Biomedicine & Pharmacotherapy 159 (2023) 114225

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Single nucleotide polymorphisms in *ADAM17*, *IL23R* and *SLCO1C1* genes protect against infliximab failure in adults with Crohn's disease

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ARTICLE INFO

Keywords:
Crohn's disease
Infliximab
Long-term response
Pharmacogenomics
Single nucleotide polymorphisms

ABSTRACT

Background: To predict primary failure of infliximab (IFX) therapy in Crohn's disease (CD) and to identify patients who maintain long-term effectiveness to IFX is currently not feasible. Some genetic variations are proposed as potential biomarkers.

Aim: We assessed a set of single nucleotide polymorphisms (SNPs) in genes related to the IFX mechanism of action and the presence of HLA-DQA1 * 05 allele on the primary response and long-term durability in CD patients.

Methods: A multi-centre cross-sectional study of IFX-exposed adult patients with CD was undertaken. Treatment persistence and time to failure were co-primary endpoints. DNA from the 131 patients was genotyped. Association between SNPs and clinical variables with IFX persistence was assessed.

Results: Failure to IFX was documented in 65 (49.6%) out of 131 patients. IFX persistence was associated either with carrying the TT genotype in *ADAM17* rs10929587 (ORa=0.2; 95%CI=0.1–0.8; p = 0.021), or the CC genotype in *SLCO1C1* rs3794271 (ORa=0.2; 95%CI=0.1–0.7; p = 0.008), according to multivariate logistic regression. In contrast, previous bowel resection increased the risk of IFX failure (ORa=2.8; 95%CI=1.1–7.3; p = 0.025). Cox regression analysis confirmed these findings and also identified *IL23R* rs10489629-TT (HRa 0.41; 95%CI=0.22–0.75; p = 0.004) and concomitant immunosuppressants (HRa 0.46; 95%CI=0.27–0.77; p = 0.003) as protection from IFX failure. However, no association between HLA-DQA1 * 05 allele and persistence of IFX therapy was found, with similar failure rates among carriers and non-carriers (52.8% *vs.* 47.4%, respectively; p = 0.544).

Conclusions: SNPs rs10929587-TT in *ADAM17*, rs10489629-TT in *IL23R* and rs3794271-CC in *SLCO1C1*, together with no previous bowel surgery and concomitant immunosuppression, were identified as protection from failure to IFX.

Abbreviations: CD, Crohn's disease; CI, confidence interval; HRa, adjusted hazard ratio; IBD, inflammatory bowel disease; IQR, interquartile range; IFX, infliximab; ORa, adjusted odds ratio; NF-kβ, nuclear factor kappa-light-chain-enhancer of activated B cells; SNP, single-nucleotide polymorphism; SSO, sequence-specific oligonucleotide typing; TDM, therapeutic drug monitoring; TNFα, tumour necrosis factor α.

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https://doi.org/10.1016/j.biopha.2023.114225
Received 14 November 2022; Received in revised form 3 January 2023; Accepted 4 January 2023
Available online 6 January 2023

1. Introduction

Crohn's disease (CD), one of the two main forms of inflammatory bowel disease (IBD), is a chronic inflammatory condition that may affect any part of the digestive tract, and is usually diagnosed in young people. While the specific aetiology of IBD remains unknown, CD involves a complex impairment in the immunity of the gut mucosa due to a combination of several genetic and environmental factors [1]. With more than 300 per 100,000 persons affected in developed countries [2], symptoms of CD may be either constant or alternate between periods of limited disease activity and flares with remarkable presence of symptoms. As CD is associated with significant morbidity and disability [3], pharmacological treatment is required, aimed at reducing the inflammatory response and attenuating the activity of the immune system. In the moderate and severe forms of the disease, therapy is usually based on immunosuppressant and/or biological drugs.

Infliximab (IFX) was the first biological drug approved to treat CD patients. IFX is a chimeric monoclonal antibody used against tumour necrosis factor α (TNFα) that has proved to be effective in inducing and maintaining remission in IBD and other immune-mediated disorders (mainly rheumatoid arthritis, psoriasis, and ankylosing spondylitis) [4]. Apart from the originator IFX (Remicade®), several biosimilar drugs are currently available with comparable efficacy and safety but at a lower cost [5]. IFX however continues to be used as a common first-line biological therapy for CD [6], despite other biological drugs being available (adalimumab, vedolizumab, ustekinumab).

Yet some patients do not respond to IFX induction (primary non-responders) and others can lose response during maintenance (secondary non-responders) [7]. Causes of the lack of response are largely unknown, but one of the most accepted explanations is the immunogenicity of IFX, which triggers the development of neutralising antibodies in some patients [8]. Combination therapy of IFX with immunosuppressant drugs (such as azathioprine) reduces immunogenicity, thus improving IFX pharmacokinetics and extending effectiveness [9].

The search for clinical variables and biomarkers that could predict the response to IFX (and to other alternative biological drugs) in advance has not yielded desirable results so far and there are no guidelines recommendations as yet. Among potential tools able to predict IFX response, genetic variations have attracted special attention and several studies have analysed single-nucleotide polymorphisms (SNPs) as potential markers [10,11]. These mostly include genes involved in the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kβ) pathway, TNFα receptors and downstream signalling, release of inflammatory mediators (such as certain types of interleukins), apoptosis and autophagy [12].

Carrying the HLA-DQA1 * 05 allele has recently been associated with an increased risk of immunogenicity to anti-TNFα drugs in CD [13] and other immune-mediated disorders [14]. Whether this could lead to early loss of response to these drugs remains controversial, however, with some data supporting this hypothesis [15], while others describing the risk suggest it could be overcome by adding immunosuppressive therapy [13] or by avoiding sub-therapeutic drug concentrations [16].

This work evaluates the carrier status of a broad set of SNPs in genes involved in the mechanism of action of IFX and HLA-DQA1 * 05 on the primary response to this biological drug in patients with CD, and its long-term durability. From this we aim to identify potential clinical variables and genetic markers capable of predicting a sustained response to IFX therapy.

2. Methods

2.1. Study design and patients

This was a cross-sectional study carried out on a prospectively-followed cohort of adult patients with CD, recruited at the IBD Units of two collaborative sites, *Hospital General Universitario Gregorio Marañón* (HGUGM) and *Hospital General de Tomelloso* (HGT).

One hundred and thirty-one patients aged over 18 years, diagnosed with CD and who were treated at any time with IFX, were recruited [17]. These included both patients who had stopped IFX therapy after primary or secondary failure, as well as patients who were continuing on IFX as maintenance therapy at the time of data analysis (October 31, 2021). All patients provided consent to obtain blood samples.

The following clinical and demographical variables were collected for all patients: date of birth, sex, hospital, smoking status, date of CD diagnosis, CD location, CD-related comorbidities, bowel resection prior to IFX therapy onset, fistula surgery prior to IFX onset, line of anti-TNF for IFX treatment, need for intensification during IFX treatment, concomitant treatment with immunosuppressant drugs at any time during IFX therapy, date of IFX treatment initiation, and date of failure to IFX treatment or date of end of follow-up.

The co-primary endpoints were failure to respond to IFX treatment and therapy length up to point of failure (treatment persistence). IFX therapy failure was defined as follows: withdrawal of IFX, and/or switching to another drug due to loss of effectiveness according to clinical, biochemical and endoscopic data, or the need for abdominal surgery related to CD progression. Time-to-failure was not available for one patient referred from another hospital.

The study was conducted in accordance with the principles of the Declaration of Helsinki, with approval by the corresponding local Ethics Committees. All patients provided written informed consent for pharmacogenetic analysis.

2.2. Single nucleotide polymorphisms analysed

A literature review was performed to search for candidate SNPs associated with anti-TNF response, by searching in PubMed and the National Library of Medicine SNP database. These included genes of the TNF pathway, genes up- or down-regulated by anti-TNF, genes related to anti-TNF response or involved in TNF signalling in any inflammatory or autoimmune disease, as well as allelic variants. In this search that included research published until end of 2020, 66 SNPs were selected for genotyping in our patients (Supplementary Table 1).

2.3. DNA isolation and genotyping

DNA was isolated using the NucleoSpin® Tissue kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. All DNA variants, except for HLA-DQA1 * 05 rs2097432, were genotyped using KASP™ technology. DNAs were sent to LGC Biosearch Technologies and genotypes were performed using their genotyping service (LGC Biosearch Technologies, London, UK).

The SNP rs2097432 was genotyped in-house using rhAMP (IDT, Neward, NJ, USA). Briefly, 1 μL of DNA (10 ng) was amplified using 2.5 μL rhAMP genotyping master mix, 0.15 μL rhAMP reporter mix and 0.125 μL probe in 5 μL of final volume. Cycling conditions were: 25ºC for 30 s; denaturation at 95ºC for 10 min; 40 cycles of amplification (or 50 for undetermined samples) - 95ºC for 10 s, 60ºC for 30 s, 68ºC for 20 s; and 25ºC for 30 s. Genotyping was done in a QuantStudio 3 and analysed using QuantStudio Design and Analysis software (Applied Biosystems, Waltham, MS, USA).

2.4. HLA-DQA1 sequence-specific oligonucleotide (SSO) typing

HLA-DQA1 typing was performed by using the LIFECODES HLA-DQA1/B1 SSO Typing Kit (Immucor, Waukesha, WI, USA) following manufacturer protocol. Briefly, DNA amplification was obtained by using a PCR mixture prepared with 6 μL of the LIFECODES Master Mix (Immucor), 80 ng of genomic DNA, and 1 U Taq polymerase in a final volume of 20 μL. The thermal cycler conditions were: denaturation at 95 °C for 3 min; 40 cycles of amplification (12 cycles: 95 °C for 15 s, 60 °C

for 30 s, 72 °C for 30 s, and 28 cycles: 95 °C for 10 s, 63 °C for 30 s, 72 °C for 30 s); and extension at 72 °C for 1 min. Hybridisation was performed using a mixture of 15 μL of probe mix and 5 μL of the PCR product under the following conditions: 97 °C for 2 min, 47 °C for 10 min, and 56 °C for 8 min. Each sample was then immediately mixed at 56 °C with 170 μL of the 1:200 pre-diluted streptavidin-phycoerythrin solution and analysed within 30 min by using the Luminex 200 system (Luminex Corporation, Austin, TX, USA). HLA-DQA1 alleles were assigned by using MatchIT DNA program v1.2.4.2 (Immucor) with IMGT/HLA Sequence Database Release 3.43.

2.5. Statistical analysis

Median and interquartile range (IQR) were used for continuous variables and qualitative variables were presented as absolute and relative frequencies. Comparisons between groups were performed with $\chi 2$ test (or Fisher's exact test wherever appropriate) for categorical variables and Mann-Whitney *U* test for continuous variables.

To analyse the IFX persistence, a multivariate logistic regression analysis, including SNPs with a p-value < 0.1 in the univariate analysis and four relevant clinical variables, was performed. Odds ratio adjusted by age of IFX onset (ORa) and 95% confidence intervals (CI) were calculated. Finally, the association between genotypes and long-term response to IFX was analysed using Kaplan-Meier curves (time-to-failure curves), with p-value calculated by the Mantel-Cox method (log rank). Hazard ratios adjusted by age of IFX onset (HRa) and their 95%CI were calculated using multivariate Cox regression analysis.

Analyses were carried out using PASW 18.0 statistical analysis software (SPSS Inc, Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was considered when $p < 0.05$.

3. Results

3.1. Demographic characteristics

Failure to IFX was documented in 65 (49.6%) of the 131 patients. Demographic and clinical variables of the study population are described in Table 1. Median (IQR) time until IFX failure was 3.4 (1.1–5.9) years while median follow-up for patients with IFX persistence was 9.7 (8.0–12.1) years.

A higher IFX failure rate was documented in HGUGM, as a subset of 38 patients who had all failed to respond to IFX before 2015 were recruited at this hospital, five out of whom were primarily failures. Remaining clinical variables showed no differences between patients with IFX persistence and those who failed to respond to IFX. IFX line therapy (first- or second-) and concomitant immunosuppression were close to the significance level: patients naive to anti-TNFα (IFX as first-line) presented higher treatment persistence than those who previously had failed to respond to adalimumab (52.9% *vs.* 25%, $p = 0.065$). In addition, concomitant immunosuppressant treatment at any point during IFX treatment tended to provide higher persistence of IFX therapy compared to monotherapy (58.6% *vs.* 43.3%, $p = 0.080$).

Table 1
Characteristics of the patients included in the study, classified according to IFX persistence or failure.

		Overall (n = 131)	IFX persistence (n = 66)	IFX failure (n = 65)	p
Gender	Female	65 (49.6%)	29 (44.6%)	36 (55.4%)	0.190
	Male	66 (50.4%)	37 (56.1%)	29 (43.9%)	
Recruiting centre	HGUGM	104 (79.4%)	47 (45.2%)	57 (54.8%)	**0.020**
	HGT	27 (20.6%)	19 (70.4%)	8 (29.6%)	
Smoking	Never	66 (50.4%)	31 (47%)	35 (53%)	0.544
	Former	38 (29%)	22 (57.9%)	16 (42.1%)	
	Current	27 (20.6%)	13 (48.1%)	14 (51.9%)	
CD localisation	Ileal	51 (38.9%)	24 (47.1%)	27 (52.9%)	0.700
	Colonic	17 (13.0%)	10 (58.8%)	7 (41.2%)	
	Ileocolonic	63 (48.1%)	32 (50.8%)	31 (49.2%)	
CD-related comorbidities	Yes	95 (72.5%)	44 (46.3%)	54 (53.7%)	0.131
	No	36 (27.5%)	22 (61.1%)	14 (38.9%)	
Previous intestinal resection	Yes	35 (26.7%)	14 (40%)	21 (60%)	0.151
	No	96 (73.3%)	52 (54.2%)	44 (45.8%)	
Previous fistula surgery	Yes	15 (11.5%)	10 (66.7%)	5 (33.3%)	0.180
	No	116 (88.5%)	56 (48.3%)	60 (51.7%)	
Line of anti-TNFα	1st line	119 (90.8%)	63 (52.9%)	56 (47.1%)	0.065
	2nd line	12 (9.2%)	3 (25%)	9 (75%)	
Need of IFX intensification	Yes	60 (45.8%)	30 (50%)	30 (50%)	0.486
	No	64 (48.9%)	36 (56.3%)	28 (43.8%)	
	Unknown	7 (5.3%)	-	7	-
Concomitant treatment with IS	Yes	68 (51.9%)	40 (58.6%)	28 (41.4%)	0.080
	No	60 (45.8%)	26 (43.3%)	34 (56.7%)	
	Unknown	3 (2.3%)	-	3	-
Type of IS	AZA	54 (79.4%)	34 (63%)	20 (37%)	0.085
	MTX	7 (10.3%)	1 (14.3%)	6 (85.7%)	
	6-MP	4 (3.1%)	3 (75%)	1 (25%)	
	MPA	3 (2.3%)	2 (66.7%)	1 (33.3%)	
Age at IFX onset (years)		36.4 (27.7–46.0)	36.7 (27.2–48.2)	35.9 (28.4–45.1)	0.878
Years of CD until IFX onset		5.3 (1.7–10.4)	4.2 (1.0–10.4)	5.8 (2.4–10.6)	0.275
Months of IFX until failure				41.1 (12.8–70.6)	-
Months of IFX until end follow-up			116.1 (95.6–145.6)		-

IFX: infliximab; HGUGM: Hospital General Universitario Gregorio Marañón; HGT: Hospital General de Tomelloso; CD: Crohn's disease; TNF: tumour necrosis factor; IS: immunosupressant; AZA: azatioprine; MTX: metotrexate; 6-MP: 6-mercaptopurine; MPA: mycophenolic acid. Quantitative variables were represented as median (interquartile range).

3.2. SNPs associated with IFX persistence

The association of DNA variants with persistence of IFX therapy was analysed using contingency tables in a co-dominant model (differentiating the three possible genotypes). From the 66 SNPs genotyped, six showed p-values lower than 0.1, but only one (*SLCO1C1* rs3794271) was significant at $p < 0.05$ (Table 2). Interestingly, the failure rate to respond to IFX was $\leq$ 30% for three of these SNPs: *ADAM17* rs10929587-TT, *ADAM17* rs2276338-CC and *SLCO1C1* rs3794271-CC. When dominant or recessive model analyses were applied, two more SNPs (*IL1B* rs1143634 and *IL23R* rs10489629) reached statistical significance (Table 2).

The two SNPs in the *ADAM17* gene were in linkage disequilibrium and all patients with the rs10929587-TT genotype also had rs2276338-CC. The correlation between rs10929587(T) and rs2276338(C) alleles was confirmed by using the LDpair Tool (https://ldlink.nci.nih.gov/) in European populations ($D'=1$, $R^2=0.702$, $p < 0.0001$).

Kaplan-Meier analysis was then performed in those six SNPs also considering co-dominant and the best of dominant or recessive models (Fig. 1). In the latter comparison, one SNP achieved a p-value< 0.05 (*IL23R* rs10489629), while the other five SNPs were close to significance (p-values ranging from 0.053 to 0.074) (Table 2).

3.3. HLA-DQA1 * 05 in IFX persistence

The influence of HLA-DQA1 * 05 on the long-term persistence of IFX therapy was evaluated using two approaches: (1) analysing the HLA-DQA1 * 05 carriage by sequence-specific oligonucleotide (SSO) typing, and (2) determining genetic variations in rs2097432 (TT, CT or CC). The results for each approach are showed in Table 3.

When compared, the TT genotype was present in most of the non-carriers of HLA-DQA1 * 05, while genotypes TC and CC were detected in the majority of patients with HLA-DQA1 * 05 in heterozygosis or homozygosis. Only three discrepancies were found in patients with TT genotype but being HLA-DQA1 * 05 carriers (all of them heterozygous). Therefore, the rate of concordance between both methods was 97.7%. Overall, HLA-DQA1 * 05 was present in 40.5% of the patients and rs20997432-CC/TC in 38.2%.

No association between HLA-DQA1 * 05 typing and persistence of IFX therapy was found, with failure rates being similar among HLA-DQA1 * 05 carriers and non-carriers (52.8% *vs.* 47.4%, $p = 0.544$). Similar results were also observed for rs2097432-CC/TC compared to rs2097432-TT (52.0% *vs.* 48.1%, $p = 0.668$). Likewise, time-to-failure was not significantly different for HLA-DQA1 * 05 ($p = 0.574$) and rs2097432-CC/TC carriers ($p = 0.658$) in Kaplan-Meier analyses (Supplementary Fig. 1).

3.4. Multivariate logistic regression analysis for association with IFX persistence

In a multivariate logistic regression analysis including the six SNPs selected in the univariate analysis (grouped by dominant or recessive models) and four clinical variables (patient age at IFX therapy onset, presence of CD-related comorbidities, bowel resection previous to IFX therapy and concomitant immunosuppression), two SNPs provided significant differences. In the final model of the multivariate analysis, both the presence of the TT genotype in *ADAM17*rs10929587 (ORa=0.2; 95%CI=0.1–0.8; $p = 0.021$) and the CC genotype in *SLCO1C1* rs3794271 (ORa=0.2; 95%CI=0.1–0.7; $p = 0.008$) was associated with reduced risk of IFX failure, thus having a protective effect. Therefore, presenting either one of these involved a reduced risk of IFX failure (28.6% *vs.* 57.9%; OR=0.49; 95%CI=0.28–0.86; $p = 0.003$) (Table 4).

In addition to these two SNPs, bowel resection prior to starting on IFX therapy was revealed as a risk factor for IFX failure in the multivariate model (ORa=2.8; 95%CI=1.1–7.3; $p = 0.025$). Consequently, persistence of IFX therapy was high (84.2%) when either of the two protective genotypes was present in patients without a previous bowel resection (Table 4).

3.5. Multivariate Cox regression analysis

A multivariate Cox regression analysis was carried out considering time to failure to respond to IFX and end of follow-up. The variables included were the same as those used in the multivariate logistic regression analysis. In this case, three SNPs and concomitant immunosuppressant treatment added to IFX showed statistical significance in the final multivariate model (Table 5).

When these findings were plotted in a survival curve and analysed by the Kaplan-Meier test, the presence of any of the three SNPs (*ADAM17* rs10929587-TT or *IL23R* rs10489629-TT or *SLCO1C1* rs3794271-CC) showed a protective effect against failure to respond to IFX ($p = 0.001$) (Fig. 2A). When concomitant immunosuppression was also considered, a protective effect was observed in those patients without any of the three SNPs, while no influence on IFX persistence was noticed

Table 2
Univariate and Kaplan-Meier results for those single nucleotide polymorphisms (SNPs) which showed a p-value< 0.1 in the three-genotype contingency analysis. Genotypes were ordered displaying the homozygous with higher IFX failure first. Dominant/recessive (D/R) model was applied to each SNP and p-values recalculated. P-values from univariate analysis were calculated using chi-square or Fisher's exact tests and p-values from Kaplan-Meier analysis were calculated using Mantel-Cox test.

SNP		Frequency (%)	IFX failure (%)		Univariate (p-value)		Kaplan-Meier (p-value)	
			3 genotypes	D/R model	3 genotypes	D/R model	3 genotypes	D/R model
ADAM17 rs10929587 n = 130	AA	54.6	47.9	53.0	0.059	0.055	0.052	0.074
	AT	33.9	61.4					
	TT	11.5	26.7	26.7				
ADAM17 rs2276338 n = 130	TT	42.3	49.1	53.6	0.096	0.052	0.099	0.053
	TC	42.3	58.2					
	CC	15.4	30.0	30.0				
IL1B rs1143634 n = 130	TT	5.4	57.1	61.5	0.096	**0.032**	0.181	0.065
	TC	34.6	62.2					
	CC	60.0	42.3	42.3				
IL23R rs10489629 n = 129	CC	24.0	58.1	57.8	0.076	**0.023**	0.094	**0.030**
	CT	40.3	57.7					
	TT	35.7	37.0	37.0				
SLCO1C1 rs3794271 n = 130	TT	40.0	59.6	55.1	**0.027**	**0.011**	0.094	0.053
	TC	42.3	50.9					
	CC	17.7	26.1	26.1				
TNFRSF1B rs1061624 n = 131	AA	17.5	43.5	54.7	0.076	0.057	0.065	0.062
	AG	55.0	58.3					
	GG	27.5	36.1	36.1				

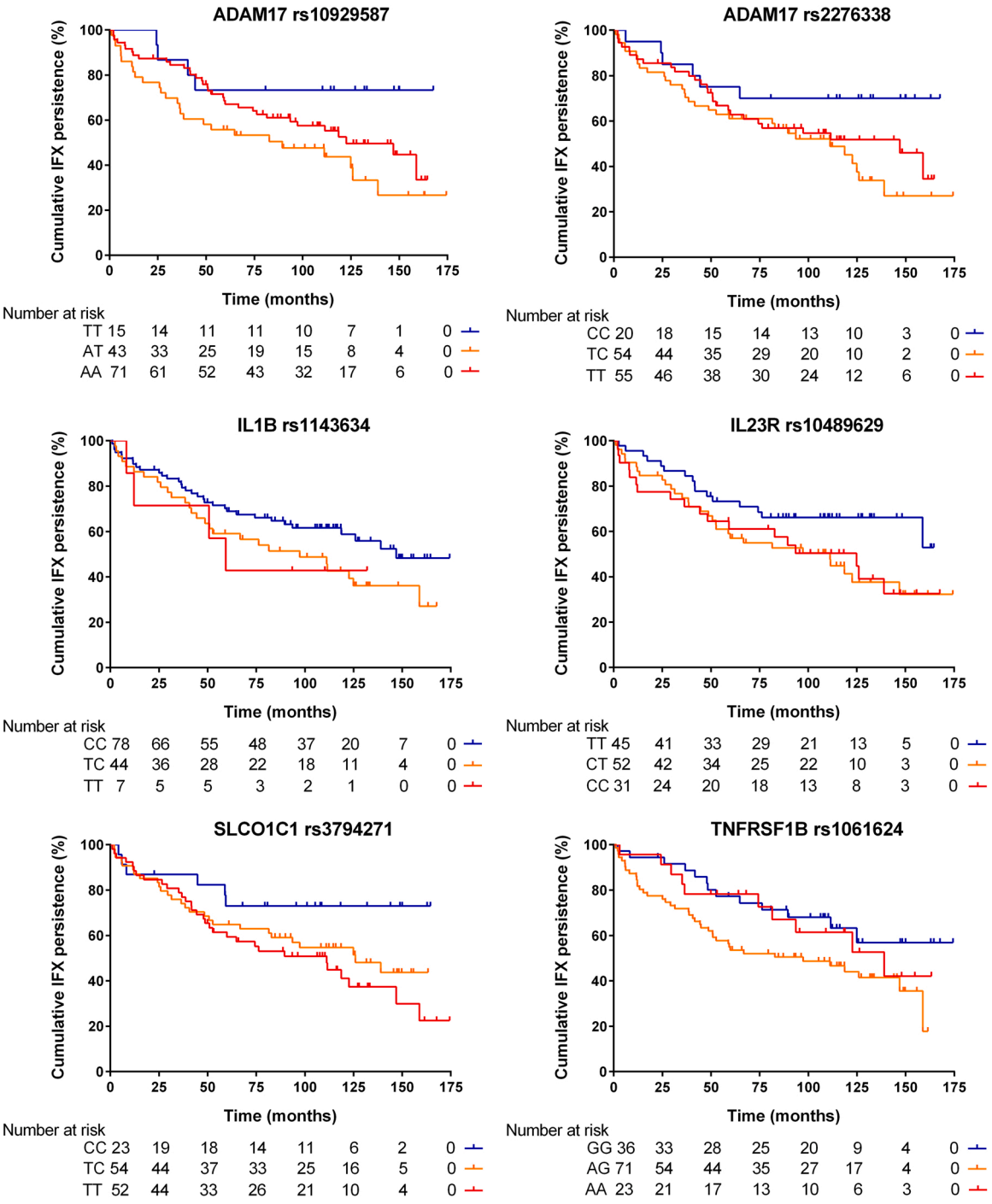

Fig. 1. Kaplan-Meier curves for the six SNPs associated with IFX persistence in the univariate analysis. Vertical lines indicate censored cases.

in patients with at least one of those SNPs (Fig. 2B). Kaplan-Meier testing displayed statistical significance when the four groups were compared ($p < 0.001$).

Similarly, contingency analyses for the presence of any of these three SNPs showed a protective effect against failure to respond to IFX (OR: 0.25; 95%CI=0.12–0.51) and a strong association with long-term persistence of IFX therapy ($p < 0.001$) (Table 6). Interestingly, a remarkably high rate of IFX failure of 88.9% was observed in the 27 patients having none of the three SNPs nor immunosuppression.

Finally, a higher persistence rate of IFX therapy was found when at least two of these genotypes were present (from 66.6% to 100%). However, the number of patients with any SNP combination was low (Supplementary Table 2).

4. Discussion

Our cohort of CD patients exposed to IFX therapy has one of the longest follow-up periods (a median of almost 10 years for patients with IFX persistence) and includes data from real-world clinical practice. This allowed us to analyse clinical variables and genetic variations associated with IFX therapy failure and sustained effectiveness more thoroughly. Of the 66 SNPs genotyped, we identified three SNPs that when present, protect from failure to respond to IFX, with immunosuppression and lack of previous bowel resection also contributing to long-term treatment persistence. The risk of failure to respond to IFX among the 55% of CD patients in our cohort who presented any of those three SNPs (*ADAM17* rs10929587-TT or *IL23R* rs10489629-TT or *SLCO1C1* rs3794271-CC)

Table 3
Results of HLA-DQA1 * 05 obtained by sequence-specific oligonucleotide (SSO) typing and by analysing the genetic variation rs2097432.

HLA-DQA1 * 05			rs2097432		
			TT	TC	CC
Homozygous n = 5 (3.8%)	05:01/05:03	1 (0.8%)			1
	05:01/05:05	2 (1.5%)			2
	05:03/05:05	1 (0.8%)			1
	05:05/05:05	1 (0.8%)		1	
Heterozygous n = 48 (36.6%)	05:01	18 (13.7%)		6	12
	05:03	1 (0.8%)			1
	05:05	29 (22.1%)	3	11	15
Overall carriers		53 (40.5%)	3	18	32
Non-carriers		78 (59.5%)	78		
Total		131	81 (61.8%)	18 (13.7%)	32 (24.5%)

Table 4
Contingency table of association to IFX persistence for the presence of either ADAM17 rs10929587-TT or SLCO1C1 rs3794271-CC and taking into account bowel resection prior to IFX therapy onset.

	Persistence	Failure	Total	p-value
Either of the two SNPs	25 (71.4%)	10 (28.6%)	35	**0.003**
Neither of the two SNPs	40 (42.1%)	55 (57.9%)	95	
Either of the two SNPs with previous BR	9 (56.3%)	7 (43.7%)	16	**0.003**
Either of the two SNPs w/o previous BR	16 (84.2%)	3 (15.8%)	19	
Neither of the two SNPs with previous BR	5 (26.3%)	14 (73.7%)	19	
Neither of the two SNPs w/o previous BR	35 (46.1%)	41 (53.9%)	76	

SNP: single nucleotide polymorphism; BR: bowel resection; w/o: without.

Table 5
Results of the multivariate Cox regression analysis. Variables in the model were ordered according to the hazard ratio (from lower to higher).

	p-value	HRa	CI 95%
ADAM17 rs10929587-TT	0.010	0.21	0.07–0.70
IL23R rs10489629-TT	0.004	0.41	0.22–0.75
*SLCO1C1*rs3794271-CC	0.049	0.43	0.18–0.99
Immunosuppression	0.003	0.46	0.27–0.77

HRa: hazard ratio adjusted by age of IFX onset; CI: confidence interval of the hazard ratio.

was 75% lower. When these SNPs are lacking, concomitant immunosuppression might contribute to the odds of longer IFX therapy persistence.

ADAM17 gene product is a metalloproteinase that belongs to the adamlysin family, which is involved in the cleavage of certain membrane proteins, such as TNFα [18]. Consequently, it plays a role in regulating the concentration of soluble TNFα and thus could be implicated in the response to IFX. This fact was first investigated by Dideberg et al. in 222 Caucasian patients with CD to evaluate early response (10 weeks) to IFX [19]. They found that rs10929587-TT was one of the 10 SNPs in a haplotype that were significantly more frequent in clinical responders. Our data indicate that rs10929587-TT could play a predominant role among those SNPs in *ADAM17* haplotype in favouring a long-term persistence to IFX.

IL23R gene encodes a receptor for interleukin-23 expressed in several immune cells, such as monocytes, T lymphocytes and dendritic cells [20]. Once IL23 joins to its receptor, an intra-cellular cascade is generated to produce an inflammatory response. *IL23R* is known for being a susceptibility gene for several immune-mediated disorders, including CD [21], and also for rheumatoid arthritis, psoriasis, uveitis or Behçet disease [22,23]. A previous study investigating two SNPs in *IL23R* found an association with response after 6 months to anti-TNFα drugs in patients with psoriasis, but the SNP identified in our research (rs10489629) was not involved [24] and therefore not previously associated with IFX response in CD. Available evidence exclusively involving the rs10489629 variant identified it as a risk factor mainly for developing CD [25], rheumatoid arthritis [22] and ankylosing spondylitis [26].

SLCO1C1 gene encodes a sodium-independent transmembrane receptor that belongs to the organic anion-transporting polypeptide family, which is involved in the active transport of different organic molecules, toxins and drugs [27]. The presence of the minor allele of SNPrs3794271 (C) was previously associated with lack of clinical response to anti-TNFα therapy in rheumatoid arthritis [28] but with improved response to these drugs in psoriasis [29]. Our results were in agreement with the findings of the latter research, as the homozygous patients for the minor allele showed better long-term persistence to IFX.

Apart from genetic variation, we also found two clinical variables that could impact IFX persistence. In the multivariate logistic regression analysis for association with IFX persistence, the absence of a bowel resection previous to starting on IFX therapy was identified as a protective factor against IFX failure. Therefore, IFX persistence rate improved for patients without previous surgery from 56% to 84%, when protective SNPs were present, and from 26% to 46% if they were absent. Our finding was in agreement with the clinical model for predicting secondary non-response to IFX in CD proposed by Xu et al., which included previous surgery as one of the four items in the model that increased the odds of failure to IFX [30]. Concomitant immunosuppressive therapy was a second clinical variable that resulted significant in the multivariate Cox regression analysis. It is generally accepted that concomitant immunosuppression in IFX treatment provides improved effectiveness over IFX monotherapy [9,31], as also observed in our multivariate model. The lack of concomitant immunosuppression in the patients of our cohort who did not carry any of the protective SNPs identified, produced a dramatically high 89% rate of failure to IFX.

In contrast, an effect of HLA-DQA1 * 05 carriage on long-term IFX persistence was not noticed. Our cohort included 40.5% of patients who carried HLA-DQA1 * 05, which is in the range of 35–55% found in previous studies, and in an almost perfect match with a mean of 41% obtained from all studies [32]. Evidence so far proved a relationship between HLA-DQA1 * 05 and increased IFX immunogenicity [13,15], although this association was not significant in another study [33]. Despite this, response to IFX is not necessarily affected, as transient antibodies against IFX at low titters may not impact on IFX effectiveness in the same way as when persistent antibodies are found [34]. Reducing immunogenicity of IFX by adding immunosuppressive drugs [13] and monitoring of IFX trough levels to keep them in the therapeutic range [16] may overcome the problem of HLA-DQA1 * 05 carriage. In our representative cohort of patients in a real-world clinical context, the use of concomitant immunosuppressant treatment (52% of patients, 55% of non-carriers and 50% of carriers) and IFX intensification when trough levels were low (46% of patients, 49% of non-carriers and 47% of carriers, following reactive monitoring strategy) seemed to suggest the influence of HLA-DQA1 * 05 in long-term IFX persistence was negligible. However, as could be observed in the survival curves for HLA-DQA1 * 05, it could have an effect on IFX persistence in the short- and mid-term, as non-carriers had higher persistence until month 45 approximately, when both curves came closer.

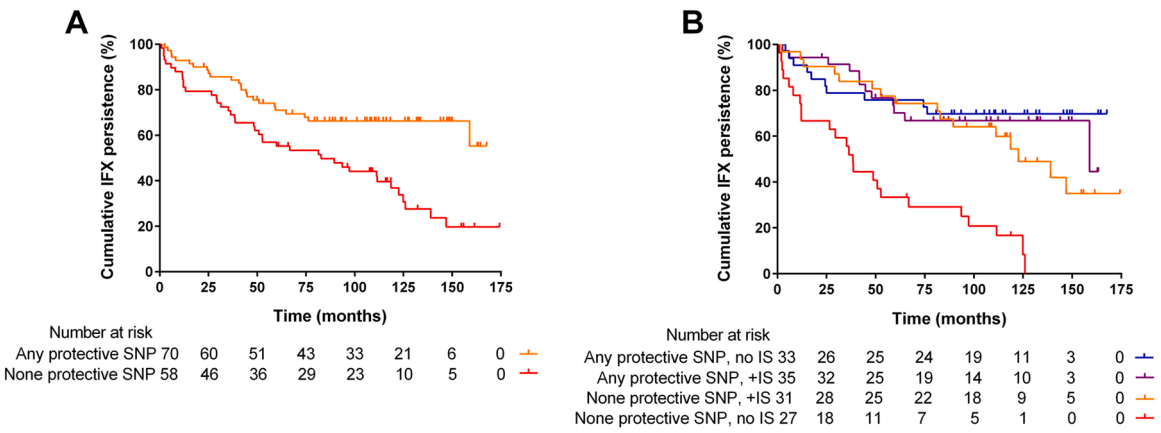

Fig. 2. Kaplan-Meier curves for IFX persistence according to the absence or presence of any of the three protective SNPs identified in multivariate Cox analysis (*ADAM17* rs10929587-TT, *IL23R* rs10489629-TT or *SLCO1C1* rs3794271-CC) (A), and also considering the concomitant immunosuppressive treatment (IS) at any time during IFX therapy (B). Vertical lines indicate censored cases.

Table 6
Contingency table of association to IFX persistence for the presence of either *ADAM17* rs10929587-TT or *IL23R* rs10489629-TT or *SLCO1C1* rs3794271-CC and taking into account concomitant immunosuppression.

	Persistence	Failure	Total	p-value
Any of the three SNPs	46 (64.8%)	25 (35.2%)	71	**< 0.001**
None of the three SNPs	18 (31.0%)	40 (69.0%)	58	
Any of the three SNPs with IS	23 (65.7%)	12 (34.3%)	35	**< 0.001**
Any of the three SNPs w/o IS	23 (69.7%)	10 (30.3%)	33	
None of the three SNPs with IS	15 (48.4%)	16 (51.6%)	31	
None of the three SNPs w/o IS	3 (11.1%)	24 (88.9%)	27	

SNP: single nucleotide polymorphism; IS: concomitant treatment with an immunosuppressant (this data was not available for 3 patients with failure to IFX); w/o: without.

Among the main strengths of our study, we would like to highlight the high number of SNPs analysed (66, including one SNP for HLA-DQA1 *05), the prospective follow-up of patients included, and the long duration of IFX treatment (close to a mean of 10 years in those patients with IFX persistence). Some of the limitations of our study include a cohort limited to adult Caucasian patients prohibiting results being extrapolated for children and patients from other ethnicities. Additionally, our results need validation in higher cohorts from other populations. Our study is also limited to IFX treatment in patients with CD who were mostly naive to biological drugs, so the influence of these SNPs in the persistence of other anti-TNF drugs or in other immune-mediated diseases or after previous failure to other biological drugs, needs to be investigated. The decision for IFX withdrawal (and thus failure to respond to IFX) was only clinical and not systematically based on pre-fixed criteria (such as clinical indexes, findings in endoscopy or laboratory parameters). In addition, although most patients were managed following a reactive therapeutic drug monitoring (TDM) strategy and thus IFX and anti-IFX levels were considered to decide IFX withdrawal, no data regarding TDM were available for the subset of 38 patients who failed to respond to IFX before 2015 in HGUGM. Similarly, treatment persistence (defined as maintenance of IFX therapy, due to sustained clinical benefit, throughout the follow-up) was the only surrogated parameter for IFX effectiveness and no additional criteria were considered. Finally, although we provided data with regard to concomitant immunosuppression, the influence of this variable on IFX persistence in the presence or absence of the protective genotypes needs to be evaluated in a more controlled study, and may be helpful to define the viability of IFX in monotherapy or combined with lower or standard dosages of immunosuppressant drugs.

In conclusion, our cohort of patients with CD and a long period of follow-up in a real clinical setting showed that the presence of the SNPs rs10929587-TT in *ADAM17*, rs10489629-TT in *IL23R* and rs3794271-CC in *SLCO1C1* protected against IFX failure. The presence of any of these SNPs, together with no previous bowel surgery, led to long-term persistence of IFX therapy. If these SNPs are not present, concomitant immunosuppressant therapy should be recommended. We hope that our findings might contribute to personalised medicine in the choice of biological drugs for CD.

CRediT authorship contribution statement

E.J. Laserna-Mendieta: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Supervision, Writing – original draft. **S. Salvador-Martín**: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **A Arias**: Formal analysis, Investigation, Methodology. **B. López-Cauce**: Data curation. **I. Marín-Jiménez**: Data curation. **L.A. Menchén**: Data curation. **L. Marín-Rubio**: Investigation. **J. Ontañón-Rodríguez**: Investigation. **L. A. López-Fernández**: Conceptualization, Funding acquisition, Writing – review & editing. **A.J. Lucendo**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Conflict of interest statement

The authors have no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

EJ Laserna-Mendieta is in receipt of a Juan Rodes grant (JR19/00005) from the Instituto de Salud Carlos III (ISCIII), Spanish Ministry of Health - Social Services and Equality, which is partly funded by the European Social Fund (period 2014–2020). Sara Salvador-Martín is in receipt of an Intramural postdoctoral grant from the Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM) (for the period 2022–2023). LA López-Fernández received financial support for the research from Instituto de Salud Carlos III (ISCIII) through the project PI19/00792 and co-funded by the European Union. We are grateful to Melanie Radcliff for the English language revision.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114225.

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