Pharmacogenetics of treatments for inflammatory bowel disease

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ABSTRACT

Introduction: Inflammatory bowel disease is a chronic inflammation of the gut whose pathogenesis is still unclear. Although no curative therapy is currently available, a number of drugs are used in induction and maintenance therapy; however, for most of these drugs, a high inter-individual variability in response is observed. Among the factors of this variability, genetics plays an important role.

Areas covered: This review summarizes the results of pharmacogenetic studies, considering the most important drugs used and in particular aminosalycilates, glucocorticoids, thiopurines, monoclonal antibodies and thalidomide. Most studies used a candidate gene approach, even if significant breakthroughs have been obtained recently from applying genome-wide studies. When available, also investigations considering epigenetics and pharmacogenetic dosing guidelines have been included.

Expert opinion: Only for thiopurines, genetic markers identified as predictors of efficacy or adverse events have allowed the development of dosing guidelines. For the other drugs, encouraging results are available and great expectations rely on the study of epigenetics and integration with pharmacokinetic information, especially useful for biologics. However, to improve therapy of IBD patients with these drugs, for implementation in the clinics of pharmacogenetics, informatic clinical decision support systems and training about pharmacogenetics of health providers are needed.

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1. Introduction

Inflammatory bowel disease (IBD) is a chronic idiopathic inflammation of the intestinal tract, characterized by relapses and remissions, that comprises two disorders, Crohn's disease (CD) and ulcerative colitis (UC). IBD develops at relatively young ages with a peak onset around 15 to 30 years of age and the incidence and prevalence of IBD, and in particular of CD, are increasing in both developed and developing countries. The pathogenesis of the disease is still unknown, however, accumulating evidence suggests that IBD is the result of complex interactions between genetics, environmental factors [1,2] and the intestinal microbioma [3]. More than 200 genetic susceptibility loci have been identified so far; however, no single variant is useful for diagnostic purposes. An association of certain genetic variants with particular IBD phenotypes has been demonstrated, testing remains however a research tool [4,5].

At present, a curative therapy does not exist for this disease, but many drugs are currently employed. Treatment depends by a number of factors, among which the type of disease (CD or UC), its location, severity, disease related complications and prognosis, and has the main objective of controlling inflammation and associated symptoms, and to prevent the occurrence of complications. Medical treatment consists in induction therapy, that aims to control inflammation and symptoms in a short time, while with maintenance therapy control of symptoms should be maintained for prolonged periods [6] (Figure 1).

The salicylates mesalazine and sulfasalazine are often used as first line agents to treat mild to moderately active colonic disease, in particular in UC; glucocorticoids (GCs) are administered for inducing remission, while the thiopurines azathioprine (AZA) and mercaptopurine (MP) are efficacious for maintaining remission. More recently, monoclonal antibodies directed toward TNFα and other cytokines have been introduced both for inducing remission and for maintenance therapy in refractory forms of the disease [6]. In cases that do not respond to these therapies, thalidomide has been recently proposed as an efficacious agent [7,8].

The efficacy and toxicities of all these drugs are characterized by a high interpatient variability that depends on a number of factors, such as the severity and localization of the disease, environmental factors and habits such as smoking. However, an important role in this variability should be ascribed to genetic factors.

The present review will be focused on the pharmacogenetics of drugs currently employed in the disease, and in particular salicylates, GCs, thiopurines, monoclonal antibodies and thalidomide.

2. Aminosalicylates

Mesalazine, also known as 5-aminosalicylic acid (5-ASA), is the first-line treatment for the induction and maintenance of

Article highlights

- The role of pharmacogenetic factors to predict therapeutic effects in IBD has been largely demonstrated but only few genetic markers have been developed to the point of clinical guidelines to tailor therapy.
- For thiopurines, administration of the drug on the basis of a multi-locus genotype considering variants in TPMT, NUDT15 and HLAs is promising in reducing adverse effects (myelosuppression and pancreatitis) and increasing efficacy of treatment.
- For all drugs and in particular glucocorticoids, based on their mechanism of action, epigenetic evaluations considering miRNAs and DNA methylation profiles are promising toward the identification of clinically useful pharmacogenetic markers.
- Biologic drugs and anti-drug antibodies concentrations are strongly associated with disease remission and proper integration of pharmacokinetic and pharmacogenetic markers may lead to the identification of strategies to prevent and overcome remission induction failure or loss of response.
- Improvement in the field of pharmacogenetics of IBD may depend on the development of large national and international collaborative groups to increase sample sizes of populations considered for analysis.
- Clinical implementation of pharmacogenetic biomarkers depend also on the development of tools that assist clinicians in handling these informations, such as informatic clinical decision support systems, and on training about pharmacogenetics of health providers.

This box summarizes key points contained in the article.

remission in mild to moderately active UC. Although the mechanism of action of this drug is not completely understood, its anti-inflammatory activity seems to be mediated by the activation of nuclear receptors involved in the control of inflammation, the γ-form peroxisome proliferator-activated receptors (PPAR-γ) [9]. This class of proteins is highly expressed in colon epithelial cells and the most important activating factor for PPAR- γ appears to be the gut microbiota [10] and a decreased gene expression of PPAR-γ was demonstrated in colonic biopsies of patients with active UC [11,12]. Moreover, a higher expression of PPAR-α, another member of the same family of nuclear receptors, in patients with UC who received only 5-ASA respect to patients receiving other therapies, was observed.

5-ASA is poorly absorbed by the gastrointestinal tract and rapidly N-acetylated by the enzymes N-acetyltransferases (primarily by NAT1 isoform) in the intestinal epithelium and liver to produce the inactive form, N-acetyl-5-ASA. Genetic polymorphisms of NAT1 affected thioguanine nucleotides (TGN) levels in young patients with IBD treated with thiopurines and aminosalicylates and could therefore influence the toxicity and efficacy of these drugs, putatively because of increased 5-ASA inactivation and consequent reduced inhibition of thiopurine metabolism [13].

Aminosalicylates are used in maintenance therapy for long periods, hence long-term toxicity is an important consideration [14]. A genome-wide association study (GWAS) published by Heap and collaborators identified genetic risk factors for the development of 5-ASA-induced nephrotoxicity in IBD patients [15]. On 151 cases, 5 were classified as definite cases, as they had a second episode of kidney injury when re-challenged with the agent and 146 as probable cases; the

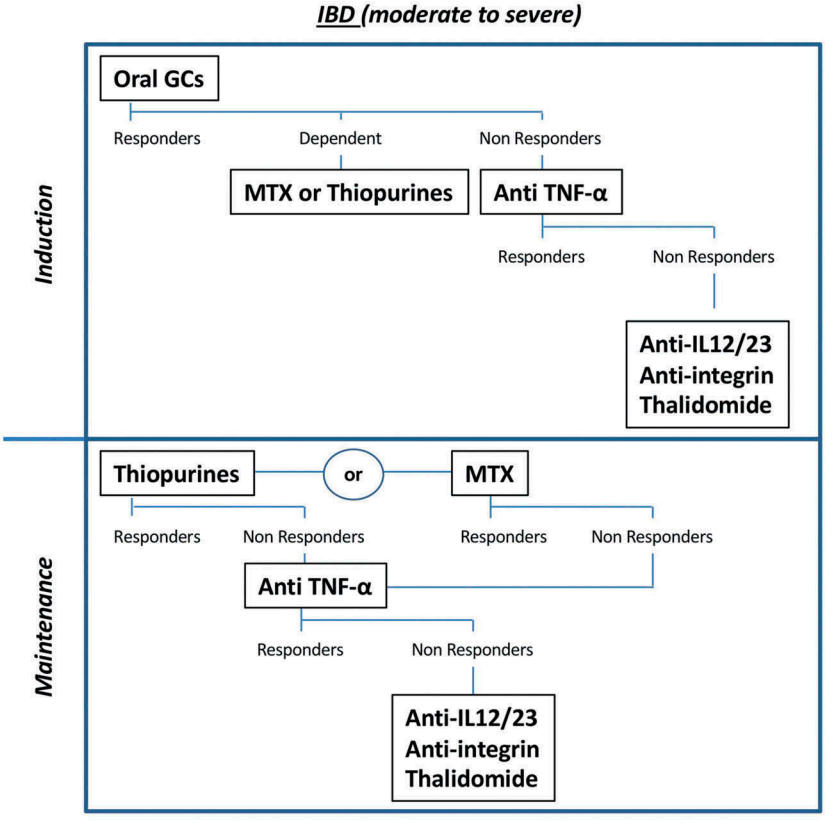

Figure 1. Simplified schematic representation of IBD treatment: though for from being complete, this figure is representative of the complexity of IBD therapeutic management. For an exhaustive overview of IBD treatment in children and adult, readers should refer to the American College of Gastroenterology (ACG) and The European Crohn's and Colitis Organisation (ECCO) guidelines [6].

control cohort consisted of 1748 CD and 2361 UC cases. The strongest association signal for the development of nephrotoxicity was in the HLA region (rs3135349) and the top single nucleotide polymorphism (SNP) detected after a dedicated HLA imputation was rs3135356.

3. Glucocorticoids

GCs remain the first-line treatment for inducing remission in moderate to severe active CD and UC. These agents are usually effective in reducing acute symptoms, but are not able to achieve mucosal healing; for this reason, they are often used to control flares until immunomodulators or biologic agents begin to work. Wide differences in the efficacy of GCs have been reported, in particular in inflammatory diseases. Steroid resistance is common in patients with IBD, with an incidence of 16–40% of subjects, in addition, a high percentage of those who initially respond cannot discontinue therapy as disease activity increases when the dosage is reduced (steroid dependent) [6,16,17].

Another problem with GC use is that even short-term use can result in side effects, including bone loss and osteoporosis, metabolic disease and increased risk of cardiovascular disease, mood disorders, narrow angle glaucoma, weight gain and many other [18].

In recent years, several studies have clarified the mechanism of action of GCs at the molecular level and the role of genetic variants in their efficacy (Table 1). These hormones passively diffuse across plasma membranes and interact with a cytosolic GC receptor (GR); the activated receptor migrates in the nucleus, and represses or induces the expression of a number of genes [19].

3.1. Pharmacogenetics

3.1.1. GC receptor

The GR is encoded by the NR3C1 (Nuclear Receptor Subfamily 3, group C, member 1) gene which is expressed in virtually all cells; a number of polymorphisms has been described in this gene but few are functionally relevant (Table 1). The most studied, that have been associated with alteration in metabolic parameters and in differences in response to GCs in IBD, are BclI (rs41423247), ER22/23EK (rs6189 and rs6190) and N363S (rs6195) polymorphisms.

The BclI polymorphism is the most clinically relevant polymorphism of the NR3C1 gene and consists in a C>G substitution, 646 nucleotides downstream from exon 2 [20]. This polymorphism has been associated with hypersensitivity to GCs in heterozygous and homozygous carriers of the G allele and with unfavorable metabolic characteristics [20]. In 119 pediatric patients with IBD (64 with CD, 55 with UC) the frequency of BclI mutated genotype was significantly higher in GC-responsive patients than in the GC-dependent group [21].

The ER22/23EK GR polymorphisms, in the N-terminal transactivation domain of the GR, involve two nucleotide changes in codon 22 and 23 of exon 2 resulting in change from glutamic acid-arginine (E-R) to glutamic acid-lysine (E-K) in the amino acid sequence. The polymorphisms have been associated with a relative GC resistance and with a reduction of GR transcriptional activity in transfected COS-1 cells and in peripheral blood mononuclear lymphocytes of homozygous carriers [22] and to higher post-dexamethasone cortisol levels and less cortisol suppression after dexamethasone suppression test in elderly healthy carriers [23]. When studying the effect of these polymorphisms in the response to exogenous steroids, no association was found in 119 pediatric patients with IBD [21].

The N363S polymorphism in exon 2 consists in an AAT>AGT nucleotide change at position 1220, resulting in asparagine (N) to serine (S) change in codon 363. In human peripheral blood mononuclear cells (PBMCs), the mutant allele exhibited a significantly higher transactivating activity [24] and was associated with increased sensitivity to endogenous GCs in a Dutch elderly population [25].

The above mentioned and other polymorphisms of the NR3C1 gene have been investigated by Mwinyi et al. [26] in

Table 1. Pharmacogenetic variants involved in glucocorticoid response in IBD patients.

GENE	PROTEIN FUNCTION	GENETIC VARIANTS	PATIENTS	INFLUENCE ON RESPONSE	REFERENCE
NR3C1	GC receptor regulates either positively or negatively, the expression of its target genes	rs41423247 (646C>G)	119 (CD = 64; UC = 55)	↑ sensitivity to GCs	[21]
		rs6189 (C>T) rs6190 (C>T) rs6195 (1220AAT>AGT)		No association with GCs response	
TNFα	Multifunctional pro-inflammatory cytokine	rs1800629 (−308G>A)	193 CD	↑ steroid-dependent disease	[33]
			386 (CD = 200; UC = 186)	↓ response to GCs in CD patients	[34]
IL1B	Proinflammatory cytokine with a central role in the regulation of inflammation in IBD	rs4149570 (−511C>T)	154 (CD = 82; UC = 72)	No association with GCs response	[38]
NLRP1	Key mediators of programmed cell death	rs12150220 (A>T)		↓ response to GCs	
ABCB1	ATP-dependent drug efflux pump for xenobiotic compounds	rs1045642 (3435C>T)	386 (CD = 200; UC = 186)	No association with GCs response	[34]
		rs2032582 G2677T/A	946 (CD = 478; UC = 468)		[42]
		rs2032583	260 CD	↑ steroid-dependent disease	[43]

Nuclear Receptor Subfamily 3 Group C Member 1 *(NR3C1)*; Tumor Necrosis Factor (*TNF*); interleukin 1 beta (*IL1B*); NLR family pyrin domain containing *1 (NLRP1)*; ATP binding cassette subfamily B member 1 *(ABCB1)*; Crohn's disease (CD); Ulcerative colitis (UC).

181 IBD patients. Among the 13 variants detected, and the resulting 17 different haplotypes, none showed a statistically significant association with GC response.

Finally, in 2012, a meta-analysis was performed on the association of ER22/23EK, N363S and BclI polymorphisms and steroid response in IBD. Nine hundred forty-two cases, from five eligible studies, were included, but no evidence of an association with steroid response was found, although the analysis was underpowered [27].

3.1.2. Pro-inflammatory cytokines

Pro-inflammatory cytokines are involved in the pathogenesis of IBD and other chronic inflammatory diseases. Patients with non-responsive inflammatory diseases often show high levels of local and/or systemic pro-inflammatory cytokines [28] and *in vitro* data suggest that cytokines could interfere with the GC receptor signaling [29,30]. Polymorphisms in the cytokine regulatory regions might therefore result in variability in the level of inflammation and response to GCs (Table 1).

The pro-inflammatory cytokine TNFα, released by cells of the immune system, has been particularly studied [31]. The −308G>A (rs1800629) SNP of the TNFα gene [32] is located in a binding site for the transcription factor AP1 and a higher transcriptional activity, with increased TNFα production *in vitro* has been demonstrated for the A allele. This polymorphism has been correlated with steroid response in several diseases and in IBD. A first study by Louis et al. [33] evaluated the −308G>A polymorphism in a cohort of 193 CD patients, showing that the frequency of the mutated allele was significantly higher in steroid dependent patients. In a subsequent study the same polymorphism was evaluated in 386 pediatric IBD patients, and the mutated allele was significantly associated with steroid resistance in the subset of 200 CD subjects, but not in UC [34].

Although TNFα has a major role in IBD, other cytokines are also overexpressed, among which interleukin-1 (IL1). IL1 is constituted by two distinct polypeptides, IL1α and IL1β; the latter is highly expressed in the intestinal mucosa of healthy subjects and IBD patients and promotes the expression of matrix metalloproteinases and the synthesis of prostaglandins, and other inflammatory mediators such as IL6, IL8 and TNFα, amplifying the inflammation cascade. IL1β is therefore very important in starting and maintaining inflammation [35] and polymorphisms in the IL1β gene, such as the −511C>T in the promoter region, associated with an increased secretion of the cytokine [36,37], might be important for GC sensitivity. This SNP was studied in 154 IBD pediatric patients, but no correlation was found with steroid response [38].

IL1β is released as a precursor (pro IL1β) by monocytes and macrophages in response to inflammatory stimuli, and it is cleaved to mature cytokine by caspase-1 [39,40]. Caspase-1 is part of multiprotein cytoplasmic complexes, called NALP1 (NACHT leucine-rich-repeat protein 1) and NALP3 (NACHT leucine-rich-repeat protein 3) inflammasomes. In pediatric IBD patients, homozygous carriers of the NALP1 rs12150220 mutated genotype, resulting in the leucine to histidine amino acidic variation in position 155, exhibited a higher probability of non-response to GC therapy [38].

3.1.3. GC transport

Polymorphisms in genes involved in GC transport could also play a role in the variability in efficacy and toxicity of these hormones. The drug efflux pump P-glycoprotein (P-gp), is a membrane transporter that extrudes from cells a number of structurally unrelated substances, among which GCs, reducing their intracellular concentration. The protein is encoded by the highly polymorphic ABCB1 gene, and most variants result in alteration of the protein expression and function, although with small effects (Table 1) [41]. In 2007, Cucchiara and colleagues studied the C3435T polymorphism in 200 pediatric Italian CD and 186 UC patients treated with GCs, and demonstrated that this SNP was not associated with response to therapy [34]. In accordance with these results, a large study in an adult Italian IBD population did not find any association between the C3435T and G2667T/A polymorphisms and clinical response [42]. On the contrary, a retrospective study in a pediatric population of 260 CD patients demonstrated that a tag SNP rs2032583 was significantly associated with GC dependence [43].

3.2. Pharmacoepigenetics

Pharmacoepigenetic is a new promising tool for therapy personalization. The main epigenetic mechanisms include histone modifications, DNA methylation, and non-coding RNAs expression, resulting in activation or silencing of gene expression [44]. The study of epigenetic factors in predicting GC response in IBD is a rapidly growing field, and it is hypothesized that GC resistance or dependence in IBD patients could be attributable to epigenetic changes as described in other diseases [45–47].

The study published by Tahara and colleagues has been the first in which a candidate methylation marker was associated with the response to treatment [48]. In particular, in colonic mucosa of UC patients, higher methylation of the Protease-Activated Receptor 2 (PAR2) gene, a protein involved in pro-inflammatory responses and in IBD pathogenesis, has been associated with GC-dependence and resistance phenotypes.

The possible correlation between long non-coding RNAs (lncRNAs) or microRNAs (miRNAs) expression and variability in GC response in IBD patients [49,50] has been recently evaluated, and a role for the lncRNA growth arrest-specific 5 (GAS5) has been demonstrated [51,52]. Pediatric IBD patients with unfavorable steroid response had higher GAS5 levels in comparison with sensitive group, suggesting that GAS5 may predict GCs ineffectiveness [53].

Recently, Heier and colleagues identified four serum miRNAs (miR-146a, miR-320a, miRNA-486 and miR-146b) modified by GC treatment in children with IBDs using quantitative real time PCR [54]. Moreover, miRNAs profile significantly deregulated by steroid treatment in PBMCs of IBD patients responding to GCs was recently published [55]. In particular, among the 18 GC sensitive miRNAs identified, miR-144, miR-142 and miR-96 could putatively recognize the 3′UTR of the GR gene, while miR-363, miR-96 and miR-142 contained positive GC responsive elements (GREs) sequences, thereby potentially enabling a direct regulation

by the GR [55]. Further validation in a larger number of patients affected by IBD is however needed.

4. Thiopurines

The thiopurines mercaptopurine (MP) and its pro-drug azathioprine (AZA), alone or in combination with other drugs, are commonly used for the maintenance of remission and steroid sparing in patients with IBD. Because of their slow time to clinical response, between 8 and 12 weeks, these agents are not effective for rapid induction of remission [6]. More recently, these immunosuppressants are also employed with biological therapies, as they can reduce the immunogenicity of biologics [6].

Thiopurines themselves are inactive and require intracellular metabolism, catalyzed by multiple enzymes, to the active thioguanine nucleotides (TGN), responsible for causing immunosuppression [56]. MP can be released from AZA both enzymatically by glutathione transferases and spontaneously after reaction with thiols (e.g. glutathione) [57,58] and is transported inside cells by sodium-coupled nucleoside transporters such as SLC28A2 (CNT2) and SLC28A3 (CNT3), and by equilibrative nucleoside transporters like SLC29A1 (ENT1) and SLC29A2 (ENT2) [59,60]. After the uptake, MP is converted into thioinosine monophosphate by the enzyme hypoxanthine guanine phosphoribosyl transferase. Thioinosine monophosphate can be converted into thioguanine monophosphate (TGMP) enzymatically in two steps and, subsequently, TGMP can be converted into thioguanine diphosphates (TGDP) and triphosphates (TGTP). MP is inactivated to thiouric acid by the enzyme xantine oxidase and to methyl mercaptopurine (MMP) by thiopurine methyl transferase (TPMT) [61].

Cytotoxic effects of thiopurines are achieved through several pathways: incorporation of thiopurine active metabolites such as TdGTP or TGTP into DNA or RNA alters the function of several enzymes involved in DNA replication and repair such as RNase H, Topoisomerase II, and DNA ligase, leading to apoptosis [62]; methylmercaptopurine nucleotides (MMPN), especially methylthioinosine monophosphate, are potent inhibitors of *de novo* purine synthesis [63]. In addition, TGTP contribute to the immunosuppressive effects also through the inhibition of signaling by GTPase Rac1. Thiopurine metabolites suppress the activation of Rac1 target genes such as mitogen-activated protein kinase kinase, NFκB, and bcl-xL, leading to a mitochondrial pathway of apoptosis in activated lymphocytes [64], which may be particularly relevant in the intestinal tissue.

4.1. Pharmacogenetics

Interindividual variability in AZA and MP efficacy and adverse effects has been reported in patients with IBD. Indeed, up to 40% of patients does not respond [65,66] and between 10 and 25% of subjects presents adverse effects that can lead to withdrawal or discontinuous treatments and sometimes to permanent sequelae. These side effects can be dose dependent (in particular myelotoxicity) and dose independent [67]: the most common are leukopenia, drug allergy, nausea and pancreatitis. The variability in therapeutic response and susceptibility to adverse effects is influenced by differences in genetic profiles in thiopurine biotransformation or cellular transport and immune system regulatory proteins (Table 2).

4.1.1. TPMT

The enzyme TPMT catalyzes the S-methylation of thiopurines to MMP with S-adenosyl-L-methionine acting as the S-methyl donor. Weinshilboum and Sladek, in a study on 298 adult blood donors, have demonstrated that TPMT activity has a trimodal distribution: approximately 89% of subjects has high enzyme activity, 11% intermediate and 0.3% is deficient

Table 2. Pharmacogenetic variants involved in thiopurine response and toxicity in IBD patients.

GENE	PROTEIN FUNCTION	GENETIC VARIANTS	PATIENTS	INFLUENCE ON RESPONSE	REFERENCE
TPMT	Inactivation of thiopurine drugs via S-methylation	*2 rs1800462 (238G>C) *3A rs1800460 (460G>A) rs1142345 (719A>G) *3B rs1800460 (460G>A) *3C rs1142345 (719A>G)	609 (CD=356; UC=253)	↑ risk of leukopenia	[74]
NUDT15	Degradation of oxidized purine nucleoside triphosphates by dephosphorylation	rs116855232 (415C>T)	978 CD	↑ risk of leukopenia	[79]
ITPA	Conversion of inosine triphosphate into inosine monophosphate	rs1127354 (94C>A)	48 (CD=19; UC=29)	Putative risk factor for leukopenia	[85]
HLA	Major Histocompatibility Complex	rs2647087 (A>C)	360 (CD=234; UC=126)	Risk factor for AZA-induced pancreatitis	[88]
GSTM1	Conversion of AZA into MP	Whole gene deletion	199 (CD=111; UC=88)	↓ 6-MMPR levels → reduced risk of AZA induced side effects – necessity of ↑ AZA dose	[92]
PACSIN2	Modulation of TPMT expression and degradation	rs2413739 (C>T)	334 (CD=244; UC=90)	No influence on adverse effects in IBD patients	[95]
ABCC4	Efflux transporter of nucleoside monophosphate analogs	rs3765534 (2269G>A)	235 (CD=78; UC=157)	Putative risk factor for leukopenia	[98]

Thiopurine methyltransferase (*TPMT*); Nudix hydrolase 15 (*NUDT15*); Inosine triphosphate pyrophosphatase (*ITPA*); Human leukocyte antigen (*HLA*); Glutathione S-transferase Mu 1 (*GST-M1*); Protein kinase C and casein kinase substrate in neurons protein 2 (*PACSIN2*); ATP-binding cassette sub-family C member 4 (*ABCC4*); Azathioprine (AZA); 6-methylmercaptopurine riboside (6-MMPR); Crohn's disease (CD); Ulcerative colitis (UC).

[68]. However, even if a trimodal distribution is traditionally described, approximately 15% of patients have a TPMT activity above the usual normal range and 1–2% have ultra-high activity [67,69]. More than 40 allelic variants of the TPMT gene have been reported [70], most related to loss of activity: TPMT*2 (238G>C, rs1800462, Ala80Pro), *3A (460G>A, rs1800460, Ala154Thr; 719A>G, rs1142345, Tyr240Cys), *3B (460G>A, rs1800460, ala154Thr), *3C (719A>G, Tyr240Cys) are the most common and well documented variant alleles, associated with a decreased enzymatic activity. These heterozygous or homozygous variant genotypes translate to a very unstable protein, with reduction of enzyme activity, resulting in decreased MMP production and a corresponding increase in TGNs, which can cause severe leukopenia [71,72]. Clinical guidelines have been developed to implement TPMT genotyping to guide therapy with thiopurines, and limit adverse events in patients with reduced activity [72]. These guidelines indicate to consider an alternate agent or extreme dose reduction of AZA for patients with low or deficient TPMT activity and to start at 30–70% of target dose (e.g. 1–1.5 mg/kg) for patients with intermediate enzyme activity. A recent prospective clinical trial demonstrated a 10-fold reduction in hematologic adverse drug reactions among variant carriers treated with a reduced dose, compared with variant carriers who did not, without differences in treatment efficacy [73,74].

Before starting thiopurine therapy TPMT genotype is generally preferred, given its strong association with TPMT activity [72], however complementary phenotype laboratory tests can be helpful adjuncts to genotyping tests [75].

Recently, age has been demonstrated to affect the activity of TPMT and the efficacy of thiopurines in pediatric IBD patients, with effects particularly strong in early onset patients (age < 6 years old), which had increased TPMT activity, reduced production of active TGN metabolites per unit of AZA dose and need for increased AZA dose [76]. Further studies are needed to investigate the molecular mechanism of this age-dependency in TPMT activity and its clinical implications.

Among patients with CD and myelosuppression during AZA therapy, only 27% had a TPMT genotype associated with enzyme deficiency, so myelosuppression could be caused by other factors, including other genetic variants [77].

4.1.2. NUDT15

Although East-Asian populations have a lower frequency of TPMT mutations compared to Caucasians (around 3% and 10%, respectively) [78], they have a significantly higher probability to develop leukopenia during AZA treatment [79]. A GWAS was conducted by Yang et al. to screen for genetic variants responsible of this side effect. Nine hundred seventy-eight Korean CD patients have been analyzed for more than 400,000 SNPs and a nudix (nucleoside diphosphate linked moiety X)-type motif 15 (NUDT15) gene variant (rs116855232, Arg139Cys) was identified as responsible of leukopenia in CD patients [80]. NUDT15 encodes for a nucleoside triphosphate diphosphatase, which degrades oxidized purine nucleoside triphosphates by dephosphorylation to prevent their potentially toxic incorporation into DNA; thio-dGTP, structurally similar to the natural substrates of NUDT15, could be hydrolyzed to the inactive thio-dGMP or thio-dGDP metabolites [79]. NUDT15 rs116855232 variant is rare in Caucasians, but has been associated with leukopenia also in IBD patients of this ancestry [80]. Recent sequencing studies of all exonic regions of this gene identified additional variants (Arg139Cys, Arg139His, Val18Ile, Val18_Val19insGlyVal; p.Arg34Thr, Lys35Glu, and Gly17_Val18del) that may contribute to thiopurine sensitivity. These coding variants resulted in 74.4–100% loss of nucleotide diphosphatase activity [81,82]; interestingly the 6 bp in-frame deletion (rs746071566) was recently confirmed also in patients with IBD as strongly associated with thiopurine-induced myelosuppression [83]. A recent study [84] conducted in 2630 Japanese patients with IBD confirmed the association of the Arg139Cys with thiopurine-induced leukopenia and alopecia, and with digestive symptoms, supporting that this is a good candidate for the prediction of these toxicities in Japanese patients with IBD.

4.1.3. ITPA

Inosine triphosphate pyrophosphohydrolase (ITPA) is a pyrophosphatase that converts inosine triphosphate (ITP) into inosine monophosphate (IMP), preventing an abnormal accumulation of ITP and deoxyITP (dITP) in cells. ITPase deficiency has a genetic basis. In healthy controls, homozygotes for the 94C>A missense mutation (Pro32Thr, rs2711354) had zero erythrocyte ITPase activity, whereas heterozygotes averaged 22.5% of the controls [85]. Another ITPA SNP, localized in intron 2, is IVS2 + 21A>C (rs7270101); this variant in homozygosis leads to a 60% enzyme activity compared to the wild type. Both variants together resulted in a 13% ITPA activity compared to wild type [85]. Variants in ITPA have been associated with AZA-induced adverse events [86]. In Japanese patients with IBD the incidence of leukopenia in subjects with the 94C>A was significantly higher than that in those without ($P < 0.05$) [87]. Recently, in pediatric patients with immunological diseases such as IBD and autoimmune hepatitis treated with AZA or MP, ITPA activity was not associated with occurrence of adverse reactions, but a relationship between high ITPA activity and γ-globulin, a marker of inflammation, was found in children with IBD [88].

4.1.4. HLA

Pancreatitis affects around 4% of AZA-treated IBD patients and leads to AZA withdrawal; however, there are no predictive tests in clinical practice to identify patients at risk. A GWAS was conducted in IBD patients and a strong association between the Class II HLA gene region polymorphism (A>C, rs2647087) and pancreatitis [89] was identified. Another recent study confirmed that this HLA is an important marker of AZA-induced pancreatitis: patients with AC or CC alleles for rs2647087 SNP had a 2.5- and 5- fold higher risk, respectively, to develop this side effect compared to wild-type [90].

4.1.5. Other genes

GST are a class of metabolic enzymes that have been shown to be relevant in the biotransformation of AZA to MP [91]. The GSTM1 isoform is highly polymorphic: around 50% of the Caucasian and Asian populations carries the homozygous gene deletion that results in the complete absence of enzyme activity. Stocco et al. [92] have shown that IBD patients with

wildtype GSTM1 genotype presented increased probability of developing adverse effects and increased incidence of lymphopenia during AZA treatment. The same authors reported that, in young IBD patients, GSTM1 deletion was associated with lower TGN/dose ratio, higher azathioprine dose requirement and reduced response to therapy [93]. Recently GSTM1 delition was associated with MMPN concentration during AZA but not MP treatment in patients with IBD [94].

Protein kinase C and casein kinase substrate in neurons protein 2 (PACSIN2) [95] is involved in the modulation of TPMT [96]. In a recent study, rs2413739 in PACSIN2 was significantly associated with severe thiopurine-related gastrointestinal toxicity in acute lymphoblastic leukemia (ALL) [96], but not in patients with IBD [97].

ABCC4 (MRP4) is an efflux transporter of nucleoside monophosphate analogs, including all major thiopurine metabolites such as TGNs. The minor allele of ABCC4 SNP 2269G>A (rs3765534, E857K) encodes for a fivefold less expressed variant, resulting in marked MP accumulation and subsequent cytotoxicity in an embryonic kidney cell line. ABCC4 2269A is rare in Caucasians and Africans, but is more frequent in Japanese (15–22%) and in Han Chinese (8.3%). In 130 IBD patients treated with thiopurines, the white blood cell count was significantly lower in patients with the MRP4 variant than in wild type patients. TGN levels were also significantly higher in patients with the variant, suggesting that the SNP might influence thiopurine sensitivity in Japanese patients [98].

Therapeutic drug monitoring of thiopurines can be useful to improve therapeutic outcomes with these medications: several studies have reported thresholds of efficacy for TGN concentrations, confirmed also by meta-analyses [99,100] while some studies associate high MMPN concentrations with the risk of hepatotoxicity [101]. The caveat of therapeutic drug monitoring for thiopurines is that, given the long half-life of TGN and MMPN, steady state concentrations are reached only after several weeks of therapy [102]. Genotyping therefore is preferred to identify preventively, before starting therapy, patients at risk of severe adverse events such as patients inheriting inactive alleles of TPMT and NUDT15 or HLA variants predisposing to pancreatitis [103]. However, particularly in patients with no risk genotype for thiopurine induced adverse event, therapeutic drug monitoring may be helpful in improving efficacy of thiopurines in IBD, also by allowing a better assessment of patients compliance [104].

In patients with unfavorable profile of thiopurine metabolism, in particular with reduced TGN and increased MMPN, allopurinol has been used to increase TGN concentration and improve therapeutic outcomes [105,106]. Some clinical guidelines suggest administration of low dose AZA and allopurinol to improve therapeutic outcomes [67]. More studies could be useful to evaluate the pharmacogenetic factors affecting efficacy and adverse events of the combination of thiopurines with allopurinol in patients with IBD [107,108].

5. Biologics

5.1. Anti TNFα agents

TNFα is a pro-inflammatory cytokine produced as a 26 kDa transmembrane protein (tmTNF), arranged in stable homotrimers. It is cleaved by TNFα converting enzyme (TACE) in the soluble form (sTNF). sTNF and tmTNF are biologically active and preferably interact with TNF receptor (TNFR)1 and TNFR2, respectively. TNFR1 pathway involves caspase (CASP)-dependent death signaling and pro-survival NFkB activation while TNFR2 activates exclusively pro-inflammatory and pro-survival signaling pathways [109]. Anti-TNFα agents are effective in patients with IBD who do not adequately respond to GCs and immunosuppressants, and have a rapid onset of effect, usually within 2 weeks after initiation of therapy. These agents, and in particular infliximab (IFX), are often used in combination with an immunomodulator, increasing their efficacy and reducing anti-drug antibody formation (ADA) [6]. Three full-length IgG1 monoclonal antibodies (mAb) IFX, adalimumab (ADM), golimumab and one Fab' antibody fragment certolizumab-pegol, are at present available in clinics. IFX is a chimeric mAb with variable regions of a mouse anti-human TNF mAb fused with the constant portion of a human IgG1; ADM and golimumab are fully human mAb and certolizumab-pegol is a humanized Fab of a mAb conjugated to polyethylene glycol. The different drug structures influence their ability to bind sTNF and tmTNF, resulting in variable therapeutic response [110]. Anti-TNFα bind with high affinity the sTNF and tmTNF forms of TNFα and inhibit binding with its receptor. They act mainly through induction of lamina propria T cell apoptosis, a form of direct antibody-dependent cytotoxicity induced by all antibody-derived anti-TNFα agents. The full-length mAbs act also by binding tmTNF on activated T cells and the Fc is recognized by Fc receptors expressed by monocytes triggering their differentiation to wound-healing macrophage. This mechanism is Fc-dependent and is therefore absent in the case of certolizumab [111]. These cellular effects at the tissue level result in restoring also the balance between matrix metalloproteinases and tissue inhibitors of metalloproteinases [112].

5.1.1. Pharmacogenetics

Anti-TNFα agents are widely used in the treatment of IBD but approximately 30% of patients initially responds to therapy but then relapse. An important determinant of therapeutic failure with these agents, and in particular with IFX, is the development of ADA [113].

Several studies have considered the possibility that this variability is caused by genetic variants in genes involved in immune processes, inflammation, autophagy and apoptosis (Table 3). Two SNPs (rs1800629; rs361525) located in the TNFα gene promoter seem to be involved in its expression and anti-TNFα response, particularly in patients with rheumatological conditions [114]. The rs1800629 SNP is a −308G>A substitution and influences the regulation of TNFα synthesis; in particular, allele A confers a major transcriptional activation and increased TNFα production comparing to the common allele G. AA and GA genotypes were correlated with non-response to anti-TNFα treatment: the combination of TNF −308A allele and the CC genotype in the SNP rs763110 of the FAS ligand (FASL) gene gives an additive effect compared to each SNP separately, and CD patients with these variants had nearly five-fold higher odds of being non-responders [115]. The TNFα rs361525 SNP (−238G>A) and genetic variations in other genes involved in NFκB-signaling pathway such as rs4149570 in TNFR1, rs976881, rs1061622 and rs652625

in TNFR2 and rs2430561 in interferon gamma (IFNG) gene seem to be also important predictors for good IFX response.

Polymorphisms in other cytokine genes could influence the predisposition to IBD and therapeutic response. The rs2275913 SNP in IL17A confers a poor response to treatment with anti-TNFα drugs [116]. Fujino et al. have shown that mRNA expression and serum levels of IL17 are increased in patients with IBD and suggested that IL17 might be associated with altered immune and inflammatory responses [117].

The rs10889677 variant in the 3-untraslated region (UTR) of the IL23R gene enhances IL23R mRNA levels and protein production, through the loss of microRNA regulation [118]. Jurgens et al. have demonstrated that this SNP increased the probability to respond to IFX in UC Caucasian patients [119].

Bank et colleagues found that the TC/CC genotypes for rs10499563 in IL6 and the GA/AA genotypes for rs4848306 in IL-1β induce a decrease of these pro-inflammatory cytokines, promoting a better response to anti-TNFα and also found a correlation with several SNPs in Toll Like Receptor (TLR)-related genes and anti-TNFα therapies efficacy in Danish IBD patients [116]. In the same study, the rs1816702CT/TT and rs3804099TC/CC genotypes in TLR2, associated with an increased expression of receptor and to a decreased expression of TNFα, IL1β and IL6, were associated with a good response to treatment while the rs4696480TT and rs11938228CA/AA genotypes in TLR2, the rs1554973TC/CC in TLR4 and rs352139AA in TLR9 were associated with non-response. TLR9 rs187084TC genotype induced a decreased expression of TLR9 and a decrease of NFkB signaling pathway activation, leading to a good anti-TNFα response [116]. Autophagy Related 16 Like 1 (ATG16L1) plays an important role in autophagy and, in an autophagy-independent manner,

Table 3. Pharmacogenetic variants involved in anti-TNFα response and toxicity in IBD patients.

GENE	PROTEIN FUNCTION	GENETIC VARIANTS	PATIENTS	INFLUENCE ON RESPONSE	REFERENCE
TNFα	Cytokine involved in systemic inflammation and regulation of cell proliferation, differentiation and apoptosis	rs1800629 (−308G>A)	121 CD	AA genotype → ↓ response to anti-TNF agents	[115]
FASL	Induction of apoptosis	rs763110 (−843C>T)	121 CD	TT genotype → ↓ response to anti-TNF agents	[115]
			287 CD	TT genotype → ↓ response to IFX	[123]
TNFR1	Activation of caspase-dependent death signaling and NFkB signaling pathway	rs4149570 (609G>T)	738 (CD = 482; UC = 256)	TT genotype → ↑ response to anti-TNF agents	[116]
IFNG	Induction of cellular response to viral and microbial infections	rs2430561 (874T>A)	738 (CD = 482; UC = 256)	AA genotype → ↑ response to anti-TNF agents	[116]
IL17A	Pro-imflammatory cytokine that regulates the activities of NFkB and mitogen-activated protein kinases	rs2275913 (197G>A)	738 (CD = 482; UC = 256)	AA genotype → ↓ response to anti-TNF agents	[116]
IL23R	IL-23 receptor interacts with IL-23 promoting inflammation and immune system's response	rs10889677 (2199A>C)	90 UC	CC genotype → ↑ response to IFX	[119]
IL6	Pro-inflammatory cytokine: inhibitory effects on TNF and IL1 and activation of IL-10	rs10499563 (−6331T>C)	738 (CD = 482; UC = 256)	−6331C and −3737A → ↑ response to anti-TNF agents	[116]
IL1B	Mediator of the inflammatory response, cell proliferation, differentiation, and apoptosis.	rs4848306 (−3737G>A)			
TLR2	Pathogen recognition and activation of innate immunity	rs1816702 (C>T)		CT/TT genotype → ↑ response to anti-TNF agents	
		rs3804099 (597 T>C)		TC/CC genotype → ↑ response to anti-TNF agents	
		rs4696480 (*−16934A>T*)		TT genotype → ↓ response to anti-TNF agents	
		rs11938228 (C>A)		CA/AA genotype → ↓ response to anti-TNF agents	
TLR4		rs1554973 (*C>T*)		TC/CC genotype → ↓ response to anti-TNF agents	
TLR9		rs352139 (1174A>G)		AA genotype → ↓ response to anti-TNF agents	
TLR9		rs187084 (−1486T>C)		TC genotype → ↑ response to anti-TNF agents	
ATG16L1	Induction of autophagy and regulation of mitochondrial *IFN-I* production and NOD1- and NOD2-driven inflammatory cytokine response	rs10210302 (C>T)	102 CD	TT genotype → ↑ response to ADM	[120]
			121 CD	NO association with anti-TNF response	[115]
FCGR3A	Removal of antigen-antibody complexes from the circulation, and antibody-dependent responses	rs396991 (559A>C)	103 (CD = 80; UC = 23)	AC/CC genotype → ↓ response to anti-TNF agents	[121]
			102 CD	CC genotype → ↑ response to IFX	[122]
CASP9	Induction of apoptosis	rs4645983 (93 C>T)	287 CD	TT genotype → ↑ response to ADM	[123]
ADAM17	Modulation of the signaling activity of a variety of cytokines, receptors and growth factors	rs1056204 (A>C)	186 CD	haplotype → ↑ response to IFX	[126]
		rs12469362 (T>C)			
		rs10495565 (A>G)			
		rs4464248 (A>G)			

Tumor Necrosis Factor (*TNF*); FAS ligand (*FASL*); Tumor Necrosis Factor Receptor (*TNFR*); Interleukin (IL); Toll Like Receptor (TLR); Autophagy Related 16 Like 1 (*ATG16L1*); Fc gamma receptor (*FCGR*); Caspase9 (*CASP9*); A Disintegrin And Metalloproteinase 17 (*ADAM17*); Adalimumab (ADM); Infliximab (IFX); Crohn's disease (CD); Ulcerative colitis (UC)

suppresses inflammatory cytokines regulating mitochondrial antiviral signaling (MAVS)-dependent type I IFN production and Nucleotide-Binding Oligomerization Domain (NOD)1 and NOD2-driven inflammatory cytokine response. Genetic variations in ATG16L1 gene influence the clinical response in IBD patients. Koderet al. have demonstrated a strong association with rs10210302 SNP and ADM response in CD Slovenian patients. Patients with the T allele had reduced C reactive protein levels and a better response to ADM after 12, 20, and 30 weeks of treatment compared to the CC genotype [120]. This correlation was not confirmed by Netz and colleagues: in CD Caucasian patients, the rs10210302 and rs2241880 SNPs, linked to diminished autophagy, were not associated with anti-TNFα response. No significant difference was observed also for SNPs in IBD5 (rs2522057), TNFα (rs361525) and FCGR3A (rs396991) genes [115]. Romero-Cara et al. have studied the role of the non-synonymous polymorphism in the receptor for the Fc portion of IgG (FCGR)3A (V158F, rs396991) in Caucasian IBD patients treated with IFX and ADM. Since FCGR receptors are involved in the degradation of IgG, the authors hypothesized that the polymorphism could be involved in the production of ADA. The rs396991T allele encodes phenylalanine (F) while the G allele encodes valine (V). In this study, VV genotype correlated with patients producing ADA [121]. However, different results were obtained by a Japanese study that reported that the FCGR3A V158 was correlated with a better response to anti-TNFα therapy at week 8 in CD patients [122].

Since the efficacy of anti-TNFα, and in particular of IFX, depends on their ability to induce apoptosis, genetic variation in apoptosis genes (FASL, CASP9) could influence the treatment response. FASL is a member of the TNF family of proteins that act as membrane-bound or soluble homotrimers to aggregate their receptors into functional signaling units. FASL is involved in the induction of apoptosis and in the immune system regulation. Hlavaty et al. reported that the rs763110 SNP is associated with IFX response in Caucasian CD patients. The -843TT genotype exhibited an association with non-response in comparison to the CC genotype at week 4 [123]. Caspase-9 (CASP9), encoded by the CASP9 gene, is critical to the apoptotic pathway [124]. The rs4645983 variant in CASP9 has been associated to response only in patients with luminal CD: patients with CASP9 93TT genotype showed better response to ADM treatment compared with patients with C allele [123].

ADAM metallopeptidase domain 17 (ADAM 17), also known as TACE, is a disintegrin-metalloproteinase that acts as a sheddase, modulating the signaling activity of a variety of cytokines, receptors and growth factors; the enzyme cleaves many membrane proteins, including TNFα [125]. Dideberg et al. [126] have identified several variants in ADAM17 gene implicated in IFX response. The rs1056204, rs12469362, rs10495565 and rs4464248 haplotypes were associated with good IFX response in CD Caucasian patients.

5.1.2. Pharmacoepigenetics

Recent studies have evaluated the role of serum biomarkers (protein and miRNA) in IFX response in IBD patients. Heier et al. identified three miRNAs (miR-146a, miR-320a and miR-146b) and 18 proteins (SERPINA1, IGFBP1-2, RETN, CCL23, P4H4, CNDP1, CDH3, CCL25, SPOCK2, ADGRE2, CHRDL1, IFNA2, NCAM1, AGT, IgM, CNTF and CD36) in serum of pediatric IBD patients responsive to IFX and prednisone. Serum markers regulated by NFkB (miR-146a, miR-320a, miR-146b, SERPINA1, IGFBP1-2, RETN and CCL23) decreased after IFX treatment, while markers that increased are involved in resolving inflammation [54]. Fujioka et al. have demonstrated the association of serum miRNA levels with IFX response in CD adult patients. In particular authors showed increased levels of five miRNAs (let-7d, let-7e, miR-28-5p, miR-221 and miR-224) during the induction therapy. Only let-7d and let-7e, that seem to play an important role in the regulation of apoptosis, were associated with IFX efficacy and clinical remission [127].

In 22 patients of the ACT1 trial of IFX in UC a transcriptomic biomarker, consisting of 109 probe sets that corresponded to 81 genes, was related to endoscopic and histologic response to IFX [128]. The biomarker was subsequently validated retrospectively in biopsies obtained from patients of the PURSUIT golimumab study [129]. When a refined 13 genes signature was subsequently used in a recent prospective phase 2a open label study in IBD patients treated with golimumab, mucosal healing was predicted with high sensitivity (87%); however, the specificity was low (34%), hence limiting the clinical usefulness of the signature [130]. These results highlight the difficulties inherent in the use of biomarkers for the prediction of the effect of these drugs and of their toxicities.

5.2. Anti IL12/23 antibodies

Among inflammatory cytokines involved in IBD pathogenesis, IL23 plays a central role because of its effects both on Th17 cells expansion and maintenance and on innate lymphocytes [131]. IL23 is a heterodimeric molecule composed of a p40 and a p19 subunit: the former chain (also known as IL12B) is shared with IL12, another important pro-inflammatory cytokine whose production is enhanced in CD, whereas the latter chain is an IL23 specific subunit (IL23A). GWAS meta-analysis established IL12B as an IBD susceptibility gene and identified SNPs rs10045431, rs6871626 and rs6556412 as major susceptible loci for increased CD risk in European ancestry populations [5,132]. Two fully human IgG1 mAbs, ustekinumab and briakinumab, have been designed to bind with high affinity and specificity the p40 chain and thus neutralize both IL12 and IL23 mediated activities. Although similar in their mechanism of action, a meta-analysis study revealed that ustekinumab, but not briakinumab, was better than placebo in achieving remission and reducing symptoms of active CD [133]. In November 2016, ustekinumab has been approved by the European Medicines Agency (EMA) for the treatment of adult patients with moderate to severe CD who showed an inadequate response to conventional or TNFα antagonist therapy.

Pharmacogenetic studies on ustekinumab response in IBD are currently missing. However, some genetic predictive markers of drug efficacy have been identified in cohorts of adult patients with psoriasis using a candidate gene approach: significant associations have been identified for variants in HLC-C, IL1b, IL12B and genes related to the TNFα and toll-like

receptor pathways [134–136]. It is an open question whether the above-mentioned candidate SNPs could play a role also in IBD patients.

5.3. Anti integrin antibodies

Anti-adhesion therapies have been recently developed and are progressively more important in IBD [137]. The clinical use of biologicals like natalizumab and vedolizumab has gained considerable interest since both mAbs regulate the capacity of T cells to localize to the gut and modulate the inflammatory process. Natalizumab targets integrin $\alpha_4\beta_1$ which is found at high levels on the surface of almost all leukocytes and prevents its binding with vascular cell adhesion molecule-1 required for leukocyte adhesion, firm attachment and transmigration across vascular barriers. Vedolizumab has a gut-specific mechanism of action because it is developed against integrin $\alpha_4\beta_7$ present on T cells in the lamina propria: impairment of $\alpha_4\beta_7$ interaction with mucosal vascular addressin cell adhesion molecule 1, which is expressed on endothelial cells, prevents T cells infiltration on the gastrointestinal mucosa [137]. Vedolizumab is now employed as a pharmacotherapeutic option before surgery for UC patients who do not respond to anti-TNFα therapy [138]. Again, no pharmacogenetic data on anti-adhesion therapies in IBD are available in literature.

6. Thalidomide

Despite thalidomide's tragic history, the discovery of its anti-angiogenic and immunomodulatory properties renewed interest in the drug. Thalidomide has been successfully used in inflammatory chronic conditions, in particular, it appears to be a valuable treatment option for IBD patients refractory to standard therapies [7,8]. Thalidomide inhibits selectively TNFα expression and suppresses TNFα-induced NFκB activation, preventing its DNA binding activity [139]. *In vitro* studies suggest that thalidomide blocks TNFα production by enhancing mRNA degradation, even though the exact mechanisms have not yet been elucidated [140]. In patients with CD who responded to thalidomide therapy, production of TNFα and IL12 was decreased in PBMCs and intestinal lamina propria mononuclear cells, whereas IL1 and IL6 concentrations did not change significantly [141]. Thalidomide inhibits phagocytosis and blocks neutrophil chemotaxis [142] and inhibits vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), both involved in the mechanisms of neovascularization [142]. Moreover, it has been shown that thalidomide binds and inactivates cereblon, a substrate recognition component of a complex that mediates proteasomal degradation of proteins, resulting in antiangiogenic and immunomodulatory effects [143], but it is still unclear whether this mechanism is implicated in the effect of the drug in IBD patients.

Thalidomide-induced peripheral neuropathy (TiPN) is one of the most frequent adverse events in IBD patients and is a common cause of treatment discontinuation. It occurs in 20% of IBD patients in treatment with thalidomide and is reversible after drug suspension. The development of this side effect is closely linked to the daily dose: the risk seems to be negligible for doses less than 25 mg per day and increases significantly if the daily dose exceeds 75 mg [144]. A recent study conducted on pediatric IBD patients, demonstrates that SNPs in ICAM1 (rs1799969) and SERPINB2 (rs6103) reduce the risk of neuronal damage and favor TiPN resolution [145], proving that polymorphisms in genes involved in neuronal inflammations may be protective.

Clinical efficacy of thalidomide has also been related to genetic polymorphisms, although not in IBD. In prostate cancer patients treated with thalidomide and docetaxel, polymorphisms in three genes (PPARδ, SULT1C2 and CHST3) were associated with clinical outcome measures whereas polymorphisms in eight genes (SPG7, CHST3, CYP2D6, NAT2, ABCC6, ATP7A, CYP4B1 and SLC10A2) were associated with toxicity [146].

7. Conclusion

Treatment of IBD comprises several agents used to induce and maintain remission. For these agents a number of studies considering genetic and epigenetic variability influencing therapeutic outcome and drug toxicity have been performed, however, only for thiopurines evidence is sufficient to provide clear guidelines for therapy personalization, while for the other agents more studies are needed. In particular, many studies present inconsistent results or consider small patients' cohorts. To further improve pharmacogenetics of IBD, great expectations rely on the study of epigenetics and integration with pharmacokinetic information, especially useful for biologics.

8. Expert opinion

Pharmacological therapy of IBD is important to induce and maintain disease remission, improve patients' quality of life and to reduce the need for surgery. Besides the effective classical immunomodulating small molecular agents, the introduction of antibodies targeting pro-inflammatory cytokines has revolutionized and greatly increased outcomes of therapy. However, significant interindividual variability is present and some patients do not achieve a successful clinical remission, lose response after initial successful treatment and may also develop severe drug induced adverse events.

For several medications such as thiopurines and GCs, a clinically relevant contribution of genetic variability in genes encoding for enzymes involved in drug biotransformation or for drug targets has been clearly demonstrated. In particular for AZA and MP, therapy personalization based on TPMT genetic variants may increase efficacy and reduce the incidence of myelosuppression in patients with variants associated with reduced enzymatic activity. However, a significant proportion of patients with normal TPMT activity may develop adverse effects from thiopurines. Thanks to agnostic genome-wide studies, important additional genetic variants associated with AZA and MP induced adverse effects have been identified, in particular NUDT15 for myelosuppression and HLA for pancreatitis.

For GCs, even if a single genetic determinant of drug efficacy and adverse effect has still to be identified, encouraging insights have been obtained from the study of innovative epigenetic markers, such as microRNAs and DNA methylation profiles: given the mechanism of action of these agents that modulate immune response through a direct action on gene expression, further development of these studies may be encouraging in identifying molecular markers useful for therapy personalization.

Several studies have evaluated the association between genetic variants and the effects of monoclonal antibodies, with some variants involved in proteins important for antibodies degradation, such as FCGR, or in drug targets, such as TNFα for IFX and ADM, have shown encouraging results, even if more studies are needed to identify clinically useful pharmacogenetics biomarkers. Even for anti-TNFα agents, preliminary data on association between epigenetic markers and treatment efficacy have been collected, however, problems in the replication of results have been observed. For these agents, it is clear that pharmacokinetics, in particular drug concentrations, are strongly associated with disease remission and proper integration of pharmacokinetic and pharmacogenetic markers may lead to the identification of strategies to prevent and overcome remission induction failure or loss of response.

Pharmacogenetic studies are often limited by relatively small sample sizes, which reduce the relevance and applicability of the associations identified. To improve this field, it will be important to develop larger national and international collaborative studies. Recently, significant breakthroughs have been obtained by the application of agnostic genome-wide analysis to the adverse effects of thiopurines, thanks to collaborative international groups and similar approaches could be applied also to the other medications. The great challenge will be to carefully and uniformly record and collect the study data across several collaborative centers, considering also all the potential environmental factors and additional biomarkers associated with clinical features of IBD.

For pharmacogenetic markers identified as clinically relevant, besides the development of evidence-based clinical guidelines, for a proper clinical implementation and application by physicians, tools for interpreting the genetic information, such as informatic clinical decision support systems and training about pharmacogenetics of physicians, nurses, pharmacists and other health providers should be performed.

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