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Polymorphisms indicating risk of inflammatory bowel disease or antigenicity to anti-TNF drugs as biomarkers of response in children

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ABSTRACT

Few genetic polymorphisms predict early response to anti-TNF drugs in inflammatory bowel disease (IBD), and even fewer have been identified in the pediatric population. However, it would be of considerable clinical interest to identify and validate genetic biomarkers of long-term response. Therefore, the aim of the study was to analyze the usefulness of biomarkers of response to anti-TNFs in pediatric IBD (pIBD) as long-term biomarkers and to find differences by type of IBD and type of anti-TNF drug. The study population comprised 340 children diagnosed with IBD who were treated with infliximab or adalimumab. Genotyping of 9 selected SNPs for their association with early response and/or immunogenicity to anti-TNFs was performed using real-time PCR. Variants C rs10508884 (*CXCL12*), A rs2241880 (*ATG16L1*), and T rs6100556 (*PHACTR3*) (p value 0.049; p value 0.03; p value 0.031) were associated with worse long-term response to anti-TNFs in pIBD. DNA variants specific to disease type and anti-TNF type were identified in the pediatric population. Genotyping of these genetic variants before initiation of anti-TNFs would enable, if validated in a prospective cohort, the identification of pediatric patients who are long-term responders to this therapy.

1. Introduction

Pediatric inflammatory bowel disease (pIBD) comprises a group of chronic conditions characterized by inflammation of the gastrointestinal tract. The most common forms of pIBD are Crohn's disease (CD) and ulcerative colitis (UC) [1], both of which can have a significant impact

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on a child's physical and emotional well-being and can be difficult to manage.

The development of IBD is affected by genetic and environmental factors, and certain genetic biomarkers may be associated with an increased risk of developing the condition [2]. The genes involved affect the innate and adaptative immune systems and are responsible for epithelial abnormalities in the gut mucosa [3].

Children with IBD should be under the care of a pediatric gastroenterologist and other specialists who can help them manage their condition and determine the most appropriate treatment, which may involve a combination of medications, dietary changes, and various additional approaches [1]. pIBD is often treated using anti-tumor necrosis factor (TNF) drugs, which work by blocking the action of TNF, a protein that plays a key role in the inflammatory response. Anti-TNF drugs have proven effective in reducing inflammation and improving symptoms in many cases of pIBD [4,5]. In addition, recent studies show an improvement in IBD in children when anti-TNF drugs are used as the first-line approach [6].

However, there are limitations to the use of anti-TNF drugs in the treatment of pIBD. For example, they may not be effective for all children, and some children may experience adverse effects [7,8].

Among other clinical and demographic factors, individual genetic variants have proven to be of extraordinary relevance in predicting failure of therapy in pIBD. Our group identified genetic polymorphisms in the *IL10, IL17*, *IL6*, and *LY96* genes that were associated with the response to anti-TNF agents in pIBD [9]. Other authors have postulated that risky genetic variants in IBD could also be important in predicting the response to anti-TNF drugs. In this sense, Dubinsky et al. [10] tested 28 known IBD susceptibility loci and assessed primary non-response to anti-TNF drugs and found six variants associated with both risk and response (rs2241880 *ATG16L1*, rs2188962 *IRF1-AS1*, rs6908425 *CDKAL1*, rs762421 *ICOSLG*, rs2395185 *HLA-DQA1*, rs2836878 *BRWD1*) in datasets from adults and children with IBD [10]. Additionally, they identified 65 variants associated with primary non-response to anti-TNF in a cohort of 94 children with IBD; of these, three were included in models for prediction of primary response (rs975664 *TACR1*, rs4855535 *TAFA4* [previously known as *FAM19A4*], and rs6100556 *PHACTR3*).

Some IBD patients who are treated with biologics such as infliximab and adalimumab can develop antidrug antibodies (ADA), which can bind to the drugs and reduce their effectiveness, leading to loss of response or treatment failure [11]. Some genetic variants are associated with formation of ADAs in IBD. The most widely studied single-nucleotide polymorphism (SNP) is rs2097432, also known as *HLA-DQA1*:05 [12]. In addition to *HLA-DQA1_05* only the SNP rs10508884 has been associated with immunogenicity to biological drugs after GWAS analysis, although there is no information about its association with response to anti-TNF drugs in children [13].

Our group recently demonstrated an association between two *HLA* genetic variants, one of which was included in the Dubinsky study (rs2395185 *HLA-DQA1*), and long-term response to anti-TNF drugs [14]. The other *HLA* SNP we tested, rs2097432, was primarily associated with the development of ADAs against anti-TNF drugs [12] and with long-term response.

Identifying children with IBD who respond to anti-TNF drugs over several years could be very significant for the treating physician and facilitate tailored therapy. Accordingly, with the aim of advancing the personalization of anti-TNF therapy, we analyzed the association between all these genetic variants (except rs2395185 in *HLA-DQA1*) associated with IBD risk and anti-TNF response by Dubinsky et al., (rs2241880 *ATG16L1*, rs2188962 *IRF1-AS1*, rs6908425 *CDKAL1*, rs762421 *ICOSLG*, rs2836878 *BRWD1)*, the three involved in prediction models of primary response to anti-TNF in children with IBD (rs975664 *TACR1*, rs4855535 *TAFA4*, and rs6100556 *PHACTR3*) and the *CXCL12* variant associated with ADA formation with long-term response to anti-TNF drugs in pIBD.

2. Materials and methods

2.1. Study design and patient characteristics

We performed an observational, multicenter, ambispective, and longitudinal study between May 2017 and March 2022. The study population comprised 340 patients aged under 18 years from 19 pIBD units in Spain. The inclusion criteria were diagnosis of IBD and treatment with infliximab or adalimumab at any time.

Failure of biologic treatment was defined as a change in the anti-TNF drug used due to loss of response according to immunogenic, pharmacokinetic, and pharmacodynamic criteria established by the clinician. We also collected the following patient demographic and clinical variables: sex, age at diagnosis, age at initiation of treatment, time from diagnosis to initiation of anti-TNF drug, type of IBD, type of anti-TNF drug, line of treatment, and concomitant immunomodulators.

2.2. DNA Isolation and Genotyping

Genomic DNA was obtained from 200 μl of whole blood collected in EDTA tubes and purified using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The DNA concentration was measured using a Q5000 spectrophotometer (Quawell Technology Inc, San Jose, CA, USA) and diluted to 10 ng/μl for genotyping.

Genotyping of the nine selected SNPs (rs2241880, rs2188962, rs6908425, rs762421, rs2836878, rs975664, rs6100556, rs4855535 and rs10508884) was performed in a real-time PCR LightCyler® 480 II device (Roche Diagnostics) and analyzed using LightCycler 480 SW 1.5 software. The polymorphism rs4855535 was genotyped using a KASP on Demand probe (LGC Genomics, Berlin, Germany), while the others were assessed using rhAMP probes (Integrated DNA Technologies, Coralville, IA, USA), both following the manufacturer's instructions. Genotyping was successful in all the SNPs assessed. The Hardy–Weinberg equilibrium was analyzed to detect deviations in genotype frequency. All SNPs satisfied the Hardy-Weinberg equilibrium.

2.3. Statistical analysis

The demographic and clinical variables of the study population were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at Hospital Gregorio Marañón [15]. Quantitative continuous variables were expressed as median and interquartile range (IQR); qualitative variables were expressed as frequency and percentage.

The statistical analysis was performed using IBM SPSS Statistics v.26 (IBM Corp., Armonk, NY, USA). The chi-squared test (or Fisher exact test, as appropriate) and Mann-Whitney test were used to compare qualitative and quantitative variables, respectively.

Kaplan-Meier curves were used to analyze the association between genotypes and long-term response to anti-TNF drugs. For SNPs with a p value < 0.05, the hazard ratios (HR) adjusted using Cox regression was calculated based on sex, anti-TNF treatment, and type of IBD as covariates with the 95% confidence interval (CI). A p value less than 0.05 was considered statistically significant.

2.4. Ethical considerations

The study was approved by the local ethics committees (number of protocol FG-2019–01) and conducted in accordance with the World Medical Association Declaration of Helsinki and Spanish legislation. All patients and legal guardians gave their signed informed consent to participate in this study.

3. Results

3.1. Patients

A total of 340 children diagnosed with IBD were recruited. This cohort has already been used to analyze the role of two SNPs in *HLA* genes in the long-term response to anti-TNF drugs [14]. The characteristics of the patients recruited are shown in Supplemental Table S1.

3.2. Long-term response to anti-TNF drugs in IBD

Three of the nine genetic variants in *CXCL12*, *ATG16L1,* and *PHACTR3* were associated with the long-term worse response to anti-TNF drugs (Table 1, Fig. 1).

Carriers of the rs10508884C allele in *CXCL12* responded worse to anti-TNFs in the Kaplan-Meier univariate analysis (*p* value 0.049), although significance was lost in a Cox regression analysis adjusted for sex, type of IBD, and type of anti-TNF drug (aHR, 0.309; 95% CI, 0.076–1.268, *p* value 0.103) (Fig. 1a). Homozygous carriers of the rs2241880 A allele in *ATG16L1* responded worse to anti-TNFs in the Kaplan-Meier univariate analysis (*p* value 0.030) and in an adjusted Cox regression analysis (aHR, 0.509; 95% CI, 0.288–0.899, *p* value 0.020) (Fig. 1b). Finally, homozygous carriers of the rs6100556 T allele in *PHACTR3* responded worse to anti-TNFs in the Kaplan-Meier univariate analysis (*p* value 0.031) and in an adjusted Cox regression analysis (HR, 1.929; 95% CI, 1.088–3.422, *p* value 0.025) (Fig. 1c). In all cases ancestral allele was used as reference for comparison.

A new survival curve analysis was performed using as variable the number of the three worse-response genotypes identified and the time to failure (Fig. 1d). Having haplotypes with risky genotypes increased the probability of failure to anti-TNF drugs in a long-term follow-up: 0 (low risk), 1 (intermediate risk) (p value 0.152) and 2 or 3 (high risk)(p value 0.012). Type of IBD was also a significant and independent covariate. Those children with 2 or 3 risk genotypes and diagnosed with UC were the most prone to failure of anti-TNF treatment (Fig. 1e), while children diagnosed with CD (Fig. 1f) and without any of these variants were the best long-term responders to biological therapy.

In addition, to invetigate the clinical relevance of these biomarkers, positive and negative predictive values (PPV and NPV, respectively) were calculated for the three SNPs in *CXCL12, ATG161L*, and *PHACTR3* associated with response of anti-TNF drugs (Table 2). We can observe how the absence of risky variants in *ATG161L* and *PHACTR3* predicts 81% of patients who do not fail to anti-TNF drugs with a specificity of 84–88%.

3.3. Long-term response to anti-TNF drugs in CD and UC

The only genetic variant associated with response of anti-TNF drugs in pediatric patients with CD was rs6908425 in *CDKAL1* (Table 3, Fig. 2). Kaplan-Meier analysis revealed that homozygous carriers of the rs6908425C allele responded more poorly to anti-TNF drugs (*p* value 0.022) (Fig. 2a). These associations persisted after a multivariate Cox regression analysis (aHR, 2.232; 95% CI, 1.021–4.879, *p* value 0.044). However, three genetic variants, namely, rs2241880 (*ATG16L1*), rs2188962 (*IRF1-AS1*), and rs6100556 (*PHACTR3*), were associated with long-term worse response to the anti-TNF drug in children with UC (Table 3). Kaplan-Meier analysis also revealed that homozygous carriers of the rs2241880 A allele responded more poorly to anti-TNF drugs in a Kaplan-Meier analysis (*p* value 0.012) (Fig. 2b), as did carriers of the rs2188962 T allele (*p* value 0.024) (Fig. 2c) and homozygous carriers of the rs6100556 T allele (*p* value 0.004) (Fig. 2d). All these associations persisted in a multivariate Cox logistic regression analysis adjusted for sex and type of anti-TNF drug (aHR, 0.322 [95% CI, 0.124–0.835], *p* value 0.020; aHR, 3.242 [95% CI, 1.125–9.345], *p* value 0.029; and aHR, 2.945 [95% CI, 1.336–6.492], *p* value 0.007; respectively). In all cases ancestral allele was used as reference for comparison.

Table 1
Associations between genotype and long-term failure of anti-TNFs in pIBD.

ID	Genotype	KM *p* value	HR (95% CI)	aHR (95% CI)
CXCL12 rs10508884	CC+CT*# vs TT	**0.049**	0.067; 0.269 (0.066–1.096)	0.103; 0.309 (0.076–1.268)
ATG16L1 rs2241880	AA*# vs AG+GG	**0.030**	**0.032; 0.551 (0.319–0.951)**	**0.020; 0.509 (0.288–0.899)**
PHACTR3 rs6100556	GG+GT* vs TT#	**0.031**	**0.034; 1.853 (1.047–3.280)**	**0.025; 1.929 (1.088–3.422)**

*Reference;
#failure risk allele; KM, Kaplan-Meier curve; aHR, adjusted hazard ratio; HR, hazard ratio; CI, confidence interval

3.4. Long-term response to infliximab and adalimumab in IBD

As for the response to anti-TNF drugs, two SNPs were associated with the response to infliximab and one with the response to adalimumab (Table 4, Fig. 3). Kaplan-Meier analysis revealed that homozygous patients carrying the rs2241880 A allele in *ATG16L1* or the rs6100556 T allele in *PHACTR3* had an increased risk of failure of infliximab (*p* value 0.021 and 0.003, respectively). These associations were maintained in a multivariate Cox regression analysis adjusted for sex and type of IBD (rs2241880, aHR, 0.374 [95% CI, 0.186–0.755], *p* value 0.006; and rs610056, aHR, 2.672 [95% CI, 1.365–5.233], *p* value 0.004). For those patients treated with ADL, only rs2241880 in *ATG16L1* was associated with long-term worse response in a Kaplan-Meier analysis (*p* value 0.033). A multivariate Cox logistic regression analysis adjusted for sex and type of IBD showed that children carrying the GG rs2241880 genotype had a higher risk of failure of adalimumab (aHR, 2.388 [95% CI, 1.109–5.143], *p* value 0.026). In all cases ancestral allele was used as reference for comparison.

4. Discussion

Failure of biological therapies is multifactorial. Clinical and biochemical parameters may play a crucial role, and effectiveness may be diminished by the development of ADAs. Studies have shown that certain genetic variations can increase the risk of ADAs and treatment failure. Furthermore, genetic polymorphisms can play a role in the risk of IBD, and this risk could worsen the response to treatment. In this work, we analyzed genetic variants previously associated with formation of ADAs or with risk of developing IBD and studied their association with long-term response to infliximab and adalimumab. Furthermore, we compared these variants between UC and CD.

We showed that specific genotypes for rs10508884 (*CXCL12*), rs2241880 (*ATG16L1*), and rs6100556 (*PHACTR3*) were associated with long-term worse response to anti-TNF therapy in children diagnosed with IBD. Additionally, rs6908425 (*CDKAL1*) in CD and rs2241880 (*ATG16L1*), rs2188962 (*IRF1-AS1*), and rs6100556 (*PHACTR3*) in UC were associated with long-term worse response to anti-TNF drugs. Finally, rs2241880 (*ATG16L1*) was independently associated with long-term worse response to infliximab and adalimumab, while rs6100556 (*PHACTR3*) was associated with long-term worse response to infliximab.

CXCL12 is a ubiquitously expressed chemokine with several roles in the immune system. The implication of this chemokine and its receptors (CXCR4 and CXCR7) in IBD have been discussed [16]. Thus, CXCL12 is overexpressed in inflamed intestinal mucosa [17]. In adults, genetic variants have been shown to be involved in the regulation of *CXCL12*, with the protein levels being higher in IBD patients carrying the genetic variant T rs10508884 in homozygosis. Similarly, patients with higher levels of CXCL12 had a higher risk of developing ADAs to biological therapy in autoimmune diseases [13]. The results we report for children diagnosed with IBD contrast with those reported by the above-mentioned studies, namely, individuals carrying the C allele in

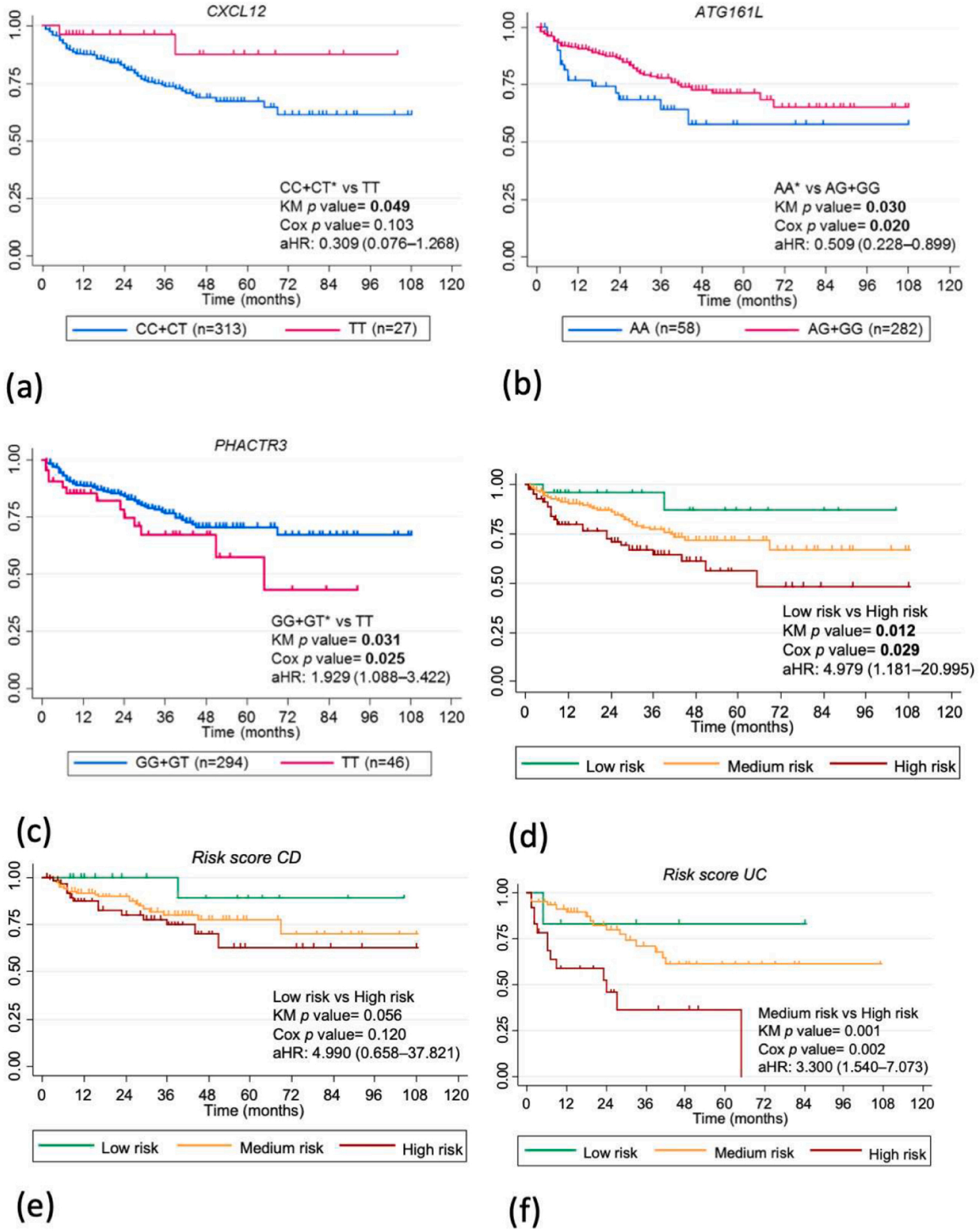

Fig. 1. Kaplan-Meier curves for the single-nucleotide variants that were significantly associated with failure of anti-TNF therapy. (**a**) rs10508884 (CC+CT genotypes) in *CXCL12*; (**b**) rs2241880 (AA genotype) in *ATG16L1*; (**c**) rs6100556 (TT genotype) in *PHACTR3*; (d) Kaplan-Meier by number of variants associated with failure (0 low risk; 1 intermediate risk, 2–3 high risk) in IBD (e) Kaplan-Meier by number of variants associated with failure (0 low risk; 1 intermediate risk, 2–3 high risk) in UC; (f) Kaplan-Meier by number of variants associated with failure (0 low risk; 1 intermediate risk, 2–3 high risk) in CD. Genotype comparisons and *p* values for the univariate analysis (KM *p* value) and multivariate analysis (Cox *p* value) are presented alongside the Kaplan-Meier curves. Significant *p* values are marked in bold. *Reference genotypes. Green, low risk; orange, intermediate risk; and red: high risk.

homo- or heterozygosis have a higher risk of failure of anti-TNF therapy than those with a TT genotype. Differences in response to anti-TNF drugs between adults and children diagnosed with IBD have been reported [14].

ATG16L1 is involved in autophagy, an essential biological process in innate immunity that also regulates the inflammatory response in IBD [18]. Some genetic variants in *ATG16L1*, such as rs2241880, are involved in the risk of developing CD [19]. This variant regulates inflammatory responses by modulating TLR- and NLR-mediated signaling and regulates commensal microbiota of the intestine [20,21]. Unexpectedly, the G allele was associated with susceptibility to CD [22], and the A allele was associated with failure of infliximab in patients with IBD

Table 2
Clinical utility of genotyping SNPs in *CXCL12, ATG161L* and *PHACTR3*.

	CXCL12, % (95% CI)	*ATG161L*, % (95% CI)	*PHACTR3*, % (95% CI)
PPV	21.73%	29.31%	32.61%
NPV	92.59%	81.21%	81.29%
Sensitivity	97.14%	24.29%	21.43%
Specificity	9.26%	84.81%	88.52%

PPV, positive predictive value; NPV, negative predictive value

Table 3
Associations between genotype and long-term failure of anti-TNFs in pediatric CD and UC.

ID	Genotype	KM *p* value	HR (95% CI)	aHR (95% CI)
CDKAL1 rs6908425	TT+TC* vs CC[#]	**0.022**	**0.027; 2.410 (1.107–5.250)**	**0.044; 2.232 (1.021–4.879)**
ATG16L1 rs2241880	AA*[#] vs AG+GG	**0.012**	**0.018; 0.320 (0.125–0.822)**	**0.020; 0.322 (0.124–0.835)**
IRF1-AS1 rs2188962	CC* vs CT+TT[#]	**0.024**	**0.033; 3.155 (1.100–9.047)**	**0.029; 3.242 (1.125–9.345)**
PHACTR3 rs6100556	GG+GT* vs TT[#]	**0.004**	**0.006; 2.997 (1.371–6.553)**	**0.007; 2.945 (1.336–6.492)**

*Reference; [#]failure risk allele; KM, Kaplan-Meier curve; aHR, adjusted hazard ratio; HR, hazard ratio; CI, confidence interval; CD, Crohn's disease; UC, ulcerative colitis

aged under 21 [10]. In our study, the AA genotype was associated with failure of anti-TNF drugs in IBD and UC, although this association was not significant in CD. Interestingly, the A allele was associated with a poorer response to infliximab, but a better response to adalimumab. The association between presence of the A allele and a poorer response in IBD could arise from the finding that 67.1% of patients in our cohort were treated with infliximab. If this is confirmed, rs2241880 could be useful as a biomarker for personalizing therapy with infliximab or adalimumab in children with IBD.

Phosphatase and actin regulator 3, PHACTR3, is a protein that binds to actin and regulates protein phosphatase 1 [23]. A variant in this gene,

Table 4
Associations between genotype and long-term failure of infliximab or adalimumab in pIBD.

ID	Genotype	KM *p* value	HR (95% CI)	aHR (95% CI)
		IFX		
ATG16L1 rs2241880	AA*[#] vs AG+GG	**0.021**	**0.024; 0.453 (0.228–0.901)**	**0.006; 0.374 (0.186–0.755)**
PHACTR3 rs6100556	GG+GT* vs TT[#]	**0.003**	**0.004; 2.689 (1.379–5.242)**	**0.004; 2.672 (1.365–5.233)**
		ADL		
ATG16L1 rs2241880	AA+AG* vs GG[#]	**0.033**	**0.038; 2.245 (1.046–4.818)**	**0.026; 2.388 (1.109–5.143)**

*Reference; [#]failure risk allele; KM, Kaplan-Meier curve; aHR, adjusted hazard ratio; HR, hazard ratio; CI, confidence interval; IFX, infliximab; ADL, adalimumab

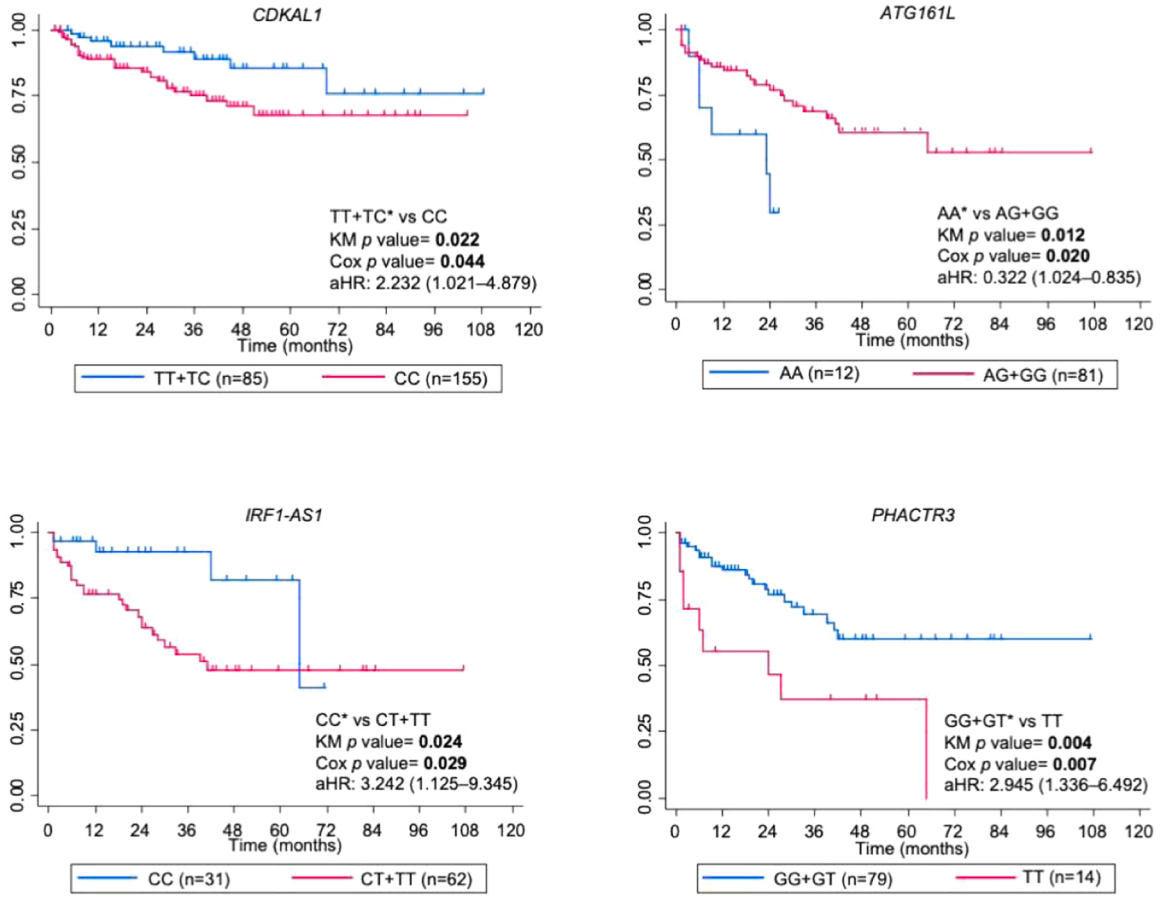

Fig. 2. Kaplan-Meier curves for the single-nucleotide variants that were significantly associated with failure of anti-TNF therapy in pediatric CD and UC. (a) rs6908425 (CC genotype) in *CDKAL1* in CD; (b) rs2241880 (AA genotype) in *ATG16L1* in UC; (c) rs2188962 (CT+TT genotypes) in *IRF-AS1* in UC; (d) rs6100556 (TT genotype) in *PHACTR3* in UC. Genotype comparisons and *p* values for the univariate analysis (KM *p* value) and multivariate analysis (Cox *p* value) are presented alongside the Kaplan-Meier curves. Significant *p* values are marked in bold. *Reference genotypes.

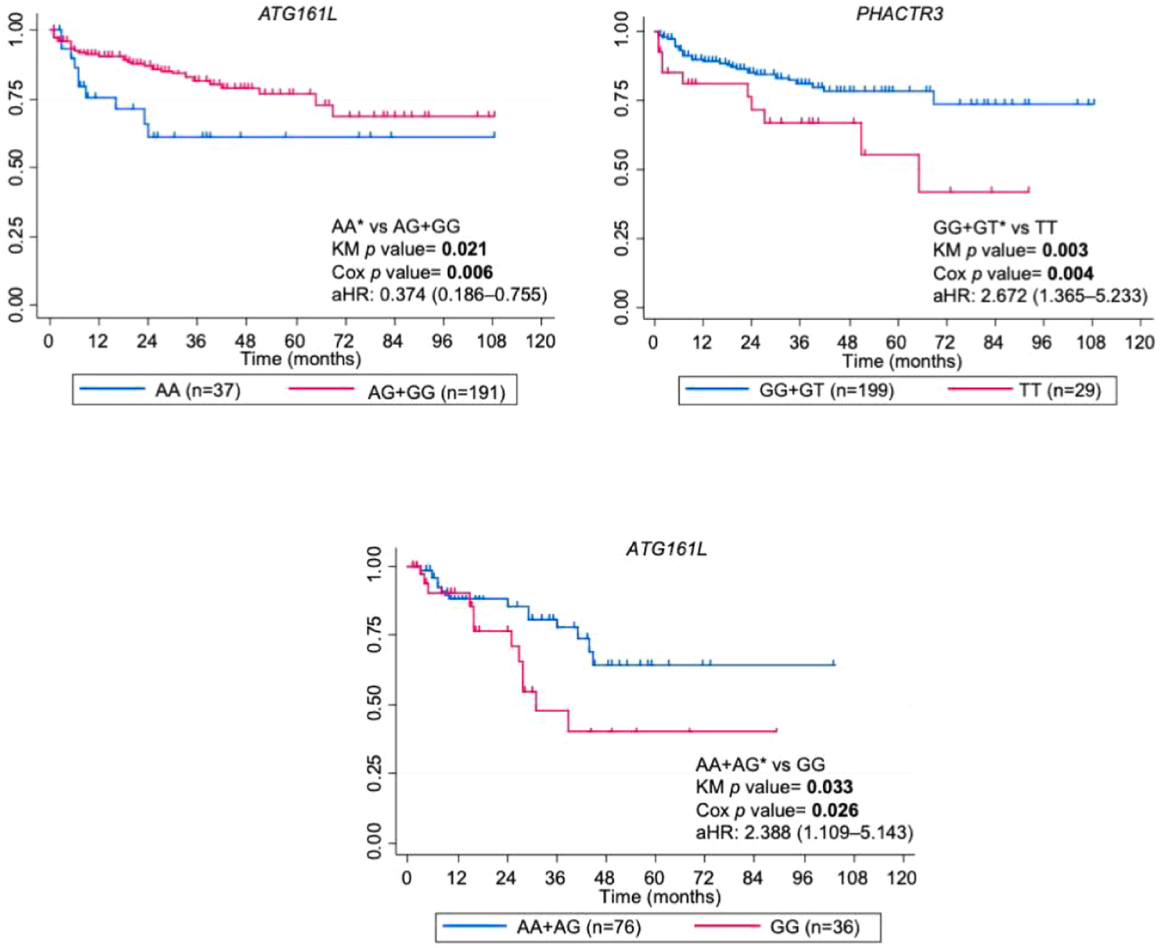

Fig. 3. Kaplan-Meier curves for the single-nucleotide variants that were significantly associated with failure of infliximab or adalimumab in pIBD. (a) Infliximab and rs2241880 (TT genotype) in *ATG16L1*; (b) Infliximab and rs6100556 (AA genotype) in *PHACTR3*; (c) Adalimumab and rs2241880 (GG genotype) in *ATG16L1*. Genotype comparisons and *p* values for the univariate analysis (KM *p* value) and multivariate analysis (Cox *p* value) are presented alongside the Kaplan-Meier curves. Significant *p* values are marked in bold. *Reference genotypes.

rs6100556, was found to be associated with primary non-response to anti-TNF therapy in patients diagnosed with IBD aged under 21 years [10]. Homozygous carriers of the T allele for this variant experienced a worse primary response to infliximab than patients with any other genotype. We also demonstrated that in the long term, children diagnosed with UC carrying the T allele in homozygosis showed a higher risk of failure to anti-TNF drugs. Similarly, pediatric patients diagnosed with IBD, treated with infliximab, and carrying the TT genotype for rs6100556 had a higher risk of failure than carriers of other genotypes during a long follow-up period. Thus, this genetic variant seems to be a good biomarker for predicting long-term response in pIBD.

CDK5 regulatory subunit–associated protein1 like 1, *CDKAL1*, is a gene of unknown function which has been associated with type 2 diabetes mellitus, psoriasis, and CD [24,25]. The genetic variant rs6908425 (C variant in homozygosity) is associated with risk of CD and prediction of disease course [26,27]. Accordingly, we observed a worse response to anti-TNF drugs in children with CD who carry the C variant in homozygosis. However, in persons who are CC homozygous for this variant, the response to anti-TNF agents in psoriasis is better [28]. The same is true of patients with IBD aged under 21 [10].

IRF1 antisense RNA (IRF1-AS1) is a long non-coding RNA which is involved in another immune-mediated disease, juvenile ankylosing spondylitis [29], and in susceptibility to CD [27,30]. The T allele of rs2188962 in this gene confers a higher risk of CD [27,30]. In parallel, we found a worse response to anti-TNF in pediatric UC patients who were carriers of the T allele, although this finding in a long-term follow-up is not coincident with the short-term response observed by Dubinsky et al. [10].

Regarding the clinical utility of the identified biomarkers, the most promising are those in *ATGL161L* and *PHACTR3*. Genotyping both variants we can predict 81% of patients who will not fail to anti-TNF drugs with a specificity of 84–88%. This data may be of extraordinary help in the personalization of biologic therapy in pIBD.

It is important to note that the development of ADAs is not the only cause of failure of these drugs and that other factors such as disease activity, dosage, and drug interactions should also be considered when evaluating the effectiveness of treatment. A limitation of the study is that in our cohort, very few patients developed ADAs owing to proactive monitoring in practically all the participating hospitals. However, we show how SNPs previously associated with the development of ADAs are related to response to anti-TNF drugs in a context of proactive monitoring. This finding might be extremely useful in clinical practice.

Finally, while the search for biomarkers of response to anti-TNFs in IBD might not be restricted to genetic variants, it is important to identify them in order to develop a prediction score that can help us to personalize biologic treatments, especially in children. In this sense, gene expression profiles have also been associated with the response to anti-TNF drugs in adults and in children with IBD [31–33]. The inclusion of other parameters, such as trough serum level of the anti-TNF drug, fecal calprotectin, and albumin could facilitate the design of a predictive score.

A limitation of the study is the possible influence of highly penetrant

monogenic defect in a subset of the participants. As over 80 genes have been linked to very early onset-IBD, most of participants do not have this information and they are considered to have non-very-early onset IBD [34].

Although the IBD cohort analyzed in the present study is the largest in Spain and one of the largest in the world, our findings should be validated prior to implementation of these genetic tests in clinical practice.

5. Conclusions

We identified DNA variants in children with IBD. These were associated with worse long-term response to anti-TNF drugs (rs10508884C *CXCL12*, rs2241880 A *ATG16L1*, and rs6100556 T *PHACTR3*) and specific to disease type (rs6098425C *CDKAL1*, rs2241880 A *ATGL161*, rs2188962 T *IRF1-AS1*, and rs6100556 T *PHACTR3*) and anti-TNF type (rs2241880 *ATG16L1* and rs610056 T *PHACTR3*). Genotyping of these SNPs before initiation of anti-TNF therapy would identify pediatric patients who are long-term responders to this therapy.

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CRediT authorship contribution statement

Conceptualization, S.S.-M. and L.A.L.-F.; Methodology, P.Z.-C., S.S.-M., and L-A.L-F.; Formal analysis, P.Z.-C.; Data acquisition and curation, Investigation, M.V., L.M.P., S.C., O.S., A.M.-Á., A.F.-L., B.P.-M., M.M., C. S., M.T., I.L., M-J.F., V-M.N-L., L.M, R.G.-R., R.T.-P., A.R., F.B., M-J.B., E. S., and M.S.-S.; Writing – original draft preparation, P.Z.-C., S.S.-M., and L.A.L.-F.; Writing – review & editing, all; Supervision, L.A.L.-F.; Funding acquisition, L.A.L.-F. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Hospital General Universitario Gregorio Marañón (protocol code FG-2019–01 23 September 2019).

Informed Consent Statement

Written informed consent has been obtained from the patients and/or legal guardians to develop this research.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2023.106859.

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