

The Pharmacogenomic Association of Fcγ Receptors and Cytochrome P450 Enzymes With Response to Rituximab or Cyclophosphamide Treatment in Antineutrophil Cytoplasmic Antibody–Associated Vasculitis

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Objective. The Rituximab in ANCA-Associated Vasculitis (RAVE) trial compared rituximab to cyclophosphamide as induction therapy for the treatment of antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis. We undertook the current study to determine whether known single-nucleotide polymorphisms (SNPs) for Fcγ receptors (FcγR) or cytochrome P450 (CYP) enzymes were associated with the response to treatment with rituximab and cyclophosphamide, respectively.

Methods. Functional SNPs for FcγR (FcγRIIa 519G>A, FcγRIIb 695T>C, FcγRIIIa 559T>G) and CYP enzymes (CYP2B6 1459C>T, CYP2C19 681G>A) were analyzed by direct sequencing of polymerase chain reaction–amplified genomic DNA. Each SNP was tested as a predictor of complete remission at 6 months or remission

with continued prednisone administration using logistic regression and including the covariates of baseline Birmingham Vasculitis Activity Score for Wegener’s Granulomatosis, ANCA type, and new versus relapsing disease. The associations of these SNPs with the secondary outcomes of time to complete remission, time to relapse, or time to B cell reconstitution were analyzed by Cox proportional hazard tests.

Results. No significant associations were identified between complete remission and any FcγR genotype in the rituximab group or any CYP genotype in the cyclophosphamide group. However, when the treatment groups were combined, an association was found between the 519AA genotype of FcγRIIa and complete remission ($P = 0.01$). The 519AA genotype predicted complete remission ($P = 0.006$) and a shorter time to complete remission ($P < 0.001$).

Conclusion. The finding that the homozygous FcγRIIa 519AA variant was associated with complete response and a shorter time to complete response in the RAVE trial, independent of treatment type, implies that FcγRIIa may be broadly involved in disease pathogenesis and response to therapy.

Supported by a grant from Genentech.

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Dr. Specks has received consulting fees from Genentech (less than \$10,000). Dr. Stone has received consulting fees from Genentech and Roche (less than \$10,000 each). Dr. Hoffman has received consulting fees from Roche and GACTA (less than \$10,000 each). Dr. Merkel has

received consulting fees from Genentech (less than \$10,000). Dr. Spiera has received consulting fees from Genentech (less than \$10,000). Dr. Monach has received honoraria from Medscape (less than \$10,000).

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Submitted for publication March 18, 2016; accepted in revised form July 19, 2016.

Table 1. Single-nucleotide polymorphisms tested*

Gene	Position in gene sequence	Wild-type allele	Variant allele	Variant allele frequency†	Effect of variant on protein function
FcγRIIa	519	G	A	0.48	Increases affinity of the receptor for IgG2
FcγRIIb	695	T	C	0.13	Decreases inhibitory activity of the receptor
FcγRIIIa	559	T	G	0.45	Increases affinity of the receptor for IgG1 and IgG3
CYP2B6	1459	C	T	0.05–0.15	Decreases hepatic production of the enzyme
CYP2C19	681	G	A	0.15–0.32	Creates a premature stop codon and results in an inactive enzyme

* FcγRIIa = Fcγ receptor IIa; CYP2B6 = cytochrome P450 isoform 2B6.

† See refs. (37) and (38).

Granulomatosis with polyangiitis (Wegener's) (GPA) and microscopic polyangiitis (MPA) are 2 forms of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) (1). Advances in therapy have transformed GPA and MPA from frequently fatal conditions to chronic illnesses. The Rituximab in ANCA-Associated Vasculitis (RAVE) trial expanded standard induction treatment options for patients with AAV by demonstrating that rituximab was not inferior to cyclophosphamide for induction of remission (2,3). Despite the success of the trial, its results were far from ideal, as only 58% of patients in both treatment groups achieved complete remission after 6 months without prednisone, and only 36% of patients had disease in stable remission throughout the 18 months of follow-up (2). As the medical community moves closer to the goal of treating patients using a precision medicine approach, it is plausible that therapy for AAV can be tailored to specific characteristics of individual patients.

Rituximab, a chimeric monoclonal antibody directed against the B lymphocyte-restricted cell surface protein CD20, kills B cells mainly through antibody-dependent cell-mediated cytotoxicity (ADCC) (4). ADCC is mediated through Fcγ receptors (FcγR), particularly FcγRIIIa expressed on natural killer (NK) cells. Of potential clinical relevance, single-nucleotide polymorphisms (SNPs) in FcγR genes can influence the function of FcγR (5). Studies in lymphoma (6–9), rheumatoid arthritis (10,11), and other autoimmune conditions (12,13) have shown that the response to rituximab may be affected by FcγR polymorphisms.

Cyclophosphamide has been used for induction of remission in patients with AAV for almost 4 decades. Cyclophosphamide is a prodrug that requires activation (4-hydroxylation) by the hepatic cytochrome P450 (CYP) enzymes. Several CYP isoforms contribute to cyclophosphamide metabolism including CYP2B6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 (14). Of these CYP enzymes, variants in the CYP2B6 and CYP2C19 genes

appear to have the most clinically relevant effects in conditions commonly treated with cyclophosphamide, such as cancer and systemic lupus erythematosus (SLE) (15–19).

To date there is no information about the effects of FcγR and CYP polymorphisms on how AAV responds to treatment with rituximab or cyclophosphamide. We therefore investigated whether functional FcγRIIa, FcγRIIb, FcγRIIIa, CYP2B6, and CYP2C19 polymorphisms that are relatively common in the general population (Table 1) are associated with therapeutic response or toxicity to specific drugs in patients from the RAVE trial.

PATIENTS AND METHODS

Patients. Patients were participants in the RAVE trial. Study design details of the RAVE trial have been reported elsewhere (2,3). All patients enrolled in the RAVE trial provided written informed consent for the collection and future use of biologic samples and data. Participating ethics boards approved the research. This work was approved by The Ohio State University Institutional Review Board. Disease activity was scored using the Birmingham Vasculitis Activity Score for Wegener's Granulomatosis (BVAS/WG) (20,21). The primary outcome of the RAVE trial, complete remission, was defined as a BVAS/WG of 0 and successful completion of the prednisone taper to 0 mg by month 6. Secondary outcomes included 1) remission, defined as a BVAS/WG of 0 during treatment with continued prednisone at <10 mg per day; 2) time to complete remission or remission in days; 3) time to relapse in days, defined as an increase in BVAS/WG of ≥1 point after having achieved remission; and 4) time to B lymphocyte reconstitution in days, defined as an increase in CD19+ B cells to >69 cells/mm³ after having an initial decrease to <10 cells/mm³ following treatment with rituximab.

SNPs for FcγR (FcγRIIa 519G>A, rs1801274; FcγRIIb 695T>C, rs1050501; FcγRIIIa 559T>G, rs396991) and CYP enzymes (CYP2B6 1459C>T, rs3211371; CYP2C19 681G>A, rs4244285) were analyzed by direct sequencing of polymerase chain reaction (PCR)-amplified genomic DNA. These genes and polymorphisms were chosen because they are relatively common in the general population and they can theoretically affect the function of their respective proteins (19,22–28) and thereby

Table 2. Association of Fc γ R genotypes in the rituximab group and CYP genotypes in the cyclophosphamide group with time to remission of ANCA-associated vasculitis, by Cox proportional hazard analyses*

Polymorphism, genotype (position in gene sequence)	Remission†				Complete remission‡			
	HR (95% CI)	P	Adjusted HR (95% CI)§	P	HR (95% CI)	P	Adjusted HR (95% CI)§	P
Fc γ RIIa (519)								
GG vs. GA/AA	1.02 (0.63–1.60)	0.93	1.03 (0.63–1.62)	0.89	0.86 (0.52–1.40)	0.57	0.91 (0.54–1.49)	0.73
AA vs. GG/GA	1.34 (0.83–2.11)	0.22	1.35 (0.82–2.17)	0.21	2.11 (1.26–3.46)	0.005	2.33 (1.36–3.9)	0.002
Fc γ RIIIa (695)								
TT vs. TC/CC	0.63 (0.41–1.01)	0.06	0.62 (0.39–1.01)	0.06	0.90 (0.57–1.44)	0.67	0.79 (0.48–1.32)	0.37
CC vs. TT/TC	1.23 (0.61–2.25)	0.53	1.18 (0.58–2.20)	0.61	1.25 (0.60–2.33)	0.52	1.44 (0.67–2.78)	0.32
Fc γ RIIb (559)								
TT vs. TG/GG	0.92 (0.55–1.62)	0.76	0.84 (0.49–1.51)	0.55	0.90 (0.52–1.65)	0.73	0.89 (0.51–1.66)	0.71
GG vs. TT/TG	3.96 (0.22–18.8)	0.26	3.85 (0.21–18.8)	0.27	2.76 (0.15–13)	0.38	3.34 (0.18–16.3)	0.32
CYP2B6 (1459)								
CC vs. CT/TT	1.12 (0.66–2.05)	0.67	1.13 (0.66–2.07)	0.65	1 (0.54–2.03)	0.99	1.07 (0.57–2.19)	0.83
TT vs. CC/CT	0.56 (0.03–2.58)	0.53	0.58 (0.03–2.87)	0.57	0.44 (0.02–2.03)	0.35	0.32 (0.01–1.62)	0.19
CYP2C19 (681)								
GG vs. GA/AA	0.78 (0.47–1.38)	0.39	0.78 (0.47–1.39)	0.39	0.76 (0.42–1.48)	0.41	0.80 (0.44–1.55)	0.50
AA vs. GG/GA	1.44 (0.23–4.64)	0.62	1.39 (0.21–4.97)	0.67	1.58 (0.25–5.12)	0.55	2.09 (0.31–7.82)	0.38

* Fc γ R = Fc γ receptor; CYP = cytochrome P450; HR = hazard ratio; 95% CI = 95% confidence interval.

† Birmingham Vasculitis Activity Score for Wegener's Granulomatosis (BVAS/WG) of 0 and receiving <10 mg/day prednisone.

‡ BVAS/WG of 0 and not receiving prednisone.

§ Adjusted for baseline BVAS/WG, type of antineutrophil cytoplasmic antibody (ANCA), and new versus relapsing disease.

impact the efficacy of rituximab and cyclophosphamide treatment (Table 1).

DNA samples were available from 189 of the 197 participants in the RAVE trial. Genomic DNA was extracted from peripheral blood leukocytes of EDTA-treated whole blood using Gentra Puregene Blood Kits (Qiagen). Oligonucleotide primers (Invitrogen) specifically designed to flank the 5 SNP regions were as follows: for 2C19*2, 5'-AAC-CAG-AGC-TTG-GCA-TAT-TG-3' (forward) and 5'-GTA-AAC-ACA-AAA-CTA-GTC-AAT-G-3' (reverse); for 2B6*5, 5'-TGT-GCC-CAC-ACT-GGT-GAC-CCT-CTG-T-3' (forward) and 5'-TAA-TTT-TCG-ATA-ATC-TCA-CTC-CTG-C-3' (reverse); for Fc γ RIIa, 5'-CCT-CTG-AGA-CTG-AAA-AAC-CC-3' (forward) and 5'-CTT-GGC-AGA-CTC-CCC-ATA-CC-3' (reverse); for Fc γ RIIIa, 5'-TCT-CGG-GCC-TGG-GGT-GTC-TG-3' (forward) and 5'-GGA-GGG-AAC-CAC-ATA-TGA-AGA-AG-3' (reverse); and, for Fc γ RIIb, 5'-CTC-TGT-TCC-TGC-CTG-CTC-ACA-AA-3' (forward) and 5'-GAG-GAG-CTA-CAA-ACC-TGA-AAT-CC-3' (reverse).

DNA amplification was carried out by PCR using 100 ng of DNA and a MasterCycler Gradient Thermal Cycler (Eppendorf). Amplification reactions were done in a reaction volume of 25 μ l with 50 mM KCl, 20 mM Tris HCl (pH 8.4), 1.5 mM MgCl₂, 200 μ M each dATP, dCTP, dGTP, dTTP (Invitrogen), and 0.5 units PlatinumTaq DNA polymerase (Invitrogen). The cycling conditions included an initial 2 minutes of incubation at 94°C to activate the Taq DNA polymerase, followed by denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and polymerization at 72°C for 30 seconds for a total of 35 cycles. PCR products were checked on 2% agarose gel to confirm a single product for direct sequencing. PCR products (5 μ l) were mixed with 2 μ l of ExoSAP-IT (USB) followed by incubation for 15 minutes at 37°C and then 15 minutes of inactivation at 80°C to remove excess dNTPs and primers. The purified products were then directly sequenced with BigDye Terminator Cycle Sequencing Chemistry (Applied Biosystems) using the same

PCR primers. Sequencing was performed using a 3730 DNA Analyzer (Applied Biosystems) in the Plant-Micro Genomic facility at The Ohio State University.

Care was taken in designing the primers for Fc γ RII to avoid inadvertently amplifying Fc γ RIIC by using a forward primer that mismatched with Fc γ RIIC at 2 of the 3 most 3' nucleotide residues and at 4 total residues (University of California at Santa Cruz BLAT genome search, December 2013 assembly). Using this primer under the cycling conditions indicated, particularly annealing at 60°C, results in amplification of Fc γ RIIb alone.

Statistical analysis. For comparison of proportions, chi-square tests were used if the number of elements in each cell was ≥ 5 ; Fisher's exact test was used otherwise. Logistic regression was used to assess the association of each SNP (as the predictor) with the primary outcome of complete remission or the secondary outcome of remission with continued prednisone (as responses). The time-to-event data were analyzed with Cox proportional hazard ratios (HRs). In both analyses, the following prespecified covariates were included: baseline BVAS/WG, ANCA type, and new versus relapsing disease. Model fit (calibration) was assessed using the Hosmer-Lemeshow goodness-of-fit test. When appropriate, odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Given the proportions observed and assuming an alpha error of 5% and a power of 90%, we were able to detect in the entire cohort an OR of 2.4 for a logistic regression analysis (binary outcome) with 1 predictor. Two-sided *P* values less than 0.05 were considered significant. JMP statistical software, version 10.0 (SAS Institute) was used for all data analyses.

RESULTS

DNA was sequenced from 189 RAVE trial participants, of whom 96 received rituximab and 93 were

Table 3. Association of complete remission status with Fc γ RIIa genotype in the entire RAVE cohort*

	No. of patients	Success, no. (%)	Failure, no. (%)	
Entire cohort	189	114 (60)	75 (40)	
GG/GA	137	75 (55)	62 (45)	
AA	52	39 (75)	13 (25)	0.01
<i>P</i>				
Cyclophosphamide-treated patients	93	51 (55)	42 (45)	
GG/GA	68	33 (49)	35 (51)	
AA	25	18 (72)	7 (28)	0.04
<i>P</i>				
Rituximab-treated patients	96	63 (66)	33 (34)	
GG/GA	69	42 (61)	27 (39)	
AA	27	21 (78)	6 (22)	0.11
<i>P</i>				

* Fc γ RIIa = Fc γ receptor IIa; RAVE = Rituximab in ANCA-Associated Vasculitis.

treated with cyclophosphamide. Due to technical problems, we were not able to determine the Fc γ RIIIa 559T>G genotype in 3 patients treated with rituximab.

In rituximab-treated patients, no significant differences were identified in the genotype, carrier status, or allele frequency for the Fc γ RIIa, Fc γ RIIb, and Fc γ RIIIa polymorphisms between patients who achieved remission or complete remission and those who did not (see Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at <http://onlinelibrary.wiley.com/doi/10.1002/art.39822/abstract>). In cyclophosphamide-treated patients, no significant differences were identified in the genotype, carrier status, or allele frequency for the CYP2B6 and CYP2C19 polymorphisms between patients who achieved remission or complete remission and those who did not (see Supplementary Table 2, <http://onlinelibrary.wiley.com/doi/10.1002/art.39822/abstract>).

In univariate and multivariate logistic regression analyses, no significant associations were identified between Fc γ RIIa, Fc γ RIIb, and Fc γ RIIIa polymorphisms

and remission or complete remission status in patients treated with rituximab, or between CYP2B6 and CYP2C19 polymorphisms and remission or complete remission status in patients treated with cyclophosphamide (see Supplementary Table 3, <http://onlinelibrary.wiley.com/doi/10.1002/art.39822/abstract>). No associations were identified between Fc γ RIIb, Fc γ RIIIa, CYP2B6, and CYP2C19 polymorphisms and time to remission or time to complete remission by univariate or multivariate Cox models. However, in rituximab-treated patients, the homozygous Fc γ RIIa 519AA genotype ($n = 27$ patients) was associated with a shorter time to complete remission (HR 2.11 [95% CI 1.26–3.46], $P = 0.005$), even after adjustment for baseline BVAS/WG, new versus relapsing disease, and ANCA type (HR 2.33 [95% CI 1.36–3.9], $P = 0.002$) (Table 2). The mean \pm SD time to complete remission was 171 ± 4 days for patients with the Fc γ RIIa 519AA genotype versus 212 ± 9.8 days for the combined Fc γ RIIa 519GG/519GA genotypes ($P = 0.001$).

The association of the Fc γ RIIa polymorphism with time to complete remission led us to examine this SNP in cyclophosphamide-treated patients and then in the whole cohort in an effort to demonstrate that this variant specifically affected responses to rituximab treatment. Unexpectedly, we found that a larger proportion of cyclophosphamide-treated patients with the 519AA genotype achieved complete remission, and this association became stronger when the entire cohort was considered (Table 3). Furthermore, in the entire cohort, the Fc γ RIIa 519AA genotype was associated with increased odds of achieving complete remission (OR 2.61 [95% CI 1.29–5.55], $P = 0.006$) and with a shorter time to complete remission (HR 1.93 [95% CI 1.33–2.78], $P < 0.001$), even after adjustment for covariates (Table 4).

In the entire cohort, the odds of achieving complete remission at 6 months were even higher in patients homozygous for both Fc γ RIIa 519A and Fc γ RIIIa 559G (OR 7.87 [95% CI 1.48–145], $P = 0.01$; $n = 12$ patients, 5 taking

Table 4. Association of complete remission and time to complete remission with Fc γ RIIa genotype in the entire RAVE cohort, by logistic regression and Cox proportional hazard modeling*

Fc γ RIIa (all patients)	Complete remission				Time to complete remission			
	Univariate		Multivariate†		Univariate		Multivariate†	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
GG vs. GA/AA	0.67 (0.34–1.32)	0.25	0.67 (0.34–1.33)	0.26	0.77 (0.51–1.13)	0.19	0.79 (0.52–1.17)	0.25
AA vs. GG/GA	2.48 (1.24–5.20)	0.009	2.61 (1.29–5.55)	0.006	1.85 (1.27–2.64)	0.001	1.93 (1.33–2.78)	<0.001

* Fc γ RIIa = Fc γ receptor IIa; RAVE = Rituximab in ANCA-Associated Vasculitis; OR = odds ratio; 95% CI = 95% confidence interval; HR = hazard ratio.

† Adjusted for baseline Birmingham Vasculitis Activity Score for Wegener's Granulomatosis, type of antineutrophil cytoplasmic antibody (ANCA), and new versus relapsing disease.

cyclophosphamide and 7 taking rituximab). This result remained significant after adjustment for ANCA type, new versus relapsing disease, and baseline BVAS/WG (OR 8.20 [95% CI 1.52–152], $P = 0.01$). Although these data are consistent with the Fc γ RIIA 519AA genotype results, the number of double-homozygous patients was small and the confidence intervals were wide, raising concerns over the importance of this finding.

The RAVE trial also had data on 119 patients at 18 months. Repeating these analyses at 18 months showed no significant association between the Fc γ RIIA 519G>A SNP and complete remission in either the entire cohort or any of the treatment arms (data not shown). Furthermore, the Fc γ RIIA 519AA genotype was not associated with increased odds of maintaining complete remission at 18 months (OR 1.26 [95% CI 0.56–2.9], $P = 0.56$). At 18 months, patients homozygous for both Fc γ RIIA 519A and Fc γ RIIIA 559G did not have increased odds of complete response (OR 2.65 [95% CI 0.60–18], $P = 0.20$).

No significant associations were found between Fc γ RIIA, Fc γ RIIB, Fc γ RIIIA, and CYP2B6 polymorphisms and time to AAV relapse or time to B cell reconstitution (see Supplementary Table 4, <http://onlinelibrary.wiley.com/doi/10.1002/art.39822/abstract>). The CYP2C19 homozygous 681AA genotype was associated with more rapid B cell reconstitution than were the combined 681GG and 681GA genotypes (HR 0.14 [95% CI 0.007–0.74], $P = 0.01$) in cyclophosphamide-treated patients. However, only 2 patients were homozygous, calling into question the reliability of this finding. No significant associations were identified between Fc γ R and CYP SNPs and the development of all adverse events, grade 3 adverse events, grade ≥ 2 leukopenia, grade ≥ 3 thrombocytopenia, or infections (data not shown).

DISCUSSION

This cohort study of AAV patients from the RAVE trial was done to evaluate the pharmacogenomic associations of Fc γ R and CYP enzymes with response to treatment with rituximab or cyclophosphamide, respectively. To the best of our knowledge, no previous study has assessed the role of pharmacogenomics in the therapy of AAV patients receiving rituximab or cyclophosphamide. Although we postulated that genetic background would be useful in choosing between rituximab and cyclophosphamide as the most effective induction drug for individual patients, this was not demonstrated for the polymorphisms tested. Furthermore, knowledge of patient genotypes for Fc γ R and CYP enzymes did not differentiate patients by time to B cell reconstitution or susceptibility to treatment-

associated side effects. Unexpectedly, however, the Fc γ RIIA 519AA genotype was found to predict complete response and a shorter time to complete response independently of the induction treatment used, suggesting that Fc γ RIIA may play a pathogenic role in AAV.

The Fc γ R that we studied included 2 activating receptors, Fc γ RIIA and Fc γ RIIIA, and 1 inhibitory receptor, Fc γ RIIB. Fc γ RIIA is expressed on myeloid cells, dendritic cells, and platelets, Fc γ RIIB is expressed on myeloid cells, dendritic cells, and B cells, and Fc γ RIIIA is expressed on macrophages, some monocyte subsets, and NK cells (29). The Fc γ RIIA SNP 519G>A changes an arginine to a histidine at amino acid 131, the Fc γ RIIIA SNP 559T>G changes a phenylalanine to a valine at amino acid 158, and the Fc γ RIIB SNP 695T>C changes an isoleucine to a threonine at amino acid 187. These SNPs all affect protein function, with the Fc γ RIIA 131H variant exhibiting enhanced binding to IgG2, the Fc γ RIIIA 158V variant exhibiting enhanced binding to IgG1 and IgG3, and the Fc γ RIIB 187T variant having defective lipid raft anchoring and consequently decreased inhibitory function (28,30). All 3 of these polymorphisms have been reported to influence susceptibility to another autoimmune disease, SLE or lupus nephritis, likely through differences in immune complex clearance (Fc γ RIIA, Fc γ RIIIA) or immunoregulation (Fc γ RIIB) (for review, see ref. 31).

Because the effects of rituximab are mediated in part through Fc γ R (6,32), it is plausible that Fc γ R polymorphisms may also influence the response to rituximab therapy. Indeed, rituximab efficacy has been reported to be better in patients with the Fc γ RIIIA 158VV genotype in the treatment of non-Hodgkin's lymphomas (33) and rheumatoid arthritis (11). In contrast, none of these Fc γ R SNPs influenced the efficacy of rituximab in AAV, suggesting that these Fc γ R genetic variations are not important for rituximab in vasculitis. However, analysis of secondary RAVE trial outcomes showed that the homozygous Fc γ RIIA SNP variant (519AA) was associated with a shorter time to complete remission. Surprisingly, this association also extended to the entire cohort, including patients treated with cyclophosphamide (data not shown). Furthermore, when the entire cohort was considered (rituximab- and cyclophosphamide-treated patients), the 519AA homozygous variant and the 519AA plus Fc γ RIIIA 559GG homozygous genotype were also found by regression analysis to be associated with and to predict complete remission. This association is consistent with a previous observation that patients with GPA who had a combined homozygous genotype of Fc γ RIIA 519AA and Fc γ RIIIA 559GG were relatively protected from disease relapse compared to having all other combinations of Fc γ R genotypes (34).

Fc γ RIIa 519AA appears to identify an AAV phenotype with a more favorable clinical prognosis. Exploring the mechanism of this beneficial effect is beyond the scope of this investigation, but expressing the high-affinity Fc γ R phenotype may help suppress AAV activity by enhancing and accelerating the clearance of ANCAs or other inciting antibodies/immune complexes.

Similar to our results with rituximab and Fc γ R polymorphisms, we observed no effect of CYP polymorphisms on the response of AAV patients to cyclophosphamide or susceptibility to cyclophosphamide toxicity, except for an association between the CYP2C19 homozygous 681AA genotype and more rapid B cell reconstitution. As pointed out in the Results, there were only 2 patients with this homozygous variant genotype, making this conclusion tentative. In other conditions, CYP enzyme SNPs have been found to affect the response to and the toxicity of cyclophosphamide therapy. The variant CYP2C19*2 (681G>A) results in an inactive enzyme (24), and 1 study showed that carriers of at least 1 CYP2C19*2 allele were protected against cyclophosphamide-related premature ovarian failure but that CYP2C19*2 homozygous patients with lupus nephritis who were treated with cyclophosphamide had a higher probability of developing end-stage renal disease (17). The same study demonstrated that patients receiving pulse cyclophosphamide for the treatment of lupus nephritis who were homozygous for CYP2B6*5, a haplotype that results in decreased enzyme expression by the liver (23), also had a higher probability of developing end-stage renal disease.

These findings suggest that SNPs resulting in reduced levels of the active drug decrease the efficacy of cyclophosphamide (17). However, other studies have not confirmed these pharmacogenomic associations in either lupus nephritis (35) or breast cancer (36). Thus, it remains unclear whether genotyping for CYP variants is useful in deciding whether to treat an individual patient with cyclophosphamide or an alternate drug, and this may be disease specific.

This study has limitations. It is a post hoc analysis. For some of the genotypes, there were only a few patients, which prevented statistical analyses to identify significant associations. Additionally, not all known FcR and CYP SNPs were tested, so we cannot exclude the possibility that other genetic predispositions may specifically affect response to rituximab or cyclophosphamide. Finally, the lack of association of genotype with response to a specific treatment will need to be validated in an independent AAV cohort. We are optimistic that these data can be reproduced in one or more of the AAV cohorts assembled for recent clinical trials. On the other hand, the study's main strength stems from its prospective cohort

assembled from a large multicenter randomized controlled trial of patients who were treated with rituximab or cyclophosphamide and followed up meticulously for clinical outcomes.

In conclusion, in this cohort study of AAV patients from the RAVE trial, there is no evidence to suggest that genotyping patients for several high-prevalence and functional FcR or CYP polymorphisms will be helpful in individualizing induction therapy to either rituximab or cyclophosphamide. This is relevant information for practice and suggests that choice of therapy should mainly consider the patient's clinical context. Alternatively, Fc γ RIIa variants appear to identify a subgroup of patients who may be expected to achieve remission more rapidly during induction. While there does not appear to be an overt clinical need to genotype Fc γ RIIa for the current management of AAV, determining the mechanisms of this apparent genotype–phenotype interaction could help further our understanding of how to decide which AAV patients are suitable for routine treatment and which patients may need novel therapy.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Rovin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Cartin-Ceba, Specks, Stone, Rovin.

Acquisition of data. Indrakanti, Specks, Stone, Hoffman, Kallenberg, Langford, Merkel, Spiera, Monach, St.Clair, Seo, Tchao, Ytterberg, Brunetta, Song, Birmingham, Rovin.

Analysis and interpretation of data. Cartin-Ceba, Specks, Stone, Birmingham, Rovin.

ROLE OF THE STUDY SPONSOR

Genentech had no role in the study design or in the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication. Publication of this article was not contingent upon approval by Genentech.

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