Letter to the Editor

Association of a TNFSF13B (BAFF) regulatory region single nucleotide polymorphism with response to rituximab in antineutrophil cytoplasmic antibody–associated vasculitis

To the Editor:

Rituximab is effective at inducing and maintaining remission in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).[1,2] The wide interpatient variability in the duration of B-cell depletion and time to relapse as well as the significant relapse risk after treatment, costs, and adverse event rates necessitate improved patient stratification.[3]

Several biomarkers have been explored[4,5] in diseases treated with rituximab; however, all require validation before they can enter clinical practice; among them, a role for candidate single nucleotide polymorphisms (SNPs) has been proposed.[6] However, no SNP has so far shown a reliable association with response to treatment and no such study has been performed in AAV. We tested a panel of candidate SNPs to investigate their potential association with response to rituximab in 2 large cohorts of patients with AAV.

We included patients with granulomatosis with polyangiitis (GPA, Wegener's) and microscopic polyangiitis (MPA) who received rituximab mainly for relapsing or refractory disease. We aimed at identifying associations between the tested SNPs and the rate of rituximab failure at 6 months and time to rituximab failure (relapse). For further details, see the Methods section in this article's Online Repository at www.jacionline.org.

We enrolled 213 patients in the primary and 109 in the replication cohort (see Table E1 in this article's Online Repository at www.jacionline.org). Across the primary cohort, a mean of 0.88 6 0.19 SNPs per sample could not be called and no SNP had a P value of less than .05 for departure from Hardy-Weinberg Equilibrium (HWE) (see Table E2 in this article's Online Repository at www.jacionline.org).

In the primary cohort, the TNFSF13B SNP rs3759467 was associated with time to rituximab failure ($P = 2.86 \times 10^{-4}$, $P_{corr} = .01$) (Fig 1, A; see Table E3 in this article's Online Repository at www.jacionline.org). We genotyped this SNP in the replication cohort (test for deviation from HWE $P = .7627$; rate of missing calls, 3%) where the association with time to rituximab failure was confirmed ($P = .002$) (Fig 1, B).

Because the results suggested a recessive effect for the SNP rs3759467, we used recessive models for the analysis in the replication cohort and reanalysis of the primary cohort. Meta-analyses of the 2 cohorts confirmed an association between the TNFSF13B SNP rs3759467 and the 2 end points ($P = .0065$ and 8.5×10^{-6}, respectively) (Table I).

We then compared the main clinical characteristics of the carriers of the CC genotype with the carriers of the TC and TT genotypes (see Table E4 in this article's Online Repository at www.jacionline.org) and found a higher rate of detectable peripheral B cells 6 months after rituximab in carriers of the CC genotype (50% vs 14%; $P = .0146$) as well as a smaller reduction in IgM levels (1.5 [1.4-10.92] and 1 [1-1.33]; $P = .01539$).

The haplotype analyses of the 5′ regulatory region of the TNFSF13B gene based on the genotyped SNPs confirmed an association of the risk of rituximab failure at 6 months for the haplotype including the risk allele of the SNP rs3759467 (see Table E5 in this article's Online Repository at www.jacionline.org).

In view of the small number of MPO-ANCA–positive patients and the fact that all the minor homozygous carriers for the rs3759467 SNP were PR3-ANCA positive, we reanalyzed the primary cohort according to ANCA specificity (see Table E6 in this article's Online Repository at www.jacionline.org); the association with the B-cell activating factor (BAFF) SNP rs3759467 was limited to the PR3-ANCA subgroup (see Tables E6 and E7 in this article's Online Repository at www.jacionline.org).

In the MPO-ANCA subgroup, a different association emerged in the primary cohort with the SNP rs6822844 in the IL2-IL21 area (rituximab failure risk at 6 months and time to rituximab failure, $P = 4.2 \times 10^{-4} - P_{corr} = .03$ and $P = 1.9 \times 10^{-4} - P_{corr} = .0068$, respectively) (Table E6). However, this was not replicated (rituximab failure risk at 6 months and time to rituximab failure, $P = .153$ and .172, respectively) in the context of a small sample size (19 patients), although there was a trend to an increased risk of treatment failure at 6 months for the carriers of the T allele (see Table E8 in this article's Online Repository at www.jacionline.org).

We have identified an SNP (rs3759467) in the 5′ regulatory region of the gene TNFSF13B (BAFF) able to predict response to rituximab in 2 cohorts of patients with AAV. Interestingly, the carriers of the unfavorable genotype showed in addition to a poor response to rituximab, a higher proportion of detectable B cells 6 months after infusion and a smaller reduction in IgM levels.

A BAFF level increase after rituximab treatment has been described and a central role for this cytokine in AAV pathogenesis has also been proposed.[7] The 5′ regulatory region of the BAFF gene includes several SNPs that may have a modulatory effect; the -TTTT- haplotype of this region has been associated with BAFF levels[8] and response to rituximab in rheumatoid arthritis.[6] In our study, a trend toward better response in carriers of this haplotype was also observed.

The role of the SNP rs3759467 will need to be clarified; this is part of a TAAT- binding site; the lack of a heterozygous effect may suggest that the losing of both sites is required to obtain the phenotype causing poor response. It seems likely that this SNP modulates B-cell survival and/or activity; this might be a consequence of higher baseline BAFF levels or greater BAFF increases after a B-cell–depleting event.

Interestingly, our findings were restricted to the subgroup of patients with positive PR3-ANCA probably due to power limitations in the MPO-ANCA subgroup where the reanalyses identified a different association in the IL2-IL21 area, as already described in systemic lupus erythematosus.[9]

Our study has limitations: a sample size relatively small for a genetic study and the retrospective nature of data collection may be weaknesses; however, at the time of this writing, it would not have been possible to enroll a comparable prospective cohort. We also have to acknowledge the closeness of the P value for the departure from HWE for the SNP rs3759467 in the primary cohort; however, the replication of the data in the context of a P value for the departure from HWE far from significance ($P = .7627$) is reassuring on the reliability of the overall finding.

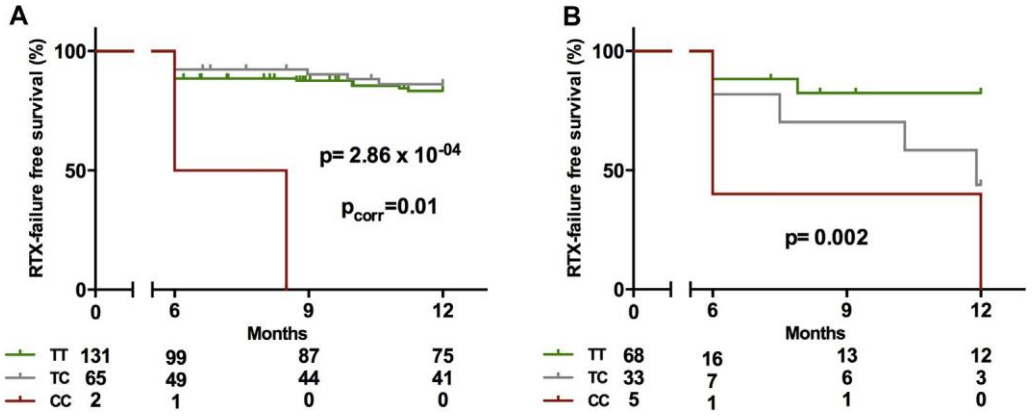

FIG 1. Rituximab (RTX) failure-free survival in an exploratory cohort of 213 patients (A) and in a replication cohort of 109 patients (B) stratified according to the genotypes of the SNP rs3759467 of the gene TNFSF13B.

TABLE I. Association results for the SNP rs3759467 after fixed-effects weighted meta-analysis for the 2 main outcomes assessed in our study assuming a recessive model

Outcome	Primary cohort		Replication cohort		Meta-analysis	
assessed	OR-HR	P value	OR-HR	P value	OR-HR	P value
Rituximab failure risk at 6 mo	9.1	.06489	8.6	.008996	8.8	.0065
Time to rituximab failure	12.4	7×10^{-204}	5.39	.0024	7.3	8.5×10^{-206}

Rituximab failure risk at 6 months has been explored using a recessive model of the Cochrane-Armitage test. Time to rituximab failure has been explored using a recessive model of Cox-proportion hazards regression model. OR and HR have been reported where appropriate.
HR, Hazard ratio; OR, odds ratio.

In conclusion, we have identified a TNFSF13B (BAFF) SNP (rs3759467) associated with response to rituximab in 2 independent cohorts of patients with AAV. This SNP may be useful in identifying patients likely to respond poorly to rituximab and for whom alternative treatments should be considered. Further studies are required to confirm this finding and to clarify its mechanism of action.

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Federico Alberici, MD[a,b,c]
Rona M. Smith, MD[a]
Mariana Fonseca, PhD[a]
Lisa C. Willcocks, PhD[a]
Rachel B. Jones, MD[a]
Julia U. Holle, PhD[d]
Stefan Wieczorek, MD[e]
Thomas Neumann, PhD[f]
Davide Martorana, PhD[g]
Gina Gregorini, MD[h]
Renato A. Sinico, MD[b,i]
Annette Bruchfeld, PhD[j]
Iva Gunnarsson, PhD[k]
Sophie Ohlsson, PhD[l]
Bo Baslund, PhD[m]
Vladimir Tesar, PhD[n]
Zdenka Hruskova, PhD[n]
Maria C. Cid, PhD[o]
Augusto Vaglio, PhD[p]
Paul A. Lyons, PhD[a]
Kenneth G. C. Smith, PhD[a]
David R. W. Jayne, MD[a]

From [a]the Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom; [b]the Nephrology and Immunology Unit, ASST Santi Paolo e Carlo, San Carlo Borromeo Hospital, Milano, Italy; [c]the Department of Clinical and Experimental Medicine, University of Parma, Parma, Italy; [d]the Department of Rheumatology, Immunology and Vasculitis Clinic, Klinikum Bad Bramstedt, Bad Bramstedt, Germany; [e]the Department of Human Genetics, Ruhr-University Bochum, Bochum, Germany; [f]the Department of Internal Medicine III, Jena University Hospital, Jena, Germany; [g]the Medical Genetics Unit, University Hospital of Parma, Parma, Italy; [h]the Department of Nephrology, A.O. Spedali Civili, Brescia, Italy; [i]the Department of Medicine and Surgery, University of Milano-Bicocca (School of Medicine and Surgery), Monza, Italy; [j]the Department of Renal Medicine, Karolinska University Hospital and Karolinska Institute, Solna, Sweden; [k]the Department of Medicine, Unit of Rheumatology, Karolinska University Hospital, Karoliska Institute, Solna, Sweden; [l]the Department of Nephrology and Transplantation, Skane University Hospital, Goteborg, Sweden; [m]the Department of Infectious Diseases and Rheumatology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; [n]the Department of Nephrology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic; [o]the Vasculitis Research Unit, Department of Systemic Autoimmune Diseases, Hospital Clinic, University of Barcelona, Barcelona, Spain; and [p]the Nephrology Unit, University Hospital of Parma, Parma, Italy. E-mail: federico.alberici@gmail.com.

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METHODS

Eligibility criteria were a clinical diagnosis of granulomatosis with polyangiitis (GPA, Wegener's) or microscopic polyangiitis (MPA) defined according to the European Medicines Agency (EMEA) algorithm[E1] and the administration of rituximab for either relapsing or refractory disease or remission induction in few patients. Patients with a clinical diagnosis of eosinophilic granulomatosis with polyangiitis (formerly known as Churg-Strauss syndrome) were not included in this study. The primary cohort included patients enrolled at European centers with expertise in vasculitis and members of the European Vasculitis Genetics Consortium and/or of the European Vasculitis Society (Table E9); the replication cohort was enrolled at the Vasculitis and Lupus Clinic, Addenbrooke's Hospital (Cambridge, United Kingdom). All patients gave written informed consent before participation. Disease severity was assessed using the Disease Extent Index (DEI)[E2]; B-cell return was defined as a B-cell count of 0.01 3 10^9/L or more.

Eligibility criteria

Patients affected by GPA or MPA for whom there was indication to the beginning of treatment with rituximab as follows:

- Relapsing disease: disease flare defined as a DEI score of more than 2, physician assessment of relapsing disease, and need for immunosuppression escalation.
- Refractory disease: persistent activity of disease despite treatment with intravenous and/or oral steroids and other immunosuppressive agents and physician assessment of refractory disease for at least 3 months.
- Other indications:
  - First presentation of the disease.
  - Contraindication to standard treatment (eg, recurrent infection and fertility preservation).
  - Grumbling disease: low-grade disease activity according to physician assessment that is not formally fulfilling the inclusion criteria in terms of disease activity scoring.
  - Patients unable to taper the prednisolone below 15 mg/d on their previous therapeutic regimen.
  - Need for steroid-free regimen.

End points

The end points of the study were to identify associations between the tested SNPs and the rate of rituximab failure at 6 months and time to rituximab failure (relapse) within 12 months of the first rituximab administration. We defined rituximab failure as active vasculitis requiring escalation of immunosuppressive treatment. SNPs showing an association with at least 1 of the 2 end points in the primary cohort were analyzed in the replication cohort for confirmation and then meta-analyses of the results were performed. Subgroup analyses were performed after merging the 2 cohorts.

Genotyping

Eighteen candidate SNPs were chosen according to a biological rationale or previous reports (Table E2).[E3-E8] DNA was extracted from peripheral blood using the Qiagen DNA extraction kit; genotyping was performed using TaqMan and Sequenom platforms with the exception of the FCGR2B SNP rs1050501, which was genotyped via a "modified" TaqMan approach as previously described.[E9]

Statistics

Statistical analysis was performed using the software R (http://www.r-project.org) and the packages coin,[E10] survival,[E11] SNPassoc,[E12] and hapassoc.[E13]

For exploratory purposes, the primary cohort has been analyzed using the Cochrane-Armitage test with log-additive model. As a result of the exploratory analysis (recessive mechanism for the SNP rs3759467), the replication cohort has been analyzed immediately using a recessive model of the same test. We have then reanalyzed the primary cohort with the same model and a meta-analysis has been performed.

The time to rituximab failure has been assessed by Kaplan-Meier survival analysis and a log-rank test has been used to compare populations. Cox-proportional hazards regression model has been used for reanalyses of the time to rituximab failure in the primary and replication cohort using a recessive model and the results have been used for meta-analyses for this end point.

Delta of reduction of IgG and IgM levels has been assessed as the ratio between the baseline value and the value at the time point of interest.

Results are expressed as value and percentage for categorical variables and median and interquartile range or mean and SEM for continuous variables when appropriate.

In view of the observation of an association in the 59 regulatory region of the gene TNFSF13B, we decided to study the haplotypes of this region because 4 of the SNPs included in the study were in strong linkage disequilibrium and organized in well-renowned haplotype blocks. We used the R package hapassoc[E13] for the identification of the haplotypes and the Cochrane-Armitage test to explore association between haplotypes and the risk of treatment failure 6 months after treatment with rituximab.

Meta-analyses were performed via a fixed-effects weighted method using the Linux version of the software metal (http://csg.sph.umich.edu/abecasis/Metal/download/). Bonferroni corrections for multiple testing were performed, with corrected P (P_{corr}) values of less than .05 considered significant.

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TABLE E1. Patients' characteristics

Characteristic	Primary cohort (n 5 213)	Replication cohort (n 5 109)
Age (y)	53.3 (40.2-64.7)	57.1 (41.9-65)
Sex: male	111 (52)	48 (44)
Diagnosis		
GPA	185 (87)	94 (86)
MPA	29 (13)	15 (14)
ANCA specificity		
PR3	148 (70)	78 (72)
MPO	29 (13)	19 (17)
Negative	36 (17)	12 (11)
Prior disease duration (mo)	56.3 (12.8-113.8)	48.2 (16.5-138)
Indication for rituximab		
Relapse	108 (51)	53 (49)
Refractory disease	76 (36)	15 (14)
Other*	26 (13)	41 (38)
Rituximab dose for induction regimen		
375 mg/m^2 weekly for 4 wk	68 (32)	28 (26)
1 g 2 wk apart	143 (67)	79 (72)
Other†	2 (1)	2 (2)
No. of previous immunosuppressive agents	2 (1-3)	3 (2-3)
Cyclophosphamide	175 (82)	94 (86)
Cumulative dose (g)	10 (4-28)	10 (4-27)
Methotrexate	92 (43)	28 (26)
Azathioprine	91 (42)	76 (70)
MMF	49 (23)	60 (55)
Immunosuppression at study entry‡	1 (0-1)	1 (0-1)
Cyclophosphamide	35 (16)	15 (14)
Methotrexate	30 (14)	8 (7)
MMF	18 (9)	17 (16)
Azathioprine	14 (8)	11 (10)
IV methylprednisolone	8 (4)	17 (16)
Oral prednisolone dose	25 mg (12.5-50)	12.5 mg (10-20)
Organ involvement at study entry		
ENT	132 (62)	60 (46)
Lungs	88 (41)	21 (19)
Joints	83 (39)	26 (24)
Kidneys	82 (39)	24 (22)
Eye	59 (28)	12 (11)
Peripheral nervous system	25 (12)	9 (8)
Central nervous system	11 (5)	3 (3)
Gastrointestinal	4 (2)	0 (0)
Cardiac	4 (2)	0 (0)
DEI	5 (3.75-7)	3 (2-5)
ANCA status at study entry		
Positive by ELISA	174 (82)	73 (67)
PR3	148 (70)	59 (54)
MPO	26 (12)	14 (13)
Negative	39 (18)	36 (33)

Results are expressed as n/N (%) or median (IQR) when appropriate.
ENT, Ear, nose and throat; IV, intravenous; MMF, mycophenolate mofetil.
*Other. Includes first presentation of disease, contraindication to standard treatment, grumbling disease, patients unable to taper the prednisolone dose, need for steroid-free regimen.
†Other. Includes 500 mg 2 weeks apart (2 cases), administration of a single dose of 1 g of rituximab (1 case), administration of 3 doses of rituximab at the dose of 375 mg/m^2.
‡Excluding oral steroids, but including oral immunosuppressive agents continued for at least 3 months after the administration of the first rituximab dose.

TABLE E2. SNPs tested, minor allele frequency (MAF) observed, and test for deviation from HWE in the primary cohort of 213 patients with AAVs treated with rituximab

SNP	Alleles	Gene	MAF	HWE-P
rs396991	G/T	FCGR3A	0.439	0.407
rs1050501	T/C	FCGR2B	0.11	0.716
rs1224141	G/T	TNFSF13B	0.235	0.839
rs16972216	A/G	TNFSF13B	0.173	1.000
rs1224147	T/C	TNFSF13B	0.221	0.680
rs10508198	C/G	TNFSF13B	0.339	0.058
rs12583006	A/T	TNFSF13B	0.231	0.554
rs8181791	A/G	TNFSF13B	0.307	0.327
rs172378	A/G	C1QA	0.38	0.303
rs9514828	C/T	TNFSF13B	0.442	0.888
rs1801274	C/T	FCGR2A	0.495	0.407
rs1800795	C/G	IL6	0.359	0.881
rs6822844	G/T	IL2-IL21	0.127	0.542
rs3759467	T/C	TNFSF13B	0.172	0.057
rs28362491	DEL/ATTG	NFKB1	0.383	0.102
rs1800471	C/G	TGFB1	0.09	1.000
rs1041569	A/T	TNFSF13B	0.166	1.000
rs9514827	T/C	TNFSF13B	0.318	0.626

The test for deviation from HWE has been calculated using the R package SNPasso,[E12] and threshold for significance has been established to $P < .05$.
MAF, Minor allele frequency.

TABLE E3. Association of 18 candidate SNPs with the 2 main outcomes explored in the study: Rituximab failure risk at 6 months and time to rituximab failure

			Rituximab failure risk at 6 mo		Time to rituximab failure	
SNP	Alleles	Gene	P value	P_{corr}	P value	P_{corr}
rs396991	G/T	FCGR3A	.275	1	.495	1
rs1050501	T/C	FCGR2B	.399	1	.089	1
rs1224141	G/T	TNFSF13B	.006*	0.216	.268	1
rs16972216	A/G	TNFSF13B	.328	1	.521	1
rs1224147	T/C	TNFSF13B	.01*	0.36	.366	1
rs10508198	C/G	TNFSF13B	.295	1	.083	1
rs12583006	A/T	TNFSF13B	.625	1	.129	1
rs8181791	A/G	TNFSF13B	.489	1	.968	1
rs172378	A/G	C1QA	.987	1	.441	1
rs9514828	C/T	TNFSF13B	.854	1	.943	1
rs1801274	C/T	FCGR2A	.949	1	.605	1
rs1800795	C/G	IL6	.574	1	.735	1
rs6822844	G/T	IL2-IL21	.184	1	.853	1
rs3759467	A/G	TNFSF13B	.911	1	2.86×10^{204}*	0.01*
rs28362491	-/ATTG	NFKB1	.012*	0.432	.245	1
rs1800471	C/G	TGFB1	.727	1	.978	1
rs1041569	A/T	TNFSF13B	.461	1	.88	1
rs9514827	T/C	TNFSF13B	.577	1	.825	1

P_{corr}, P corrected for multiple testing according to Bonferroni.
*Statistically significant.

TABLE E4. Main clinical characteristics of patients at time of study entry and 6 months after rituximab according to the different genotype for the SNP rs3759467 of the gene TNFSF13B in the overall study population (primary 1 replication cohorts merged)

Characteristic	Genotype TT 1 TC	Genotype CC	P value
Diagnosis (GPA)	260 of 301 (86%)	7 of 7 (100%)	.2951
Historical ANCA specificity (PR3)	209 of 294 (71%)	7 of 7 (100%)	.2453
DEI	4 (2-6)	4 (3.5-4.5)	.6925
Age (y)	54.2 (40.8-65)	54.8 (37.5-58.5)	.5965
Indication for rituximab (active flare)*	233 of 299 (78%)	6 of 7 (86%)	.6229
B-cell return at 6 mo	22 of 161 (14%)	3 of 6 (50%)	.0146†
ANCA positivity at 6 mo	137 of 244 (56%)	6 of 7 (86%)	.12
IgG delta at 6 mo†	1.06 (0.96-1.34)	0.97 (0.76-0.97)	.09389
IgM delta at 6 mo†	1.5 (1.04-10.92)	1 (1-1.33)	.01539†
DEI score at rituximab failure	4 (2-5)	4 (2.5-4.75)	.703

Results are expressed as n/N (%) or median (IQR) when appropriate.
B-cell return has been defined as B-cell count ≥ 0.01 3 10^9/L.
*Active flare defined as rituximab given either for relapsing or for refractory disease.
†Statistically significant.
‡Delta has been calculated as the ratio between the value at the time of rituximab administration and the value at the time point of interest. Associations have been tested using the Mann-Whitney rank sum test.

TABLE E5. Haplotypes of the 59 regulatory region of the gene TNFSF13B and their association with risk of TF 6 months after treatment with rituximab according to a log-additive and recessive models

		Rituximab failure risk at 6 mo			
		Log-additive model		Recessive model	
Haplotype	Frequency	P value	P_{corr}	P value	P_{corr}
TTAC	34%	.635	1	.58	1
CTAT	31%	.73	1	.699	1
TCAC	18%	.028*	0.28	2.6×10^{-205}*	2.6×10^{-204}*
TTTT	13%	.028*	0.28	.386	1
Pooled	4%	.475	1	.741	1

*Statistically significant.

TABLE E6. Association of 18 candidate SNPs with the 2 outcomes explored in our study (rituximab failure risk at 6 months and time to rituximab failure) according to the historical ANCA specificity

			PR3 – AAVs (n 5 148)				MPO – AAVs (n 5 29)			
			Rituximab failure 6/12		Time to rituximab failure		Rituximab failure 6/12		Time to rituximab failure	
SNP	Alleles	Gene	P value	P_{corr}	P value	P_{corr}	P value	P_{corr}	P value	P_{corr}
rs396991	G/T	FCGR3A	.150	1	.815	1	.388	1	.25	1
rs1050501	T/C	FCGR2B	.073	1	.401	1	.433	1	.808	1
rs1224141	G/T	TNFSF13B	.007	0.53	.208	1	.380	1	.672	1
rs16972216	A/G	TNFSF13B	.164	1	.806	1	.667	1	.705	1
rs1224147	T/C	TNFSF13B	.012	0.84	.253	1	.360	1	.675	1
rs10508198	C/G	TNFSF13B	.775	1	.206	1	.546	1	.824	1
rs12583006	A/T	TNFSF13B	.980	1	.162	1	.966	1	.702	1
rs8181791	A/G	TNFSF13B	.645	1	.829	1	.625	1	.603	1
rs172378	A/G	C1QA	.733	1	.481	1	.551	1	.671	1
rs9514828	C/T	TNFSF13B	.390	1	.533	1	.434	1	.782	1
rs1801274	C/T	FCGR2A	1	1	.401	1	.194	1	.562	1
rs1800795	C/G	IL6	.574	1	.392	1	.135	1	.287	1
rs6822844	G/T	IL2-IL21	.987	1	.874	1	4.2×10^{204}*	0.03*	1.9×10^{204}*	0.0068*
rs3759467	A/G	TNFSF13B	.667	1	4.8×10^{204}*	0.017*	.452	1	.825	1
rs28362491	-/ATTG	NFKB1	.044	1	.263	1	.212	1	.268	1
rs1800471	C/G	TGFB1	.609	1	.148	1	.626	1	.363	1
rs1041569	A/T	TNFSF13B	.306	1	.648	1	.899	1	.668	1
rs9514827	T/C	TNFSF13B	.111	1	.598	1	.706	1	.534	1

Rituximab failure rates at 6 mo were compared using a Cochrane-Armitage test with log-additive model and time to rituximab failure using log-rank test.
*Statistically significant.

TABLE E7. Association results for the SNP rs3759467 in the subgroup of patients with PR3-ANCA after fixed-effects weighted meta-analysis for the 2 outcomes assessed in our study assuming a recessive model for the SNP

	Primary cohort (n 5 148)		Replication cohort (n 5 78)		Meta-analysis	
Outcome assessed	OR/HR	P value	OR/HR	P value	OR/HR	P value
Rituximab failure risk at 6 mo	8.2	.0853	9.2	.0089	8.8	.007
Time to RTX-failure	11.6	.0012	6.2	.002	8.2	8.7×10^{206}

Rituximab failure risk at 6 months has been explored using a recessive model of the Cochrane-Armitage test. Time to rituximab failure has been explored using a recessive model of Cox-proportion hazards regression model.

TABLE E8. Genotype distribution for the SNP rs6822844 of the gene IL2-IL21 in the subgroup of patients MPO-ANCA positive of the replication cohort

	Response at 6 mo		
Genotype rs6822844	Positive response	TF	TF percentage
GG	15	1	6%
GT	2	1	33%
TT	0	0	0%

The difference is not statistically significant (P 5 .1722, Cochrane-Armitage test, log-additive model) although there was a trend toward an increased risk of TF in the carriers of the T allele in small sample size (19 patients).
TF, Rituximab failure.

TABLE E9. Primary cohort recruitment according to country

Country	No. of patients
Germany	53
Italy	49
Sweden	46
Denmark	41
Czech Republic	13
Spain	11