The Pharmacogenetics of Rituximab: Potential Implications for Anti-CD20 Therapies in Multiple Sclerosis

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Abstract

There are a broad range of disease-modifying therapies (DMTs) available in relapsing-remitting multiple sclerosis (RRMS), but limited biomarkers exist to personalise DMT choice. All DMTs, including monoclonal antibodies such as rituximab and ocrelizumab, are effective in preventing relapses and preserving neurological function in MS. However, each agent harbours its own risk of therapeutic failure or adverse events. Pharmacogenetics, the study of the effects of genetic variation on therapeutic response or adverse events, could improve the precision of DMT selection. Pharmacogenetic studies of rituximab in MS patients are lacking, but pharmacogenetic markers in other rituximab-treated autoimmune conditions have been identified. This review will outline the wider implications of pharmacogenetics and the mechanisms of anti-CD20 agents in MS. We explore the non-MS rituximab literature to characterise pharmacogenetic variants that could be of prognostic relevance in those receiving rituximab, ocrelizumab or other monoclonal antibodies for MS.

Key words Multiple sclerosis · rituximab · ocrelizumab · monoclonal antibody · pharmacogenetics · pharmacogenomics

Introduction

For patients with relapsing-remitting multiple sclerosis (RRMS), available disease-modifying therapies (DMTs) are key, but imperfect, in preventing relapses and disease progression. Delayed effective treatment and prolonged exposure to suboptimal therapy increases the risk of irreversible disability [1–3] and adverse events. The sources of inter-individual therapeutic variability, particularly genetic factors, are incompletely understood. Pharmacogenetic markers could optimise long-term disease outcomes by improving treatment selection for a given patient. Existing pharmacogenetic and pharmacogenomic studies of MS DMTs have not yet led to significant changes in prescribing practice, and are largely limited to examinations of interferon-beta and glatiramer acetate [4]. Some pharmacogenetic markers for these two

modestly effective agents have been replicated and show promise as predictors of therapeutic success. However, a more complete understanding of the gene-drug interactions that dictate the efficacy of DMTs, particularly higher efficacy therapies including monoclonal antibodies, is required. Rituximab, an anti-CD20 monoclonal antibody with demonstrated efficacy in RRMS and other neurological, rheumatological and haematological conditions, represents a currently unique opportunity to explore and identify these mechanisms.

Pharmacogenetics and Pharmacogenomics

The spectrum of inter-individual pharmacokinetic variability was clarified with assays able to identify those with unusually high or low plasma drug or metabolite levels. The discovery of genetic variation in the cytochrome P-450 (CYP450) family of enzymes, and other metabolic and transport pathways that underpin this unpredictability, was a key achievement [5]. Pharmacogenetics is the study of DNA variations that relate to drug response. Single-nucleotide polymorphisms (SNPs), genetic variations present in at least 1% of a population, are the focus of most genetic studies of complex traits such as drug response. In each human genome, there are approximately 4–

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5 million SNPs, one for every 1000 nucleotides [6]. Candidate genes, selected for known associations with disease phenotypes, were the initial focus of pharmacogenetics studies. The advent of next-generation sequencing (NGS) revealed the whole exome (protein-coding DNA) and genome (almost all DNA), and has resulted in an exponential increase of known rare and common SNPs in the last two decades [7, 8]. With the availability of electronic health records and associated infrastructure, gene and genome-based therapeutic personalisation has the potential to be diversely and recurrently useful throughout a lifetime [9].

Gene-drug interactions can affect pharmacokinetics (absorption, distribution, metabolism or elimination) or, less commonly, pharmacodynamics (target effects) [10]. Prescribers may therefore benefit from the genetic identification of patients at risk of therapeutic failure or drug toxicity. Although most are not screened for in routine practice, 91 to 99% of patients possess at least one common genetic variant that would prompt more vigilant surveillance, a change in dosing, or avoidance, of certain medications [11, 12]. As of September 2020, the Clinical Pharmacogenetics Implementation Consortium (CPIC) recognises 416 gene interactions with drugs or drug classes, 34 of which have established guidelines (Table 1). Although some increase enzymatic activity (e.g. *CYP2C19**17 and *CYP2D6* duplications), the majority of these variants effect a partial or complete inhibition of function [5]. Examples include allelic variants of CYP450 enzymes such as *CYP2C19* and *CYP2D6*, which predict outcomes in patients taking antidepressants or antipsychotics [13–16]. An individual's metaboliser status can be described on a range between “poor” and “ultrarapid,” with corresponding variations in drug plasma concentrations, efficacy and side effect profile [17]. Codeine and other oral opioid formulations require enzymatic bioactivation for efficacy [18]. Poor *CYP2D6* metabolisers are subjected to therapeutic inefficiency or failure, and ultrarapid metabolisers are posed a significantly higher risk of opioid toxicity [19]. Clopidogrel, an antiplatelet that similarly requires activation by *CYP2C19*, is commonly prescribed for secondary prevention of cardiovascular events. Patients who have poor or intermediate *CYP2C19* activity are subjected to significantly worse cardiovascular outcomes, such as in-stent restenosis following percutaneous coronary intervention [20]. Where gene-informed prescribing of clopidogrel is available, multiple studies have shown its superiority, with significantly lower ischaemic and bleeding events compared to usual prescribing practices [21–24].

Contemporary oncology routinely makes use of tumour sequencing or gene product assays to identify driver mutations, successfully targeting these mutations with molecular therapies [25]. Gene product targets include human epidermal growth factor receptor 2 (HER2) in breast cancer (trastuzumab), epidermal growth factor receptor (EGFR) in

Table 1 Gene-drug interactions with existing CPIC guidelines

Gene(s)	Drug(s)
<i>CFTR</i>	Ivacaftor**
<i>CYP2B6</i>	Efavirenz
<i>CYP2C19</i>	Clopidogrel Voriconazole Selective serotonin reuptake inhibitors Tricyclic antidepressants Proton pump inhibitors
<i>CYP2C9</i>	Phenytoin Non-steroidal anti-inflammatory drugs Warfarin
<i>CYP2C9</i> <i>VKORC1</i> <i>CYP4F2</i> <i>CYP2D6</i>	Atomoxetine Codeine Ondansetron, tropisetron Tamoxifen Selective serotonin reuptake inhibitors Tricyclic antidepressants
<i>CYP3A5</i>	Tacrolimus
<i>DPYD</i>	Fluoropyrimidines
<i>G6PD</i>	Rasburicase**
<i>HLA-A</i>	Carbamazepine, oxcarbazepine
<i>HLA-B</i>	Abacavir** Allopurinol Carbamazepine**, oxcarbazepine* Phenytoin
<i>IFNL3</i>	Peginterferon-based regimens
<i>RYR1</i> , <i>CACNA1S</i>	Succinylcholine and volatile anaesthetic agents
<i>SLCO1B1</i>	Simvastatin
<i>TPMT</i> , <i>NUDT15</i>	Thiopurines*
<i>UGT1A1</i>	Atazanavir

**Testing required; *testing recommended

Source: Clinical Pharmacogenetics Implementation Consortium (<https://cpicpgx.org/guidelines>)

colorectal cancer (cetuximab) and CD20 in chronic lymphocytic leukaemia and non-Hodgkin's lymphoma (rituximab). With selective cytotoxicity against CD20-positive B cells, rituximab became the first clinically used chemotherapeutic monoclonal antibody in 1997; it has since shown efficacy in various autoimmune conditions such as rheumatoid arthritis [26] and multiple sclerosis [27].

B Cells and MS

Although initially thought to be primarily driven by aberrantly activated cytotoxic (CD8+) and helper (CD4+) T cells, evidence now suggests the inflammation in MS stems from more complex and bidirectional interactions between T cells and antigen-presenting cells (APCs) such as B cells and myeloid

cells (macrophages, dendritic cells and microglia) [28–31]. The pro-inflammatory pathogenicity of B cells in MS most likely involves antibody-independent processes such as antigen presentation, cytokine production and formation of ectopic lymphoid organs in the central nervous system (CNS) [32, 33].

Antibody Production

Plasma cells derived from B cells fulfil the function of humoral immunity in the adaptive immune system. B cell receptor (BCR) binding to a specific antigen results in clonal expansion of the B cell, maturation to plasma cells and a coordinated antibody response. Antibodies have been implicated in active MS lesions, where they can be found along myelin sheaths, and within phagocytosing macrophages [34, 35]. A signature of humoral activity is the intrathecal presence of oligoclonal bands: clonally restricted antibodies, produced by plasma cells that undergo clonal expansion in the cerebrospinal fluid (CSF) [36–38]. Autoantibodies are an unlikely primary pathogenic mechanism in MS, particularly because anti-CD20 therapies—which deplete B cells, but usually not plasma cells or immunoglobulin levels—are highly effective in the disease [27, 39].

Antigen Presentation

Unlike myeloid APCs such as macrophages and neutrophils, B cells are highly selective for antigens bound to their unique BCR [40]. The antigen-BCR complex is internalised and processed, before being presented externally to CD4+ T cells (Th1 and Th17) with complementary T cell receptors. This process is dependent on major histocompatibility complex class II (MHC-II) and costimulatory molecules (CD80, CD86 and CD40). In MS, priming of T cells is caused by autoreactive B cells, which more densely express MHC-II and costimulatory molecules and demonstrate higher levels of antigen-presenting activity compared to B cells of healthy controls [41, 42].

Cytokine Production

The binding of autoantigen to BCR also causes aberrant B cells to produce effector cytokines that perpetuate the inflammatory milieu. In MS, the effector cytokine profile is abnormally inflammatory compared to controls, with elevated interleukin 6 (IL-6) and depressed IL-10 levels [43, 44]. Anti-inflammatory effects are mediated by regulatory B (B-reg) cells and cytokines such as IL-10 and IL-35; in patients with MS, these B-reg cells respond and differentiate aberrantly compared to controls [45]. IL-6 supports differentiation of CD4+ T cells into pro-inflammatory Th17 cells and diverts the pool away from regulatory T cells [46, 47], an effect that is

mitigated by B cell depletion [48]. Other B cell-secreted cytokines upregulated in MS include granulocyte macrophage-colony-stimulating factor (GM-CSF), which increases mobility and activity of myeloid populations [49], and B cell activation factor (BAFF), which supports B cell self-propagation [50].

Ectopic Lymphoid Follicle Formation

In this landscape of pro-inflammatory cytokines, chemokines and lymphotoxin signalling, B cells support the development of ectopic lymphoid follicles (ELFs), also known as tertiary lymphoid organs [51]. The presence of ELFs in the CNS of MS patients is associated with progressive phenotypes and an earlier onset of the disease [52]. Over time, the primary influence of B cells shifts from infiltrating primed cells that cause focal relapses, to the support and seeding of autonomous populations sequestered in ELFs that drive grey and white matter atrophy [53]. ELFs are similarly described in autoimmune diseases such as systemic lupus erythematosus, as well as cancers and organ transplant rejection, where they perpetuate chronic inflammation in target tissues [54]. B cells residing in transplant-associated ELFs appear relatively protected from anti-B cell therapy: this may in part be due to paracrine secretion of BAFF [55]. In MS, this is further compounded by a relative lack of drug access to the CNS [56, 57].

Anti-CD20 Therapies and Mechanisms in MS

Therapies for RRMS such as alemtuzumab, cladribine and mitoxantrone cause cytolysis of B cells. Fingolimod sequesters lymphocytes including B cells, teriflunomide is cytostatic, while natalizumab prevents their trafficking across the blood-brain barrier. However, it was the efficacy of the anti-CD20 agents in preventing relapse and progression that led to the appreciation of B cells as key to aberrant inflammation in MS [40, 58]. Anti-CD20 therapies deplete cells displaying CD20 (pre-B cells, mature and memory B cells and some plasmablasts), but not B cell progenitors (pro-B cells) and terminally differentiated plasma cells [40, 59]. Administration therefore causes selective loss of B cell lineage cells responsible for antigen presentation and cytokine production, without affecting B cell reconstitution or pre-existing humoral immunity [39]. T cells expressing CD20 are also affected [60, 61]: this could be relevant as patients with MS have an increased proportion of CD20-expressing anti-myelin T cells compared to controls [62, 63]. A negligible peripheral B cell count can be seen as early as 4 days following infusion, with radiological benefit demonstrable at 4 weeks and clinical benefit apparent at an average of 8 weeks [64]. The duration of effect is variable but thought to be typically 6 to 9 months, and is followed by repopulation with B cells that

are more naïve and less autoreactive than the mature and memory B cells they replace [65].

Upon binding to B cells, anti-CD20 agents induce both complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). In CDC, C1q binding to the Fc region of the anti-CD20 antibody results in activation of the complement cascade, complement deposition onto the B cell and apoptosis due to membrane attack complex (MAC) insertion [66]. In ADCC, immune effector cells recognise the Fc portion of the bound anti-CD20 antibody, leading to cytotoxicity or phagocytosis of the B cell [67, 68]. Although CDC is believed to be the major effector mechanism for rituximab, the anti-CD20 family now includes several agents of different constitution, each with a distinct ADCC and CDC profile (Table 2). ADCC is dependent on the binding of the Fc domain to the Ig receptor (FcγR) of effector cells such as natural killer (NK) cells, monocytes, macrophages and dendritic cells [69]. The classes of these receptors include stimulatory (high affinity FcγRI, and low affinity FcγRIIA and FcγRIIA) and inhibitory FcγRs (FcγRIIB) [70]. FcγR

binding also facilitates elimination of immune complexes by myeloid cells, but this mechanism appears to be less significant for agents with cellular targets such as CD20 [71]. Allelic polymorphisms of genes responsible for such receptors are associated with variability in the binding strength of monoclonal antibodies, as discussed next.

All monoclonal antibodies are subclasses of IgG, with metabolism mostly reliant on intracellular lysosomal degradation [72]. Cellular uptake is required for this process and is achieved by either (1) receptor-mediated endocytosis by the target cell following binding of the antibody to its target antigen, or (2) non-specific pinocytosis by phagocytes or endothelial cells of lymphatic vessels [73]. Binding of the monoclonal antibody to its target (for example, the Fab portion of rituximab to CD20 expressed on B cells) triggers endocytosis of the antibody-receptor complex. These target-mediated interactions are dependent on binding affinity and receptor expression, leading to complex non-linear kinetics that vary with repeat administration [74]. At higher doses, receptor-mediated endocytosis becomes a saturated pathway for IgG

Table 2 Characteristics of selected anti-CD20 agents

Drug	Constitution	Administration/dose in MS	Mechanism/characteristics	Indications/evidence
Rituximab	Chimeric Mouse-human IgG1	Intravenous - Loading: two 1000 mg doses, 2 weeks apart - Maintenance: 500–1000 mg every 6–12 months - Alternative induction, or in cases of disease breakthrough: 375 mg/m ² weekly, for 4 weeks	- CDC > ADCC - Enables CDC by effecting clustering of CD20 into lipid microdomains within the plasma membrane [68] - Elevated immunogenicity due to chimeric constitution	Evidence exists for: - RRMS [27], SPMS [80], PPMS [78] - RA [153, 154], ANCA vasculitis [155], CD20+ lymphoid malignancies [156, 157]
Ocrelizumab	Humanised IgG1	Intravenous - Loading: two 300-mg doses, 2 weeks apart - Maintenance: 600 mg every 6 months	- ADCC > CDC [158] - Stronger ADCC than rituximab due to enhanced binding to FcγRIIA receptors [159] - Weaker CDC than rituximab	Evidence exists for: - RRMS [91], PPMS [93] - RA [160, 161]
Ofatumumab	Human IgG1	Subcutaneous - Loading: 20 mg at days 1, 7 and 14 - Maintenance: 20 mg every 4 weeks	- CDC > ADCC - Stronger CDC with similar ADCC to rituximab [98] - May be related to the binding of a more proximal epitope, and more gradual dissociation [68] - Less profound B cell depletion and faster repletion [97]	Evidence exists for: - RRMS [99] - Chronic lymphocytic leukaemia [162]
Obinutuzumab	Humanised IgG1	Intravenous - Dose for RRMS not yet established	- ADCC > CDC - Does not cluster CD20 into lipid microdomains [163] - Direct cytotoxicity by homotypic adhesion [164]	Evidence exists for CD20+ lymphoid malignancies [165, 166]
Ublituximab	Chimeric Mouse-human IgG1	Intravenous - Dose for RRMS not yet established	- ADCC > CDC - Stronger ADCC than rituximab due to enhanced binding to FcγRIIA receptors [167]	Evidence exists for CD20+ lymphoid malignancies [168] Phase 2 data for RRMS [101] with phase 3 trials ongoing (ULTIMATE I and II)

ADCC, antibody-dependent cellular cytotoxicity; ANCA, anti-neutrophil cytoplasmic antibody; CDC, complement-dependent cytotoxicity; PPMS, primary progressive multiple sclerosis; RA, rheumatoid arthritis; RRMS, relapsing-remitting multiple sclerosis

metabolism, and non-specific pinocytosis by phagocytes and endothelial cells becomes more relevant. The neonatal Fc receptor (FcRn) is a key modulator of IgG degradation by this pathway [75]. Following pinocytosis, FcRn-bound IgG molecules are protected from intracellular catabolism, whereas unbound IgG molecules are degraded by endosomal proteases [76]. Two-thirds of IgG molecules are protected in this way after pinocytosis, and are instead recycled into circulation to contribute to the therapeutic pool and half-life [77]. The expression and binding capacity of FcRn is associated with polymorphisms in the promotor region of its gene, *FCGRT*.

In the 2008 placebo-controlled HERMES trial, rituximab, a chimeric mouse-human monoclonal antibody first trialled in lymphoid cancers a decade prior, was shown to reduce clinical and radiological disease activity in people with RRMS [27]. In primary progressive multiple sclerosis (PPMS), rituximab was shown to reduce disease progression in younger patients and patients with contrast-enhancing MRI lesions. [78]. For commercial reasons, rituximab never progressed to phase 3 studies in RRMS. Contemporary use of rituximab in MS is off-label with variable uptake worldwide; it has largely been supplanted by ocrelizumab, a humanised anti-CD20 agent [79]. Rituximab has also been used off-label for secondary progressive MS (SPMS), with reductions in disease progression shown in retrospective analyses [80]. Value has also been seen in other autoimmune neurological conditions such as neuromyelitis optica spectrum disorder (NMOSD) [81], myasthenia gravis [82] and autoimmune encephalitis [83]. First-dose infusion reactions, likely due to cytokine release accompanying B cell lysis [84], were reported in 78.3% of patients (compared to 40.3% in the placebo group) in the HERMES trial, ...although incidence dropped off significantly with repeat infusion. Hypogammaglobulinaemia, not explicitly defined in the HERMES study but commonly defined as a serum IgG level of less than 6 g/L [85, 86], was seen more frequently in the treated compared to placebo group (7.9% vs 3.0%). Urinary tract infections (UTIs) and sinusitis were more common with rituximab compared to placebo, but serious or opportunistic infective complications are only rarely reported with rituximab in MS [87] and other autoimmune conditions [88]. Hepatitis B (HBV) reactivation has been reported in rituximab-treated patients with systemic autoimmune or lymphoproliferative disorders [89]. Progressive multifocal leukoencephalopathy (PML), due to the opportunistic reactivation of John Cunningham virus in myelin-producing oligodendrocytes, has also been reported in these populations [90]. PML carries a significant risk of mortality or irreversible disability, of particular concern in this setting as the treatment effects of rituximab cannot be reversed.

The efficacy of ocrelizumab, a humanised anti-CD20 agent, was established in the OPERA trials, with treatment

resulting in a 46 to 47% lower annualised relapse rate (ARR) compared to subcutaneous interferon-beta 1a in patients with RRMS [91]. Treatment benefit was also seen for disease progression and MRI metrics, and benefit for all endpoints was sustained over most demographic and clinical stratification subgroups [92]. The ORATORIO trial demonstrated the efficacy of ocrelizumab over placebo in PPMS. The reduction in clinical and radiological progression in this trial supports the significance of B cells in the pathogenesis of progressive MS phenotypes [93]. In the OPERA trials, infusion reactions were seen in 34% of patients and lessened with subsequent infusions. Again, these are thought to be mostly due to cytokine release by lysed B cells and are usually manageable with slowed infusion rates, and administration of antihistamines and corticosteroids. In these trials, treatment with ocrelizumab was associated with a higher incidence of upper respiratory tract and oral herpes infections compared to placebo. Cellulitis and UTIs were also more common in a study of rheumatoid arthritis patients [94]. HBV reactivation [95] and hypogammaglobulinaemia [96] have been described. Given its mechanism of action, PML is expected to rarely occur.

Other anti-CD20 monoclonal agents have been the subject of MS trials. Ofatumumab is the first subcutaneously administered anti-CD20 agent approved for relapsing forms of MS. It demonstrates more effective CDC than rituximab, and patients have reduced B cell depletion and faster repletion kinetics compared to intravenous alternatives [97, 98]. Its superiority for the prevention of relapses and disability progression was recently demonstrated over teriflunomide, an oral DMT [99]. Obinutuzumab is humanised while ublituximab is chimeric; both are used in B cell lymphoid malignancies, with stronger ADCC activation compared to rituximab [100]. Phase 2 placebo-controlled data demonstrated the safety and efficacy of ublituximab in RRMS [101], and phase 3 trials are ongoing (ULTIMATE I and II).

Pharmacogenetic Prediction of Rituximab Outcomes

Genotypic differences relating to monoclonal antibody recognition, metabolism and disease-related signalling are likely to explain a significant amount of variability seen in their efficacy and toxicity [69]. To date, no pharmacogenetic studies of rituximab or ocrelizumab efficacy in MS have been performed. However, genotype-phenotype studies of rituximab response in various diseases including lymphoid malignancies and rheumatoid arthritis have yielded several positive results. The identified polymorphisms affect the FcγR family (FCγRIIIA and FCγRIIA), FcRn, BAFF, C1qA, CD20 and IL-6 (Fig. 1 and Table 3).

Fig. 1 Selected polymorphisms relevant to rituximab efficacy

The FcγR Family

Genetic polymorphisms in FcγR affect the cytotoxic function of macrophages and NK cells, which could alter monoclonal antibody efficacy (Table 4). The most studied of these is a dimorphism of the *FCGR3A* gene that modulates the strength of interaction with the lower hinge region of IgG1, characterised by either phenylalanine (F) or valine (V) at residue 158 [102]. Although monocytes and macrophages possess a combination of stimulatory and inhibitory FcγRs, NK cells only express the stimulatory FcγRIIIA. The NK cells of V/V homozygotes participate in more effective ADCC compared to those of with V/F or F/F allotypes, thought to be due to stronger binding affinity for Fc seen in the *FCGR3A-158V* genotype [103, 104]. There is also evidence of increased NK cell expression of FcγRIIIA in V/V homozygotes [105, 106].

The relationship between *FCGR3A* variants and rituximab efficacy has been mostly examined in B cell malignancies [107, 108] and rheumatoid arthritis [109]. An analysis of 212 patients with rheumatoid arthritis found a significantly higher rate of clinical response to rituximab in V/V homozygotes (89.5%), compared to the V/F and F/F groups (66% and 66.2%, respectively). Loss of response to rituximab was seen only in V/F and F/F patients (10.8% and 16.4%, compared to none in the V/V group) [110]. In patients given rituximab as part of chemotherapy for follicular lymphoma, the V/V and F/

F genotypes have been associated with 12-month response rates of 75% and 30%, respectively [111]. This relationship has been shown to be specific for rituximab-containing regimens [112, 113]. One study of 85 patients with RRMS has reported a lack of relationship between *FCGR3A-158* genotype and outcomes with alemtuzumab, an anti-CD52 monoclonal antibody [114]. Although there have been no other analyses of FcγR variant relationship to monoclonal antibody treatment response in MS patients, a key study of rituximab-treated patients with NMOSD showed that V allele carriage at *FCGR3A-158* was associated with lower relapse risk (OR 0.35, 95% CI 0.12–0.91) and longer time to retreatment [115]. The V/V genotype is associated with enhanced B cell depletion [116], whereas poorer outcomes seen with F/F homozygosity are associated with incomplete memory B cell depletion. In a study of NK cells from patients with B cell malignancies, the rituximab concentration required to lyse 50% of target cells was 4.2 times lower in for *FCGR3A-158V*, compared to F/F homozygotes [117]. It has therefore been suggested that F/F homozygotes may require more frequent dosing.

A similar relationship exists between rituximab and polymorphisms affecting FcγRIIA, another activating Fc receptor found on monocytes and macrophages. The strength of IgG binding at this receptor varies depending on a polymorphism at residue 131, with improved response to monoclonal

Table 3 Pharmacogenetic associations for rituximab efficacy

Gene	SNP	Disease	Study	Sample/method	Clinical correlation
FCGR3A	158F>V (rs396991 T>G)	Follicular lymphoma	Cartron et al. (2002) [107]	<i>N</i> = 49 (20% V/V, 45% V/F and 35% F/F) French cohort No baseline clinical or demographic differences between genotypes	- Clinical remission or partial response higher in V/V (90%), compared V/F (45%) and FF (59%) genotypes
		Follicular lymphoma	Weng & Levy (2003) [111]	<i>N</i> = 87 (15% V/V, 46% V/F and 39% F/F) US cohort No baseline clinical or demographic differences between genotypes	- Progression-free survival at 2 years higher in V/V homozygotes (45%) compared to F carriers (14%) - Predictive value enhanced in combination with FCGR2A-131 genotyping
		Follicular lymphoma	Persky et al. (2012) [112]	<i>N</i> = 30 (10% V/V, 50% V/F and 40% F/F) treated with rituximab, compared to 70 patients treated with chemotherapy alone US cohort	- 5-year overall survival higher in V/V (100%) and V/F (97%), compared to F/F (75%) - Association specific for those receiving rituximab
		DLBCL	Kim et al. (2006) [169]	<i>N</i> = 113 (47% V/V, 48% V/F and 5% F/F) treated with rituximab + CHOP, compared to 85 treated with CHOP chemotherapy alone Korean cohort Response defined as per International Working Group criteria	- Complete response rate higher in V/V (88%) and V/F heterozygotes (79%), compared to F/F (50%) - Time to clinical response faster in V/V compared to other genotypes (median 70 vs. 99 days) - Association specific for those receiving rituximab
		DLBCL	Ahlgrimm et al. (2011) [113]	<i>N</i> = 263 treated with rituximab + CHOP, compared to 249 treated with CHOP alone (29% V/V, 53% V/F and 18% F/F) German cohort	- Trend towards increased progression-free survival in V/V (80.5%) and V/F (76.1%), compared to F/F genotype (68.3%), which was not seen in the chemotherapy-only cohort
		RA	Kastbom et al. (2012) [170]	<i>N</i> = 177 (10% V/V, 44% V/F and 46% F/F) Swedish cohort DAS28 calculated between months 3 and 6 No baseline clinical or demographic differences between genotypes	- Heterozygotes had higher response rates (83%) than V/V (56%) and F/F (68%) genotypes - Response differed between sexes among V/V homozygotes (100% of males vs. 39% of females, <i>p</i> = 0.036)
		RA	Ruyssen-Witrand et al. (2012) [109]	<i>N</i> = 111 (13% V/V, 39% V/F and 48% F/F), all also treated with methotrexate French cohort DAS28 calculated at month 6 No baseline clinical or demographic differences between genotypes	- V allele carriage associated with higher response rates (91% vs 71%, OR 4.6, CI 1.5–13.6, <i>p</i> = 0.006)
		RA	Quartuccio et al. (2014) [110]	<i>N</i> = 212 (18% V/V, 48% V/F and 34% F/F) Italian cohort DAS28 calculated at months 4 and 6 Baseline DAS28 was worse in V/V compared to F/F genotypes (6.2 vs 5.5, <i>p</i> = 0.01)	- Positive response at 6 months was more frequent in V/V (89.5%) than V/F (66%) and F/F (66.2%) genotypes - Loss of response between assessments only occurred in V/F (10.8%) and F/F (16.4%) genotypes
		RA	Pal et al. (2017) [171]	<i>N</i> = 52 (16% V/V, 65% V/F and 19% F/F) Hungarian cohort Remission: DAS28 < 2.6 at month 6 Low disease activity: DAS28 < 3.2 at month 6 No baseline clinical or demographic differences between genotypes	- V allele associated with better response - Remission in 37.5% of V/V, 32.4% of V/F and 30% of F/F genotypes (no significant difference) - Low disease activity less common in F/F homozygotes (30%), compared to V/V (62.5%, <i>p</i> = 0.03) and V/F (64.7%, <i>p</i> = 0.015) genotypes

Table 3 (continued)

Gene	SNP	Disease	Study	Sample/method	Clinical correlation
FCGR2A		RA, SLE, ANCA vasculitis	Ajeganova et al. (2017) [123]	<i>N</i> = 61 (11 cases of late-onset neutropaenia selected with 50 controls) Swedish cohort	- V allele carriage was negatively associated with disease flare at 12 months (OR 0.10, 95% CI, 0.03–0.42, <i>p</i> = 0.034)
		NMOSD	Kim et al. (2015) [115]	<i>N</i> = 100 (8% V/V, 25% V/F and 57% F/F) Korean cohort No baseline clinical or demographic differences between genotypes	- Relapse risk lower in V allele carriers compared to F/F homozygotes (OR 0.35, CI 0.12–0.91, <i>p</i> = 0.04) - V allele carriage was associated with significantly longer time to rituximab re-treatment
		WM	Treon et al. (2005) [108]	<i>N</i> = 58 (17% V/V, 45% V/F and 38% F/F) US cohort Partial response: as at least 50% reduction in IgM levels (no patients had complete response)	- V allele associated with higher partial response rate compared to F/F homozygotes (36% vs. 9%)
	131R>H (rs1801274 C>T)	Follicular lymphoma	Weng and Levy (2003) [111]	<i>N</i> = 87 (23% H/H, 49% H/R and 28% R/R) US cohort Progression-free survival assessed at 2 years No baseline clinical or demographic differences between genotypes	- Progression-free survival higher in H/H group (37%) compared to R carriers (14%) - H/R and R/R genotypes had similar response rates - Predictive value enhanced when combined with FCGR3A-158 genotyping
BAFF	871C>T (rs9514828)	RA	Ruyssen-Witrand et al. (2013) [138]	<i>N</i> = 115 (34% C/C, 47% C/T and 19% T/T) French cohort DAS28 calculated at month 6	- C/C associated with a higher response rate (92%) compared to T/T homozygotes (64%) - C allele carriage independently associated with response (OR 4.1, CI 1.3–12.7, <i>p</i> = 0.017)
	2704T>C (rs3759467)	ANCA vasculitis	Alberici et al. (2016) [139]	<i>N</i> = 213 (European cohort) with replication cohort of 109 (UK) Failure defined as disease activity requiring escalation of immunosuppression	- C/C genotype associated with rituximab failure at 6 months (OR 9.1, <i>p</i> = 0.065; replication cohort OR 8.6, <i>p</i> = 0.009) compared to T carriers - C/C homozygotes more likely to have detectable B cells at 6 months (50% vs. 14%)
	TTTT haplotype: 2841T>C (rs9514827) 2704T>C (rs3759467) 2701T>A (rs1041569) 871C>T (rs9514828)	RA	Fabris et al. (2013) [140]	<i>N</i> = 152 (Italian), with validation cohort of 117 (Italian and British) DAS28 calculated at months 4 and 6 TTTT: four SNPs in the 5' regulatory region of BAFF gene—all in strong linkage disequilibrium and form four common (> 5%) haplotypes (CTAT, TTAC, TTTT and TCAC)	- TTTT haplotype (either hetero- or homozygotes) in seropositive patients significantly associated with good response, in both the first (OR 6.97, CI 0.84–58.14, <i>p</i> = 0.05) and replication (OR 18, CI 1.65–196.42, <i>p</i> = 0.01) cohorts - Rare haplotypes more prevalent among non-responders
CIQA	276G>A (rs172378)	Follicular lymphoma	Racila et al. (2008) [146]	<i>N</i> = 133 (29% A/A, 54% A/G and 18% G/G) US cohort Response defined as per International Workshop Response Criteria	- Complete response more frequent in A/A homozygotes (36%) than heterozygotes (29%) and G/G homozygotes (21%) - A/A homozygotes had significantly longer time to disease progression than G/G homozygotes (708 vs 282 days)
		DLBCL	Jin et al. (2012) [143]	<i>N</i> = 129 (27% A/A, 43% A/G and 30% G/G) Chinese cohort Response defined as per International Working Group criteria	- A/A homozygotes had a significantly higher complete response rate (89.2% vs. 51.1%) and longer overall survival (676 vs. 497 days) than G allele carriers

Table 3 (continued)

Gene	SNP	Disease	Study	Sample/method	Clinical correlation
<i>CD20</i>	Exon2-216C>T (rs2070770)	DLBCL	Ding et al. (2013) [147]	<i>N</i> = 164 (75% C/C homozygous) Chinese cohort Three SNPs identified in the sample after all six exons of <i>CD20</i> gene were examined.	- Better response was seen with C/C genotype, compared to T allele carriers - Overall (complete or partial) response: 90.6% vs. 79.5% (<i>p</i> = 0.17) - Complete remission: 67.4% vs. 47.1% (<i>p</i> = 0.036)
<i>IL6</i>	174G>C (rs1800795)	RA	Fabris et al. (2012) [149]	<i>N</i> = 158 (43% G/G, 42% G/C and 15% C/C) Italian cohort DAS28 calculated at month 6	- G carriage was associated with higher response rates (81.5%) compared to C/C homozygotes (61%) - Good response seen in 24.4% of G allele carriers, compared to 4.3% of C/C homozygotes - No definite association between genotype and baseline serum IL-6 levels

ANCA, anti-neutrophil cytoplasmic antibody; *BAFF*, B cell-activating factor; *CIQA*, complement C1q A chain; *CHOP*, cyclophosphamide/doxorubicin/vincristine/prednisone; *DLBCL*, diffuse large B cell lymphoma; *DAS28*, Disease Activity Score-28; *FCGR2A*, Fc gamma receptor 2a; *FCGR3A*, Fc gamma receptor 3a; *IL6*, interleukin-6; *NMOSD*, neuromyelitis optica spectrum disorder; *RA*, rheumatoid arthritis; *SLE*, systemic lupus erythematosus; *WM*, Waldenstrom macroglobulinaemia

antibodies predicted with homozygosity for histidine (H) compared to arginine (R) [118]. For example, the response rates to rituximab in follicular lymphoma for *FCGR2A-131* H/H homozygotes (55% at 12 months) is superior to patients with H/R or R/R genotypes (26%) [111]. This stratification is enhanced by combined *FCGR2A-131* and *FCGR3A-158* genotyping: 100% of patients with both -131H/H and -158V/V maintained clinical response at 12 months, compared to 54% of those with either -131H/H or -158V/V, and 18% of those with neither.

The distinctions in rituximab response between *FCGR3A-158* and *FCGR2A-131* genotypes appear generalisable to monoclonal antibodies with non-CD20 targets. Trastuzumab utilises FcγR for ADCC in its effect against breast cancer, and significantly poorer clinical response rates are seen with *FCGR3A-158* V/F and F/F genotypes (42% and 35%, respectively), compared to V/V homozygotes (82%) [119]. Clinical response rates and *in vitro* antibody-mediated cytotoxicity are also improved in those with the *FCGR2A-131* H/H genotype [119]. Genetic variants affecting FcγR also appear relevant to metastatic colorectal cancer patients treated with cetuximab, an anti-EGFR monoclonal antibody: one study found

progression-free survival to be significantly longer for *FCGR3A-158* V/V compared to F/F homozygotes (5.5 vs 3.0 months) [120].

FcγR family polymorphisms could be similarly useful in predicting adverse events due to rituximab (Table 5). Hypogammaglobulinaemia following rituximab is predicted by *FCGR3A-158* status in patients with non-Hodgkin's lymphoma [121]. In a study with comparable baseline immunoglobulin levels between genotype groups, post-rituximab IgG levels were significantly lower in *FCGR3A-158* F/F homozygotes compared to V allele carriers. The effect was not seen in ten controls treated with transplantation using conditioning regimens without rituximab. This relationship is contrary to what was initially hypothesised by the authors. A possible explanation is increased rituximab uptake by lymphomatous B cells in carriers of the higher-affinity V allele could lead to reduced anti-CD20 effects on non-malignant cells. Carriage of the F allele at *FCGR3A-158* was associated with a higher risk of bloodstream infections after rituximab therapy in a cohort of ABO blood group incompatible liver transplant recipients, although a significant difference in IgG levels was not seen [122]. *FCGR3A-158* status also stratifies the risk of rituximab-

Table 4 FcγR polymorphisms and rituximab binding strength

Receptor	Dimorphism	Weaker binding	Stronger binding
FcγRIIIA	SNP at residue 158 of <i>FCGR3A</i> gene	Phenylalanine (F) allele	Valine (V) allele
FcγRIIA	SNP at residue 131 of <i>FCGR2A</i> gene	Arginine (R) allele	Histidine (H) allele

FCGR2A, Fc gamma receptor 2a; *FCGR3A*, Fc gamma receptor 3a; *SNP*, single nucleotide polymorphism

Table 5 Pharmacogenetic associations for rituximab adverse events

Gene	SNP	Disease	Study	Sample/method	Clinical correlation
<i>FCGR3A</i>	158F>V (rs396991 T>G)	Non-Hodg- kin's lymphoma	Nishio et al. (2009) [121]	<i>N</i> = 24 (13% V/V, 50% V/F, 38% F/F) Japanese cohort No baseline difference in IgG levels between genotypes, including non-rituximab-treated control group (<i>N</i> = 10) Absolute IgG levels measured	Reduced IgG levels: - Post-rituximab IgG levels were significantly lower in F/F homozygotes (6.21 ± 2.66 g/L) compared to V allele carriers (10.43 ± 3.50 g/L); $p < 0.05$ - Association specific for those receiving rituximab when compared to 10 controls (10.64 ± 3.04 g/L)
		B cell lymphoma	Li et al. (2010) [126]	<i>N</i> = 56 (7% V/V, 66% V/F, 27% F/F) Taiwanese cohort Late-onset neutropaenia: blood neutrophil count $< 1.5 \times 10^9$ /L after at least 4 weeks	Late-onset neutropaenia: - Associated with V allele carriage (OR 1.47, 95% CI 1.21–1.78, $p = 0.046$)
		DLBCL	Keane et al. (2011) [124]	<i>N</i> = 115, compared to 105 healthy controls Australian cohort Late-onset neutropaenia: blood neutrophil count $< 1.0 \times 10^9$ /L after at least 6 weeks	Late-onset neutropaenia: - Higher in V/V homozygotes (50%) than hetero- zygotes (7%) and F/F homozygotes (2%), $p = 0.028$ and 0.005 respectively
		RA, SLE, ANCA vasculitis	Ajeganova et al. (2017) [123]	<i>N</i> = 61 (11 cases of late-onset neutropaenia and 50 matched con- trols) Swedish cohort Late-onset neutropaenia: blood neutrophil count $< 1.5 \times 10^9$ /L after at least 4 weeks Reduced IgM: difference between baseline and post-treatment nadir measured over time with linear mixed models	Late-onset neutropaenia: - Each V allele conferred increased risk (OR 4.0, 95% CI 1.0–16.7, $p = 0.017$) - Development of neutropaenia associated with a longer flare-free survival and lower IgM levels Reduced IgM levels: - V/V homozygotes ($\beta = -0.30$, 95% CI -0.54 to -0.06 ; $p = 0.016$). ($p = 0.016$)
		ABOi liver transplant	Sakai et al. (2017) [122]	<i>N</i> = 20 <i>FCGR3A</i> : 45% V/V, 55% F allele carriers; <i>FCGR2A</i> : 50% H/H, 50% R allele carriers Japanese cohort Bloodstream infection: symptomatic and microbiologically proven, within 3 months IgM levels compared between groups at multiple time points	Bloodstream infection and <i>FCGR3A</i> : - Higher rates of bloodstream infections in F allele carriers than V/V homozygotes ($p < 0.05$)
<i>FCGR2A</i>	131R>H (rs1801274 C>T)				Bloodstream infection and <i>FCGR2A</i> : - Higher in H/H homozygotes ($p < 0.001$) Reduced IgM levels and <i>FCGR2A</i> : - H/H homozygote IgM levels lower at 2 weeks to 3 months post-treatment, compared to R allele carriers ($p < 0.05$)
		DLBCL	Ahlgrimm et al. (2011) [113]	<i>N</i> = 263 treated with rituximab + CHOP, compared to 249 treated with CHOP alone (42% H/H, 46% H/F, 13% R/R) Finnish cohort	Anaemia (haemoglobin < 80 g/L) - Higher in H allele carriers (24.2%) compared to R/R homozygotes (7.9%), $p = 0.07$

ABOi, ABO blood group incompatible; *ANCA*, anti-neutrophil cytoplasmic antibody; *CHOP*, cyclophosphamide/doxorubicin/vincristine/prednisone; *DLBCL*, diffuse large B cell lymphoma; *RA*, rheumatoid arthritis; *SLE*, systemic lupus erythematosus

induced late-onset neutropaenia. In a cohort of patients treated for rheumatological conditions, each V allele carried conferred a fourfold increase in odds ratio for developing neutropaenia after 4 weeks [123]. Another study of diffuse large B cell lymphoma found 50% of homozygotes of the higher-affinity V allele experienced late-onset neutropaenia, compared to 7% of heterozygotes and 2% of F/F homozygotes [124]. Hypotheses for this effect include bystander neutrophil loss due to increased lysosomal enzyme release, higher risk of

anti-neutrophil autoantibody development, and greater disturbance of bone marrow homeostasis [125, 126]. Similarly, the higher-affinity H allele at *FCGR2A-131* predicts a greater risk of post-treatment anaemia compared to R/R homozygotes (24.2% vs 7.9%) [113]. The H allele is also associated with lower immunoglobulin levels and increased risk of bloodstream infections in liver transplant recipient [122]. There could be a shared mechanism for rituximab efficacy and the genesis of these adverse events; for example, late-onset

neutropaenia has been identified as a positive prognostic factor following rituximab for rheumatological and lymphoproliferative disorders, and can predict remission without need for re-treatment [123, 127]. Late-onset neutropaenia has been described in MS patients receiving rituximab, but it is unclear whether its occurrence has similar prognostic implications [128].

Interestingly, the effects of these FcγR polymorphisms are reversed for TNF-α inhibitors, a monoclonal antibody class that does not rely on ADCC for efficacy. This reversal is thought to be due to the relationship between the binding strength of these agents and their endocytic and pinocytic metabolism. The expression of higher-affinity FcγR genotypes (*FCGR3A-158V* and *FCGR2A-131H*) on monocytes, macrophages and dendritic cells results in accelerated drug clearance and reduced efficacy [69, 71]. In a meta-analysis of TNF-α inhibitors in rheumatoid arthritis, H allele carriage at *FCGR2A-131* predicted a reduced response to adalimumab therapy [129]. Infliximab non-response in rheumatoid arthritis has been associated with V allele carriage at *FCGR3A-158* (2.2%, compared to 24.1% for F/F homozygotes), and H allele carriage at *FCGR2A-131* (33.3% vs. 60% for R/R homozygotes) [130]. Similar findings were seen in rheumatoid and psoriatic arthritis patients [131]. The relationship has also been described in V/V homozygous patients with Crohn's disease, with increased rates of infliximab elimination, biochemical inflammation, clinical relapse and treatment discontinuation [132].

Neonatal Fc Receptor

The neonatal Fc receptor (FcRn) is a critical mediator of antibody metabolism that requires consideration, although no rituximab-specific pharmacogenetic data exists. Higher FcRn levels protect IgG from intracellular catabolism; FcRn-knockout mice eliminate antibodies more efficiently [133] and FcRn-blocking monoclonal antibodies are associated with a dose-dependent reduction in circulating IgG levels [134]. FcRn expression is modulated by a variable number of tandem repeats (VNTR) polymorphism within the promoter region of its gene *FCGRT*. VNTR3 (three repetitions of a 37 base-pair VNTR) homozygosity is associated with increased FcRn expression and a lower clearance rate of administered immunoglobulins compared to VNTR2/VNTR3 heterozygotes [135]. Greater infliximab and adalimumab blood levels are seen in VNTR3 homozygotes treated for inflammatory bowel disease, compared to VNTR2/VNTR3 heterozygotes [136]. For cetuximab, VNTR3 homozygosity is associated with lower distribution clearance due to increased retention in cells and interstitial tissues [137]. As FcRn status is relevant to the survival of all circulating IgG molecules, the potential impact of VNTR status (or other FcRn variants) on the risk of

hypogammaglobulinaemia associated with anti-CD20 agents also warrants further investigation.

B Cell Activation Factor

As a B cell survival cytokine, B cell activation factor (BAFF) promotes survival of autoreactive B cells both before and after anti-CD20 therapy. A C/T polymorphism at locus 871 of the *BAFF* gene modulates rituximab efficacy and corresponds to the binding site of myeloid zinc finger 1 (MZF1), which modulates BAFF mRNA expression. Rheumatoid arthritis patients who are C/C homozygous at *BAFF-871* had a higher rate of 6-month rituximab response compared to T/T homozygotes (92% vs. 64%) [138]. At a locus in the same promoter region (*BAFF-2704*), C/C homozygous patients with ANCA vasculitis experienced a higher risk of rituximab failure, lower immunoglobulin levels and greater B cell reconstitution at 6 months [139]. The common TTTT promoter haplotype, characterised by four SNPs in the *BAFF* gene regulatory region (871 C>T, 2704 T>C, 2841 T>C and 2701 T>A), significantly predicted a positive rituximab response in patients with seropositive rheumatoid arthritis [140]. Although not yet examined for associations with rituximab outcomes, an insertion-deletion variant (*BAFF-var*) is associated with over-expression of BAFF, higher B cell and immunoglobulin levels and an increased risk of MS [141]. It has been suggested that the B cell contribution to disease may be greater in patients with *BAFF-var*, which could translate to a greater response to anti-CD20 therapies [142].

Complement C1q A Chain

The G to A polymorphism at residue 276 of the C1q A chain gene (*C1QA*) does not alter the C1q protein structure, and appears to affect rituximab outcomes by modulating C1q expression [143]. C1q is the complement cascade trigger and crucial for CDC, but also has a complex relationship with autoimmune disorders. C1q deficiency is associated with autoimmunity, suggesting a role for modulating tolerance to self-antigens [144]. Higher C1q levels and more efficient CDC are seen with G allele of *C1QA-276*, whereas the A allele is associated with lower C1q levels and a higher incidence of cutaneous lupus [145]. However, *C1QA-276* A/A homozygosity is linked to improved rates and duration of rituximab response in B cell malignancies, compared to G allele carriers [143, 146]. It has been hypothesised that, although A/A homozygosity could lead to less effective opsonisation and CDC, this results in more B cell fragments for APC recognition and a more developed and coordinated immune response [146].

CD20

One pharmacogenetic study of rituximab-treated patients with B cell lymphoma demonstrated the clinical significance of a C/T polymorphism at locus 216 in Exon2 of the *CD20* gene. Significantly improved rates of remission were reported with the C/C genotype (67%), compared to carriers of the T allele (47%). Exon2-216C/T is a synonymous SNP, with effects likely related to gene expression, splicing efficiency or mRNA stability [147]. The association did not extend to predicting progression-free survival, disease-specific survival or overall prognosis: this may be due to inadequate power, or the possibility that *CD20* polymorphisms are important determinants of initial response, but not long-term outcomes. It should be noted that *de novo* mutations of *CD20* in target tissues can also be a source of outcome variability in patients treated with rituximab for lymphoma [148].

Interleukin-6

In rituximab-treated patients with rheumatoid arthritis, G allele carriage at *IL6-174*, a locus within the promoter region for interleukin-6 (IL-6), was associated with a higher clinical response rate (81.5%) compared to C/C homozygotes (61%) [149]. Although there was no definite link between genotype and baseline serum IL-6 levels in this study, post-rituximab assays were not performed. Persistently elevated IL-6 levels in the sera and synovia have since been described in ungenotyped rheumatoid arthritis patients who failed to respond to rituximab [150]. Interestingly, homozygous carriage of the C allele at *IL6-174* is associated with increased risk for the development of MS in Asian populations [151]. IL-6 has various pro-inflammatory functions and is known to promote survival and proliferation of B cells [152]. Levels in MS patients are elevated and result in increased T cell differentiation towards the pro-inflammatory Th17 phenotype [46, 47].

Conclusion

Given the expanding range of DMTs available for use in RRMS, pharmacogenetic studies are a promising avenue towards personalised therapy. Studies of rituximab in rheumatological and haematological diseases suggest anti-CD20 therapy outcomes could be predicted by SNPs that affect ADCC, CDC and the signalling and survival of B cells. That these polymorphisms predicted efficacy but not baseline clinical or demographic differences suggests treatment response and phenotype expression are not generally linked. The effects of some of these variants appear generalisable to monoclonal agents with non-CD20 targets. Based on experiences seen with other disease examples highlighted in this review, pharmacogenetics and pharmacogenomics to predict MS

outcomes in monoclonal antibody therapy have significant potential, and could be rapidly translated to clinical care.

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