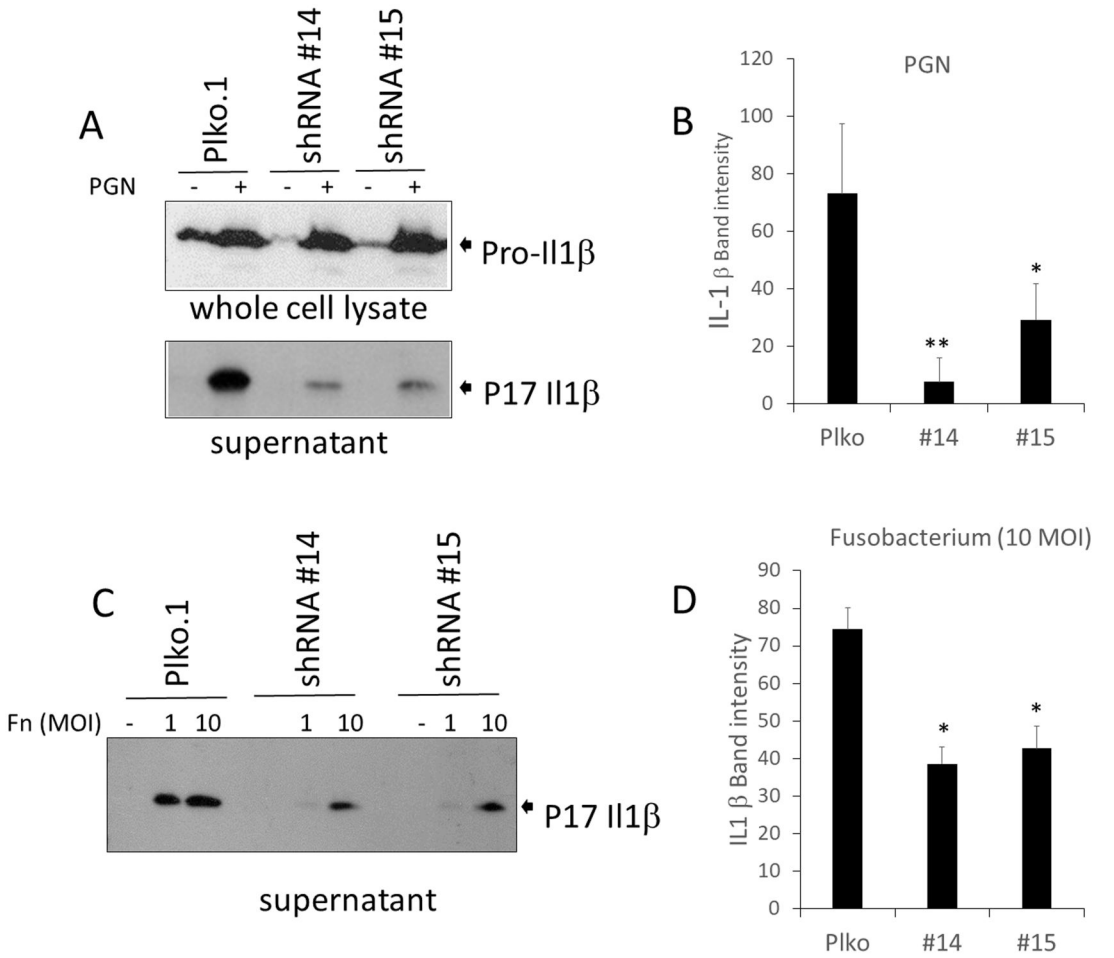

Figure 2 Effect of OCTN1 on IL-1β secretion in THP-1 cells. Supernatants from OCTN1-defective THP-#14 and THP-#15 cells and their THP-1 pLKO controls exposed to either (A) peptidoglycan (PGN) or (C) the indicated multiplicity of infection (MOI) of *Fusobacterium nucleatum* were analyzed by Western blotting with an antibody specific for interleukin (IL)-1β. Panels B and D show Il1b band densitometry from 3 independent experiments. Numbers are mean ± SD of absolute band volumes (area × intensity). Fn, *Fusobacterium nucleatum*; shRNA, short hairpin RNA. *P < 0.05; **P < 0.01 versus pLKO control.

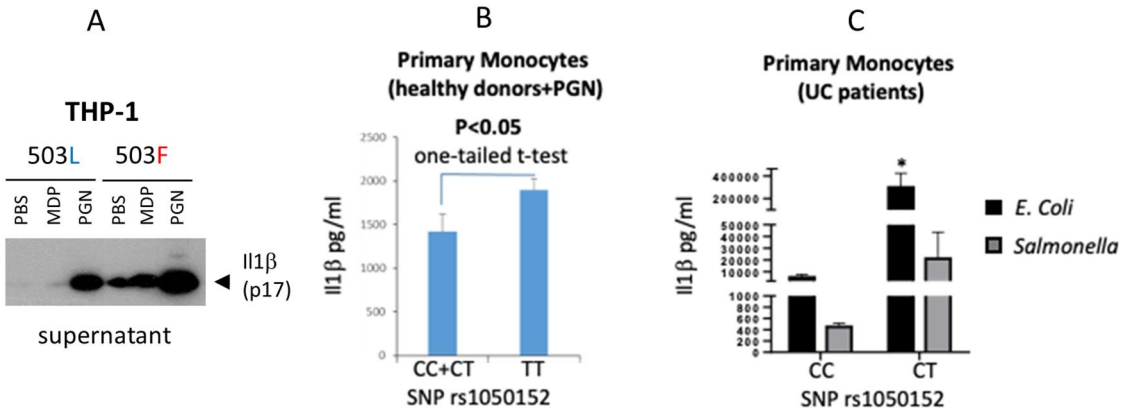

Figure 3 Effect of the OCTN1 503F variant on interleukin (IL)-1β secretion by monocytes. (A) THP-1 cells overexpressing the indicated OCTN1 variants were treated with muramyl dipeptide (MDP) (10 μg/mL) or peptidoglycan (PGN), and supernatants analyzed by anti IL-1β immunoblotting as in Figure 1. The prominent band, corresponding to the mature cytokine, is indicated by an arrow. (B) Primary monocytes isolated from the buffy coats of 22 genotyped healthy donors (4 TT and 18 CC+CT) were stimulated with PGN in vitro, and IL-1β release determined by enzyme-linked immunosorbent assay. Columns are mean ± SE. (C) IL-1β secretion by peripheral blood mononuclear cells from 22 ulcerative colitis patients of different OCTN1 genotype, stimulated in vitro with the indicated bacterial strains. Columns are mean ± SE. *$P < 0.05$ by 2-tailed t test. PBS, phosphate-buffered saline; SNP, single nucleotide polymorphism.

and *Salmonella*) than patients lacking this variant, confirming a general hyperreactivity of OCTN1 503F-expressing cells to bacterial challenges.

Overall, these data demonstrate that OCTN1 is necessary for monocyte secretion of IL-1β induced by bacterial stimuli, with the disease-associated 503F variant displaying, in the same setting, a gain of proinflammatory capacity as compared with 503 L.

General characteristics of the patient sample

As for the human study, we enrolled 105 subjects, 54.3% female, with a median age of 40 years and median age at diagnosis 31 years. The median duration of disease was 4 years, and the disease was almost equally localized as left-sided colitis and pancolitis (44% and 56%, respectively). OCTN1 molecular analysis disclosed a wild-type 503 L genotype in 34 cases, heterozygous in 49, while the 503F mutated genotype was the least frequent, with 22 cases. No significant finding emerged in baseline characteristics among the 3 genotypes and at pairwise comparisons. All the data are reported in Table 1.

Assessment of potential predictors of 1-year steroid-free clinical remission and endoscopic response

We focused on demographic, genetic, and biochemical markers potentially associated with the defined endpoints, encompassing 1-year therapy persistence, steroid-free clinical remission, and endoscopic improvement. All data are reported in Tables 2 and 3 and Tables S1 and S2.

At univariate analysis, logistic regression models disclosed a significant association of mutated 503F genotype with therapy persistence, 1-year steroid-free remission (SFR), and endoscopic response (odds ratios [ORs]: 16.58, 4.86, and 6.52, respectively). Interestingly, with regard to endoscopic response, the heterozygous genotype was also associated with positive endpoint (OR, 2.89; $P = .031$), suggesting a positive effect induced by even a single mutation. Conversely, the use of adalimumab and the presence of extraintestinal manifestation (EIM) showed a negative effect on 1-year SFR.

At the multivariable analysis, the presence of the mutated 503F genotype resulted was associated with 1-year therapy persistence (OR, 17.71; $P = .009$), SFR (OR, 4.82; $P = .019$) and endoscopic response (OR, 7.88; $P = .001$). Interestingly, the multivariate analysis confirmed the role of heterozygous genotype in determining endoscopic response (OR, 3.24; $P = .021$). Conversely, no significant association was found between genotype and EIM resolution. Of note, the use of adalimumab was associated with reduced rates of 1-year therapy persistence (OR, 0.24; $P = .03$), while on the contrary, it was associated with increased probability of EIM resolution (OR, 5.76; $P = .025$).

To complement the classical statistical approach, we performed an exploratory machine learning analysis aimed at identifying the variables most strongly associated with each clinical endpoint. Using Orange Data Mining, we applied both a neural network classifier (ReLU activation, Adam solver, 500 iterations) and a LASSO (L1) logistic regression model. For machine

Table 1 General characteristics of the study population (n = 105).

	Overall	CC (n = 34)	CT (n = 49)	TT (n = 22)	P_{trend}
Age at enrollment, y	40 (25-49)	41 (30-52)	40 (23-51)	36.5 (23-44)	.536
Age at diagnosis, y	31 (20-44)	32 (20-46)	31 (20-42)	32 (19-41)	.771
Sex					.738
Female	48 (45.7)	14 (41.2)	24 (49.0)	10 (45.5)	
Male	57 (54.3)	20 (58.8)	25 (51.0)	12 (54.5)	
Disease duration, y	4 (1-8)	4.5 (2-9)	4 (2-8)	4 (1-7)	.768
Disease extension at diagnosis (n = 100)					
Left-sided colitis	44 (44)	16 (48.5)	20 (44.4)	(36.4)	.680
Pancolitis	56 (56)	17 (51.5)	25 (55.6)	14 (63.6)	
Therapy					*.980*
IFX	75 (71.4)	23 (67.7)	36 (73.5)	16 (72.8)	
ADA	15 (14.3)	6 (17.6)	6 (12.2)	3 (13.6)	
GOL	15 (14.3)	5 (14.7)	7 (14.3)	3 (13.6)	
Previous therapy (vedolizumab)	4 (3.8)	4 (11.7)	0	0	.011
Combination therapy (AZA/MTX)	13 (12.4)	2 (5.9)	9 (18.3)	2 (9.1)	*.24*
1-y therapy persistence (yes)	74 (70.5)	19 (55.9)	34 (69.4)	21 (95.5)	.004[a]
1-y steroid-free remission (yes)	52 (49.5)	14 (41.2)	21 (42.9)	17 (77.3)	.014[a]
Endoscopic response (yes) (n = 101)	51 (50.5)	9 (29.0)	26 (54.2)	16 (72.7)	.006[a]
EIM resolution (n = 45)	13 (28.9)	5 (27.8)	6 (28.6)	2 (33.3)	1.000

Values are median (intrquartile range or n (%). Either chi-square test or Fisher-Freeman-Halton's exact test was applied to qualitative data, while either 1-way analysis of variance or Kruskal-Wallis test on quantitative data, with pairwise comparisons assessed by either Student's *t* test or Mann-Whitney *U* test, with false discovery rate correction.

Abbreviations: AZA, azathioprine; EIM, extraintestinal manifestation; GOL, golimumab; IFX, infliximab; MTX, methotrexate.

[a] Significant finding ($P < .05$).

Table 2 Logistic regression on long-term 1-year therapy persistence (n = 105).

	Univariable analysis			Multivariable analysis
	Yes	No	OR (95% CI), *P*	OR (95% CI), *P*
Age at diagnosis, y	41 (25-52)	35 (23-48)	1.02 (0.98 to 1.04), .316	—
OCTN1				
CC (WT) (Ref.)	15 (44.1)	19 (55.9)	—	—
CT	15 (30.6)	34 (69.4)	1.79 (0.72 to 4.44), .210	1.70 (0.67 to 4.36), .264
TT	1 (4.6)	21 (95.5)	16.58 (1.99 to 137.70), .009[a]	17.71 (2.05 to 152.54), .009[a]
Therapy				
IFX (Ref.)	57 (76.0)	18 (24)	—	—
ADA	7 (46.7)	18 (53.3)	0.28 (0.08 to 0.87), .028[a]	0.24 (0.07 to 0.87), .030[a]
GOL	10 (66.7)	5 (33.3)	0.63 (0.19 to 2.09), .452	0.63 (0.18 to 2.21), .466
Female (Ref.: male)	34 (70.8)	14 (29.2)	1.03 (0.44 to 2.39), .941	—
Disease duration, y	4.5 (2-8)	3 (1-8)	1.01 (0.94 to 1.08), .799	—
Localization				—
Left colitis (Ref.)	32 (77.7)	12 (27.3)	—	
Pancolitis	37 (66.1)	19 (33.9)	0.73 (0.30 to 1.73), .476	
Previous AZA/MTX (yes)	20 (71.4)	8 (28.6)	1.06 (0.41 to 2.76), .897	—
Previous vedolizumab (yes)	1 (25.0)	3 (75.0)	0.13 (0.12 to 1.28), .080	NS
Concomitant mesalamine therapy (yes)	65 (74.7)	22 (25.3)	2.53 (0.76 to 8.34), .127	NS
Concomitant steroid therapy (yes)	45 (68.2)	21 (31.8)	0.63 (0.25 to 1.63), .346	—
Combination therapy with AZA/MTX (yes)	9 (64.3)	5 (35.7)	0.72 (0.22 to 2.38), .597	—
Baseline MES	3 (2-3)	3 (2-3)	0.92 (0.50 to 1.71), .800	—
EIM (yes)	22 (61.1)	14 (38.9)	0.51 (−0.21 to 1.22), .131	NS

Values are median (interquartile range) or n (%).
Abbreviations: AZA, azathioprine; CI, confidence interval; EIM, extraintestinal manifestation; GOL, golimumab; IFX, infliximab; MES, Mayo endoscopic score; MTX, methotrexate; NS, not significant; OR, odds ratio.
a Significant finding ($P < .05$).

learning purposes, the OCTN1 genotype was dichotomized into homozygous mutated (TT) vs all other variants (CT+CC). In the full-variable analysis of SFR, the neural network achieved an AUC of 0.627, while the corresponding logistic regression yielded an AUC of 0.683 (Figures 4A and 4B) To reduce model complexity and evaluate clinically meaningful parameters, we repeated the analysis using a restricted variable set selected based on clinical relevance. In this reduced model, the neural network achieved an AUC of 0.688 and the logistic regression an AUC of 0.700 (Figures 4C and 4D) We next evaluated the remaining endpoints using LASSO logistic regression. The model showed moderate discrimination for endoscopic response (AUC 0.601) and therapy persistence (AUC 0.717) (Figures 4E and 4F).

Across all models, the variable-ranking analysis repeatedly positioned the OCTN1 genotype among the relevant predictors, suggesting a possible contribution to the evaluated clinical outcomes, although not as the predominant determinant.

Discussion

This exploratory study highlights a potential role for the OCTN1 rs1050152 polymorphism (L503F) in modulating both innate immune responses and long-term clinical outcomes in UC patients treated with anti-TNFα agents. We found that the homozygous 503F genotype (TT) was associated with better outcomes, including higher rates of steroid-free clinical remission, greater endoscopic response, and increased 1-year therapy persistence, with heterozygous carriers (CT) showing an intermediate phenotype. These observations were consistent across univariate and multivariable analyses and were further supported by an exploratory machine learning evaluation. In this latter analysis, OCTN1 (dichotomized as TT vs CT+CC) repeatedly ranked among the relevant predictors of clinical endpoints across LASSO logistic regression and neural network models, although not as the dominant determinant. The machine learning component, strengthened by parameter tuning and regularization to reduce overfitting, reinforces but does not overstate the contribution of OCTN1 to outcome variability.

Mechanistically, our in vitro findings showed that monocytes carrying the 503F variant produce significantly higher IL-1β upon stimulation with bacterial components. This suggests a gain-of-function phenotype in early innate signaling, potentially amplifying T helper 1–skewed responses and possibly augmenting downstream TNFα production. Such hyperresponsiveness may increase susceptibility to inflammation but also enhance sensitivity to TNFα blockade. To reduce confounding, we excluded patients with anti-drug antibodies detection during the observation phase or allergic

Table 3 Logistic regression on long-term 1-year steroid-free remission (n = 105).

	Univariable analysis			Multivariable analysis
	Yes	No	OR (95% CI), *P*	Or (95% CI), *P*
Age at diagnosis, y	32.5 (20-44)	31 (20-43)	1.00 (0.97-1.03), .961	—
OCTN1				
CC (WT) (Ref.)	14 (41.2)	20 (58.8)	—	—
CT	21 (42.9)	28 (57.1)	1.07 (0.44-2.60), .879	0.87 (0.33-2.25), .775
TT	17 (77.3)	5 (22.7)	4.86 (1.45-16.3), .010[a]	4.82 (1.29-17.9), .019[a]
Therapy				
IFX (Ref.)	41 (54.7)	34 (45.3)	—	—
ADA	3 (20.0)	12 (80.0)	0.21 (0.05 to 0.79), .022[a]	0.27 (0.06-1.25), .095
GOL	8 (53.3)	7 (46.7)	0.95 (0.31-2.88), .925	0.92 (0.28-3.05), .904
Female *(Ref: male)*	24 (50.0)	24 (50.0)	1.04 (0.48-2.23), .929	—
Disease duration, y	4 (1.5-7)	4 (1-9)	0.99 (0.93-1.05), .645	—
Localization				—
Left colitis (Ref.)	22 (50.0)	22 (50.0)	—	
Pancolitis	28 (50.0)	28 50.0)	1 (0.45-2.2), 1.000	
Previous AZA/MTX (yes)	16 (57.1)	12 (42.9)	1.52 (0.63-3.62), .348	—
Previous vedolizumab (yes)	0 (0.0)	4 (100.0)	1 (—), —	—
Concomitant mesalamine TP (yes)	47 (54.0)	40 (46.0)	2.64 (0.76-9.24), .128	NS
Concomitant steroid TP (yes)	31 (47.0)	35 (53.0)	0.66 (0.29-1.52), .332	—
Combination TP with AZA/MTX (yes)	4 (28.6)	10 (71.4)	0.36 (0.10-1.22), .101	NS
Baseline MES	2.5 (2-3)	3 (2-3)	0.98(0.56-1.69), .933	—
EIM (yes)	10 (27.8)	26 (72.2)	0.25(0.10-0.59), .002[a]	0.30 (0.12-0.79), .015[a]

Values are median (interquartile range) or n (%).
Abbreviations: AZA, azathioprine; CI, confidence interval; EIM, extraintestinal manifestation; GOL, golimumab; IFX, infliximab; MES, Mayo endoscopic score; MTX, methotrexate; NS, not significant; OR, odds ratio.
a Significant finding ($P < .05$).

reactions to anti-TNFα therapy. In our real-life setting, therapeutic drug monitoring is not routinely performed, as trough level and antidrug antibody testing are generally at the patient's expense and current guidance does not systematically recommend proactive monitoring. For this reason, only patients with documented antibody-mediated failure were excluded at baseline or during the observation period, whereas among those enrolled and completing the study—including nonresponders—systematic drug-level and antibody data were not available. Consequently, we could not formally distinguish immunogenic from pharmacodynamic failure or explore genotype-specific failure patterns.

These results contribute to the growing field of precision medicine in IBD, in which biomarkers guiding drug selection are increasingly needed. Although promising, our study has limitations. In fact, the relatively small overall sample size, and particularly the limited number of TT homozygotes (n = 22), represent an important limitation of this exploratory pharmacogenetic study. The small size of this subgroup inevitably reduced statistical precision and contributed to wide confidence intervals around the estimated ORs. In this context, effect sizes may appear inflated, especially in the presence of low event numbers within genotype strata. Such imprecision limits the robustness of the magnitude of association, even when statistical significance is achieved. Therefore, the strength of the observed associations should be interpreted with caution.[14]

In addition, monocentric and ambispective design limits the external validity of our findings, as center-specific clinical practices and patient selection may have influenced outcomes. Pharmacogenetic associations are particularly susceptible to context-dependent effects and require independent replication to exclude chance findings. Future multicenter, prospectively designed studies with external validation cohorts will be essential to confirm reproducibility and to clarify the real-world clinical applicability of OCTN1 genotyping.[15]

The exploratory machine learning analysis must also be interpreted in light of important methodological constraints. The dataset size was limited for neural network modelling, increasing the risk of instability and overfitting, particularly in a small pharmacogenetic cohort. Notably, the observed AUC values (0.601-0.72) indicate only modest discriminatory ability; however, excessively high AUCs in small datasets would have raised even greater concerns for overfitting. In this context, the machine learning approach should not be viewed as a definitive predictive tool, but rather as complementary to traditional regression analyses. Its main contribution was to provide a prediction-oriented perspective and to reinforce the relative importance of OCTN1 among the evaluated variables, offering an additional layer of interpretative support rather than conclusive evidence.

Preclinical findings are also novel but preliminary, with a mechanistic connection between OCTN1 variants and proinflammatory monocyte responses still missing. In general, while focusing on a single genetic marker has merit for clinical translatability, UC pathogenesis is multifactorial, and multiomics approaches integrating microbial, genomic, and immune signatures will likely yield more powerful predictive models.

Figure 4 Machine learning models for the prediction of clinical outcomes. Neural network model (ReLU activation, Adam solver, 500 iterations) applied to the full variable set for predicting steroid-free remission (SFR). (B) LASSO (L1) logistic regression on the same full SFR dataset. (C) Neural network trained on a reduced clinically selected variable set for SFR. (D) LASSO (L1) logistic regression on the reduced SFR variable set. (E) LASSO logistic regression for predicting endoscopic response. (F) LASSO logistic regression for predicting 1-year therapy persistence. Parameter tuning (training/test proportion, regularization strength, and model complexity) was performed to maximize area under the curve. Numeric area under the curvevalues are reported within each panel. The OCTN1 genotype consistently ranked among the most influential predictors across models. AZA, azathioprine; EIM, extraintestinal manifestation; MES, Mayo endoscopic score; MTX, methotrexate.

As mentioned, future multicenter, prospectively designed studies with larger and ethnically diverse cohorts are required to validate the association between OCTN1 genotype and anti-TNF response, ensuring adequate power and external reproducibility. Independent validation datasets will be essential to confirm effect size estimates and to refine predictive modelling approaches. Integration of OCTN1 genotyping within broader multiomics and clinically annotated frameworks may ultimately clarify its role in precision-medicine strategies for UC.

In conclusion, OCTN1 rs1050152 genotyping emerges as an investigational biomarker associated with improved clinical and endoscopic responses to anti-TNFα therapy, supporting the concept of genetically defined, cytokine-driven UC endotypes that may benefit from tailored treatment strategies.

Supplementary material

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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Conflicts of interest

No conflict of interest to declare by all authors.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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