Review

Genetics of ANCA-associated vasculitides: HLA and beyond

F. Alberici[1-3], D. Martorana[4], F. Bonatti[4], A. Gioffredi[3], P.A. Lyons[1], A. Vaglio[3]

[1]*Department of Medicine, University of Cambridge, United Kingdom;*
[2]*Lupus and Vasculitis Service, Cambridge University Hospital, United Kingdom;*
[3]*Unit of Nephrology, and* [4]*Molecular Genetics Unit, University Hospital, Parma, Italy.*

Federico Alberici, MD
Davide Martorana, PhD
Francesco Bonatti, PhD
Andrea Gioffredi, MD
Paul A. Lyons, PhD
Augusto Vaglio, MD, PhD

Please address correspondence and reprint requests to:
Dr Augusto Vaglio,
Unit of Nephrology,
University Hospital of Parma,
Via Gramsci 14,
43126 Parma, Italy.
E-mail: augusto.vaglio@virgilio.it

Received on March 10, 2014; accepted in revised form on March 13, 2014.

Clin Exp Rheumatol 2014; 32 (Suppl. 82): S90-S97.

Key words: ANCA, HLA, polymorphisms, genome-wide association study

Funding: this work was supported by the grant "A tailored approach to the immune-monitoring and clinical management of viral and autoimmune diseases", funded by the Regione Emilia Romagna within the Programma di Ricerca Regione Università 2010-2012.

Competing interests: none declared.

ABSTRACT

The pathogenesis of ANCA-associated vasculitis (AAV) is multifactorial and most likely involves the interaction of environmental and genetic factors. During the past few years, a number of studies have investigated genetic associations with AAV; earlier studies explored associations with single nucleotide polymorphisms (SNPs) at genes of potential pathogenetic interest ("candidate gene" studies), whereas more recent larger studies analysed associations with SNPs covering ~90% of the human genome (genome-wide association studies – GWAS). The latter studies have significantly advanced our understanding of the genetic aspects of AAV, confirming some previously reported findings and uncovering new genetic associations. In addition, these studies have also shown that different AAV subtypes such as granulomatosis with polyangiitis (Wegener's, GPA) and microscopic polyangiitis (MPA) are underpinned by distinct genetic risk factors, with GPA being associated with HLA-DP, SERPINA1 *(encoding α1-antitrypsin),* PRTN3 *(encoding proteinase-3, PR3, the main GPA-related autoantigen) and* SEMA6A *(semaphorin 6A), whereas MPA has been mainly associated with* HLA-DQ. *Interestingly, in the European GWAS, which included both GPA and MPA patients, the* HLA-DP, SERPINA1, PRTN3 *and* HLA-DQ *SNPs were more significantly associated with ANCA-specificities (PR3 vs. myeloperoxidase, MPO) than with the clinical syndromes. In addition, the finding of GPA and PR3-positive subsets being associated with SNPs of genes encoding PR3 and α1-antitrypsin, a protease able to inactivate PR3, highlighted the central role of PR3 as an auto-antigen in AAV. This paper reviews the main genetic association studies in AAV, with particular emphasis on the two GWAS performed so far.*

Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) is a group of small-vessel vasculitides including granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg Strauss syndrome) (1). AAV has been historically classified according to the clinical phenotype. GPA and MPA are characterised by the presence of vasculitic manifestations, namely glomerulonephritis, alveolar haemorrhage, purpura and peripheral neuropathy. EGPA's main clinical features are eosinophilia, necrotising vasculitis and eosinophil-rich granulomatous inflammation (2, 3). Granulomatous involvement of ear, nose, throat (ENT) and lungs is a typical feature of GPA and may sometimes be the only manifestation (1). Whether or not the clinical syndromes making up AAV should be considered different ends of the same disease spectrum, or should be regarded as distinct diseases, is still debated. Moreover, the role of ANCA in the classification is still not completely clear. GPA and MPA are characterised by ANCA positivity in around 90% of patients; ANCA specificity by ELISA testing may be directed against neutrophil proteinase 3 (PR3 ANCA) or neutrophil myeloperoxidase (MPO ANCA) (4). Although GPA is more frequently PR3 ANCA-positive and MPA more often MPO ANCA-positive, a significant overlap does exist. For this reason, ANCA positivity, but not ANCA specificity, has been included in the latest classification criteria although PR3 and MPO specificity should not be used as surrogate diagnostic tools for the classification of AAV in GPA or MPA (5). In EGPA the proportion of ANCA-positive patients is around 40% with MPO ANCA more often represented (2, 4).

In terms of disease severity, AAV may range from life- or organ-threatening conditions to milder forms; however, regardless of their severity, a chronic-relapsing course is common resulting in the necessity for repeat immunosuppressive treatments and therefore a high burden of treatment-related damage (6, 7).

The pathogenesis of AAV is still debated. Infective triggers facilitating neutrophil activation may play a key role leading to the migration of cytoplasmic PR3 and MPO antigens to the neutrophil surface, where they become accessible targets for ANCAs. B cells also play a central role, not only being the precursors of ANCA-producing plasma cells and plasmablasts, but also through their interaction with the T cell compartment, ultimately leading to the development and activation of the T effector memory (T_{EM}) cells. Activated neutrophils coated by ANCAs and T_{EM} cells are ultimately responsible for the inflammatory vasculitic process (8). Complement activation seems to play an important role locally in the development of the inflammatory process, although C3 and C4 blood levels are often normal (9).

The report of, albeit rare, familial cases of AAV has drawn attention to the role of genetic factors in the pathogenesis of AAV (10, 11). The identification of genetic associations for AAV would allow not only a better understanding of the disease pathogenesis and risk factors, but also a clearer classification with a potential impact on the clinical management of the disease.

Genetic research in AAV has mainly focused on association studies between single nucleotide polymorphisms (SNPs) and the risk of developing disease. The two main tools used for this purpose are genome-wide association studies (GWAS) or candidate gene studies. GWASs routinely assay SNPs tagging 90% of the human genome; in order to avoid spurious associations a very high level of statistical significance ($p<5 \times 10^{-8}$) is required a significant association with disease. Such studies need large numbers of cases and controls to avoid missing potential associations due to underpowered cohorts and generally, in the field of rare diseases, this requires a multicentre approach. On the other hand, candidate gene approaches explore the possible association with disease of a few genes at most, usually highlighted by earlier studies or because they are considered of potential biological interest. Regardless of the approach taken initially, replication of the association in an independent cohort of patients is essential to ensure the finding is robust.

We will review in this paper the main genetic association studies in AAV, with particular emphasis on the two GWAS performed so far and the more robust candidate gene studies.

Genome-wide association studies in AAV

Two GWAS have been performed in AAV (12, 13). The first was run in Europe by the European Vasculitis Genetic Consortium (EVGC) (12) and involved 2,687 Caucasian GPA and MPA patients, while the second was performed on 1,020 American GPA patients of European descent by the Vasculitis Clinical Research Consortium (VCRC); the main characteristics of these two studies, including eligibility criteria and samples sizes, are summarised in Table I. No GWAS has been conducted to date in EGPA.

The EVGC GWAS (12) reported associations with AAV at three loci (*HLA DPB1*, *SERPINA 1* and *PRTN3*) that reached genome-wide significance. The finding of *HLA DPB1* being a predisposing factor was not surprising, as it confirmed one of the most robust findings of previous candidate gene studies (14). The other two associations highlight the potential role of the auto-antigen PR3, which is encoded by the gene *PRTN3,* and of the locus *SERPINA1* which encodes α1-antitrypsin, a protease inhibitor. The study population was also re-analysed after stratification for diagnosis and antibody specificity and PR3 ANCA positivity was found to be the strongest factor associated with these SNPs. Interestingly, analysis of the MPO ANCA-positive patients alone revealed a further association with the *HLA DQ* locus that had been masked during the initial analysis. The pivotal role of the MHC in the pathogenesis of AAV was confirmed in the VCRC GWAS (13), which also identified an association with the *HLA DPB1* and *HLA DPA1* loci. In addition

Table I. Comparison of the main characteristics of the Genome-Wide Association Studies (GWAS) performed by the European Vasculitis Genetic Consortium (EVGC) (12) and the Vasculitis Clinical Research Consortium (VCRC) (13).

	EVGC (12)	VCRC (13)
Study subjects	GPA and MPA	GPA
Discovery cohort	1,233 case patients, 5,884 controls	459 GPA patients, 1,503 controls (study stage 1)
Replication cohort	1,454 case patients, 1,666 controls	First independent replication (study stage 2a): 291 GPA patients, 371 controls; Second independent replication (study stage 2b): 528 patients, 1,228 controls.
Combined cohort	2,687 case patients, 6,858 controls	Combined analysis of data from stage 1 and 2a: 750 patients, 1,820 controls; Combined analysis of data from stage 1 and 2b: 987 patients, 2,731 controls
ANCA status	Positive ANCA[#]	Any
Main findings	Main associations with GPA were found with *HLA-DPB1*, with *SERPINA1* locus and the proteinase 3 gene (*PRTN3*). These associations were more evident with the antibody subtype (anti-PR3 *vs*. anti-MPO) than with the clinical syndromes.	*SEMA6A* and HLA loci (*HLA-DPB1* and *HLA-DPA1*). Additionally, significant associations were identified between single-nucleotide polymorphic (SNP) marker across the HLA region and C-ANCA-positive GPA.

[#]ANCA-positivity was either with ELISA (anti-PR3 or –MPO) or with Immunofluorescence.

this study also reported a non-MHC association at the *SEMA6A* locus, which encodes semaphorin 6A. As was the case with the EVGC GWAS, these associations were strongest in the PR3 ANCA-positive patients (Table II).

The first conclusion that can be drawn from these two studies is that, since the genetic associations are stronger with ANCA specificities rather than clinical syndrome, AAV patients would be better classified on the basis of their ANCA specificity: it was therefore provokingly proposed to separate AAV into PR3- and MPO-positive polyangiitis. This distinction already has a well-recognised clinical base, as PR3- and MPO-associated vasculitis often occur in patient populations that differ on the basis of their age at presentation, cardiovascular risk and relapse rates (4). Moreover, the confirmation of the MHC system as a risk factor, together with the auto-antigen PR3 and its main inhibitor α1-antitrypsin, confirmed the centrality of auto-reactivity in the development of AAV. Further studies are required to better clarify the role of semaphorin 6A in AAV pathogenesis.

One interesting question that still needs to be addressed is why, although the genetic background differs between PR3 and MPO AAV, the clinical phenotypes significantly overlap. One hypothesis is that the ANCA, once developed, may generate inflammation through similar mechanisms (15, 16) and that currently unrecognised shared genetic associations, environmental factors and chronic infections may contribute significantly to the final clinical phenotype.

The two GWAS performed to date have therefore contributed massively to our understanding of AAV pathogenesis and classification. The next step will be to perform separate, properly powered GWAS studies in PR3- and MPO-positive vasculitis, in order to uncover additional novel unique and shared genetic associations. A GWAS in EGPA is also warranted.

Table II. Main genetic associations with Granulomatosis with Polyangiitis (Wegener's), C-ANCA or PR3-ANCA vasculitis shown by the Genome-Wide Association Studies (GWAS) performed by the European Vasculitis Genetic Consortium (EVGC) (12) and the Vasculitis Clinical Research Consortium (VCRC) (13).

A) EVGC GWAS: associations of MHC and Non-MHC Loci with GPA

					Combined analysis (patients, n=1,683; controls, n=6,858)	
Gene	Chromosome	SNP	Location, bp	Nucleotide change	*p*-value	OR
HLA-DPB1	6	rs3117242	31177871	A>G	5.39×10^{-85}	5.39
SERPINA1	14	rs7151526	93933389	A>C	4.4×10^{-10}	0.54
PRTN3	19	rs62132295	840448	G>A	2.6×10^{-5}	0.78

B) EVGC GWAS: associations of MHC and non-MHC Loci with Proteinase 3-positive GPA

					GPA Proteinase 3 *vs*. controls (patients, n=1,433; controls, n=6,858)	
Gene	Chromosome	SNP	Location, bp	Nucleotide change	*p*-value	OR
HLA-DPB1	6	rs3117242	31177871	A>G	3.7×10^{-86}	7.51
SERPINA1	14	rs7151526	93933389	A>C	1.2×10^{-10}	0.52
PRTN3	19	rs62132295	840448	G>A	3.9×10^{-7}	0.73

C) VCRC GWAS: associations of MHC and Non-MHC Loci with GPA

					Combined analysis (patients, n=750; controls, n=1,820)	
Gene	Chromosome	SNP	Location, bp	Nucleotide change	*p*-value	OR
HLA-DPB1	6	rs9277554	33163516	T>C	1.92×10^{-50}	0.24
HLA-DPA1	6	rs9277341	33147603	C>T	2.18×10^{-39}	0.33

					Combined analysis (patients, n=987; controls, n=2,731)	
Gene	Chromosome	SNP	Location, bp	Nucleotide change	*p*-value	OR
SEMA6A	5	rs26595	115787389	C>T	2.09×10^{-8}	0.74

D) VCRC GWAS: associations of MHC Loci with c-ANCA-positive GPA

					c-ANCA-positive patients *vs*. controls (patients, n=578; controls, n=1,820)	
Gene	Chromosome	SNP	Location, bp	Nucleotide change	*p*-value	OR
HLA-DPB1	6	rs9277554	33163516	T>C	4.7×10^{-57}	0.16
HLA-DPA1	6	rs9277341	33147603	C>T	2.30×10^{-42}	0.27

Candidate gene studies

AAV is a rare disease, with an incidence of approximately 12-18 cases/million people/year, and therefore the collection of very large cohorts has proved challenging and historically most studies used a more focussed candidate gene approach. In the following paragraphs we will discuss the more interesting results of these candidate gene studies. Table III reports a detailed summary of their reported associations.

Associations with the MHC

The MHC has been found to be associated with several autoimmune diseases (57, 58). The hypothesis of an autoimmune pathogenesis for AAV, together with previously documented associations (17, 19, 46), drove attention to the role of the MHC as a potential predisposing factor to AAV. The HLA region is encoded on chromosome 6p21-31 and contains more than 200 genes divided in three classes: HLA class I (A, B, C), class II (DR, DQ and DP) and class III (59). This is an extremely polymorphic area and its study is quite complex due to the presence of strong linkage disequilibrium across the region.

A study in a cohort of 150 Northern German patients with GPA reported association with the *HLA DP* locus. Fine mapping showed that the *DPB1*0401* allele is strongly associated with the risk of developing the disease with the extended *DPB1*0401/RXRB03* haplotype found to be even more significantly associated (p=7.13x10^{-17}, odds ratio (OR) 6.41) (14). This finding was then replicated in 282 German patients and, interestingly, the association was more striking when the analysis was restricted to the ANCA-positive subgroup (p=1.26x10^{-22}). In addition, a further potential association was found in the proximity of *RING1*, a transcription modulator involved in the repression of autoimmunity in mice (18).

In a Dutch cohort of 304 patients with AAV, low-resolution genotyping documented *HLA-DR4* as risk factor for AAVs in general (OR 1.7, p<0.0001) and for GPA in particular (20). Two recent and relatively small studies showed association of AAV with *HLA-DRB1* in African American (47) and Chinese (21) populations; interestingly, a different genetic background emerged for the PR3 ANCA-positive subgroup in both studies.

In both MPO-positive vasculitis and EGPA, association studies have been strongly limited by the rarity of these diseases and consequently the small cohorts studied. In 50 MPO positive Japanese patients, an association with *HLA-DRB1*0901-HLA DQB1*0303* was found, thus replicating, in a cohort of different ethnicity, the same HLA association shown in the EVGC GWAS (12, 39, 60). An Italian study of 48 EGPA patients showed an association with *HLA DRB4* (42), which was confirmed in a subsequent study of 102 German patients (43). Interestingly, the Italian study showed that *HLA-DRB4* was particularly enriched in patients with "vasculitic" manifestations of EGPA, such as glomerulonephritis, mononeuritis multiplex, and purpura.

Non-MHC associations

Candidate gene studies have also allowed the identification of non-MHC genes potentially involved in the pathogenesis of AAV. Genetic associations, not reaching genome-wide significance, have been proposed for the protein tyrosine phosphatase N22 (PTPN22) and for the cytotoxic T-lymphocyte antigen 4 (CTLA-4); less strong but intriguing evidence has been recently provided for toll like receptor 9 (TLR9).

The *PTPN22* gene is located on chromosome 1p13.2; the encoded protein (LYP) influences the threshold of activation of the T cell receptor but is also involved in B cell activation (61). *PTPN22* has been found to be associated with several autoimmune diseases, including type 1 diabetes (62-64). The proposed mechanism of action is that an alteration of the interaction of LYP with a protein kinase involved in T cell receptor activation leads to abnormal CD4 T_{reg} function and increased humoral activity (65). Interestingly, mice with *PTPN22* knocked-out contain increased numbers of germinal centres and higher IgG levels (66). The central role of ANCA and of the B cell compartment in AAV (8) suggested a possible role for *PTPN22* also in GPA. In a cohort of 199 German patients the 620W variant at the rs2476601 SNP was significantly increased in GPA (OR 1.75, p=0.002), with a stronger association documented in the ANCA-positive subgroup (OR 2.01, p=0.0002) (36). This finding was confirmed in an independent cohort of 641 British GPA and MPA patients (53) and 344 Italian AAV cases (37). The latter study interrogated for the first time the association of *PTPN22* with EGPA with negative findings (p=0.1508), further underlying a different genetic background for EGPA (37).

The *CTLA4* gene is located on chromosome 2q33. CTLA4 is a protein whose function is still not completely clear. It is proposed to act as a T cell inhibitor, competing with the co-stimulatory protein CD28 for the binding of CD80/CD86. However, its mechanism of action is probably more complex, involving pathways not yet completely understood. Several studies, including a meta-analysis, showed associations at different *CTLA4* SNPs with AAV (29, 52, 53, 67, 68) suggesting a role for this molecule in AAV pathogenesis. Interestingly CTLA4 has also been explored as potential therapeutic target in a pilot trial of patients with non-severe GPA treated with abatacept, a monoclonal antibody including the binding region of CTLA4, with good results in terms of safety and disease response (69).

A recent large association study focused on TLR9 in AAV. The toll like receptor family is a group of proteins that recognise microbiological structures and activate the innate immune response. TLRs are usually over-expressed during infections and provide a potential link between infections and the pathogenesis and development of AAV. TLR9 stimulation in *ex-vivo* neutrophils was associated with increased MPO release and PR3 surface expression; moreover, degranulation was induced after stimulation with PR3-ANCA in neutrophils primed by TLR9 ligand. These findings did not differ between neutrophils of a small cohort of patients with AAV and controls suggesting that this could be expression of a physiological mechanism not necessarily over represented in AAV, nevertheless this pathway might be involved in the development

Table III. Main association studies between ANCA-associated vasculitis and HLA and non-HLA genes. (AAVs, ANCA-associated vasculitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; RLV, renal-limited vasculitis; OR, odds ratio).

Gene	Population origin	Cases	*p*-value	OR	Ref. (Year)
Genetic associations with GPA					
HLA-B50	North American, Caucasian	83 GPA	<0.0001	4.9	(17) (1995)
HLA-DPB1*0401	Germany	150 GPA	$<1x10^{-9}$	3.9	(14) (2004)
HLA DPB1*0401	Germany	282 GPA	$<1x10^{-7}$		(18) (2008)
HLA-DR1	UK	27 GPA	<0.001	2.3	(19) (1992)
HLA-DR1	The Netherlands	241 GPA (+30 MPA and 12 EGPA and 21 RLV)	<0.05	0.6	(20) (2009)
HLA-DR4	The Netherlands	241 GPA (+30 MPA and 12 EGPA and 21 RLV)	<0.0001	1.7	(20) (2009)
HLA-DR6	The Netherlands	241 GPA (+30 MPA and 12 EGPA and 21 RLV)	<0.0001	0.4	(20) (2009)
HLA-DR9	North American, Caucasian	76 GPA	<0.01	4	(17) (1995)
HLA-DR13 (6)	The Netherlands	241 GPA (+30 MPA and 12 EGPA and 21 RLV)	<0.0001	0.3	(20) (2009)
HLA-DRB1*1202	China	45 GPA	<0.05		(21) (2011)
AAT Z allele	Sweden	66 GPA	<0.0001	6	(22) (1994)
AAT Z allele	Scandinavia	44 GPA	<0.001		(23) (1996)
AAT Z allele	Germany	79 GPA	<0.0001	3.8	(24) (2001)
AAT Z allele	Caucasian	723 GPA (+133 MPA)	<0.0001	2.54	(25) (2011)
C3F	Sweden	34 GPA (+33 MPA)	<0.05	0.52	(26) (1999)
CD226 rs 763361	Germany	642 GPA	<0.05	1.2	(27) (2009)
CDK6	Germany	664 GPA	<0.05	1.21	(28) (2010)
CTLA 4-318T	Sweden	32 GPA	<0.05	3.26	(29) (2002)
IL-10 microsatellite	Sweden	32 GPA	<0.01	7.94	(30) (2002)
IL-10 (-1082) AA genotype	Germany	125 GPA	<0.05	0.3	(31) (2003)
FCGR2A R131 RR genotype and FCGR3A F158 FF	The Netherlands	91 GPA	<0.01	4.6	(32) (1999)
FCGR3B low copy number	France	84 GPA	<0.001		(33) (2007)
FCGR3B low copy number	UK	80 GPA	<0.01		(34) (2007)
LEPR rs8179183 C/G (Lys 656 Asn)	Germany and UK	789 GPA (+88 MPA and 196 EGPA)	<0.01	0.72	(34) (2010)
PRTN3-564G	Germany	66 GPA	<0.01	0.5	(35) (2000)
PTPN22-620W	Germany	199 GPA	<0.01	1.8	(36) (2005)
PTPN22-R620W	Italy	143 GPA (+102 MPA and 99 EGPA)	<0.01	1.91	(37) (2012)
TLR9	Germany UK and The Netherlands	919 GPA (+153 MPA and 217 EGPA)	<0.01	1.27	(38) (2013)
TNFAIP3	Germany	664 GPA	<0.05	0.83	(28) (2010)
Genetic associations with MPA					
HLA-DRB1*1101	China	107 MPA	<0.05		(21) (2011)
HLA-DRB1*0901	Japan	96 MPA	<0.01	1.90	(39) (2013)
FCGR3B low copy number	UK	76 MPA	<0.001		(33) (2007)
IL-10 (-1082) AA genotype	Germany	36 MPA	<0.001	0.3	(31) (2003)
IRF 5	Japan	177 MPA	<0.05	1.27	(40) (2013)
LILRA2 intron 6 AA genotype	Japan	50 MPA	<0.05	2.52	(41) (2008)
TLR9	Germany ,UK and The Netherlands	153 MPA (+919 GPA and 217 EGPA)	<0.01	0.44	(38) (2013)
Genetic associations with EGPA					
HLA-DRB3	Italy	48 EGPA	<0.05	0.54	(42) (2007)
HLA-DRB3	Germany	102 EGPA	<0.01	0.61	(43) (2008)
HLA-DRB4	Italy	48 EGPA	$<1x10^{-3}$	2.49	(42) (2007)
HLA-DRB4	Germany	102 EGPA	$<1x10^{-3}$	1.87	(43) (2008)
IL-10 haplotype	Germany	103 EGPA	<0.001	2.16	(44) (2008)
Genetic associations with multiple AAVs subtypes					
HLA-DPB1*0402	China	152 (44 GPA 94 MPA 14 RLV)	<0.05		(45) (2012)
HLA-DQw7	UK	34 GPA and 25 MPA	<0.0025	2.9	(46) (1992)
HLA-DR3	UK	34 GPA and 25 MPA	<0.01	0.31	(46) (1992)
HLA-DRB1*15	African-American, Caucasian	90 PR3 AAVs (+47 MPO AAVs)	$<1x10^{-8}$	73.3	(47) (2011)
HLA-DRB1*0405	China	152 (44 GPA 94 MPA 14 RLV)	<0.05		(45) (2012)
AAT Z allele	Austria	29 GPA and 2 MPA and 1 RLV	<0.0001	13.2	(48) (1994)
AAT Z allele	Italy	33 GPA and 28 MPA and 23 RLV	<0.05	3.7	(49) (1997)
CAV1	Germany and UK	641 GPA and 135 MPA	<0.05	1.83	(50) (2013)
CD18 Ava II	Germany	31 MPO AAVs	<0.005	2.56	(51) (2000)
CTLA 4 +49G	The Netherlands	50 GPA and 24 MPA and 7 EGPA and 21 RLV	<0.05	1.32	(52) (2008)
CTLA 4 rs3087243	UK	641 AAVs	<0.01	1.19	(53) (2009)
DEFB4 high copy number	China	112 AAVs	<0.01	1.49	(54) (2012)
FCGR3B high copy number	UK	556 AAVs	$<1x10^{-7}$		(55) (2008)
IL2RA rs41295061	UK	675 AAVs	<0.05	0.77	(56) (2009)
PTPN22-620W	UK	641 AAVs	$<1x10^{-3}$	1.4	(53) (2009)

of the disease in predisposed subjects (70). An association between four *TLR9* SNPs and GPA was documented in 863 German patients compared to controls but was not replicated in 426 Dutch and British patients possibly due to lack of power. MPA patients were associated with the same SNPs but the proposed risk allele was the opposite of that found in GPA. No association was found with EGPA. However, the most robust and replicated result was the significant difference in terms of association for the SNP rs352140 between cases PR3 ANCA-positive compared to MPO positive (p=0.000016 after combining the two cohorts) (38).

As already discussed above, the T-cell compartment plays a key role in the pathogenesis of AAVs. *IL2RA* encodes the high affinity IL-2 receptor mainly represented on the surface of T cells, whose stimulation is essential for T cell survival, proliferation and activation. A weak association of the *IL2RA* gene SNPs rs41295061 with AAV has been described in a cohort of 744 British patients (p=0.012) (56) but no replication of this finding has been published to date.

IL-10 is an anti-inflammatory cytokine with complex and still not completely clear functions. Two relatively small studies identified an association of one *IL-10* SNP and risk of developing GPA and MPA in 39 and 161 patients (31, 71). A larger report, however, failed to confirm this association in 403 GPA cases; the same study documented in a cohort of 103 EGPA patients an association of an *IL-10* haplotype with the ANCA negative subtype of EGPA, a potentially interesting finding but one difficult to interpret due to the sample size and the lack of replication (44).

The CD226 protein is part of the immunoglobulin supergene family and is expressed on several cell types including T lymphocytes, NK cells and monocytes. CD226 acts mainly as an adhesion molecule but also regulates leukocyte transmigration and lymphocyte co-activation. A study performed on 642 German patients showed an association of a *CD226* SNP with AAV (OR 1.23, p=0.0031); the same study tried to replicate the finding in a small cohort of 105 British patients but failed (27). A further study explored the same SNP in 641 British patients with no significant association documented (53).

Fc gamma receptors (FcγR) are a family of surface proteins that bind the Fc region of immunoglobulins. FcγRs interact directly with ANCA and therefore have been investigated as possible risk factors for the development of AAVs. These proteins are encoded on chromosome 1q23.3 in a region difficult to genotype because of very high variability as well as the presence of copy number variation (CNV). *FcγRIIa - IIIb* SNPs as well as *FcγRIIIb* CNV have been proposed as possible risk factors for AAV, although for the latter conflicting results have emerged between different cohorts that can be largely attributed to different genotyping techniques (33, 55, 72, 73). Further studies relying on modern genotyping and sequencing techniques are warranted to clarify the roles of the *FCR* variants in AAV.

An association between a SNP in the leptin receptor gene (*LEPR)* has been found in a cohort of 466 German patients with GPA and confirmed in an independent cohort of 226 cases. An analysis of the same SNP in 196 EGPA patients found an association with the complementary allele when compared to GPA (34). Although statistically significant, the rationale behind these associations is still unclear and requires further investigation.

With the exception of the MHC region, none of the candidate gene associations described above reached the statistical threshold required to report genome-wide significance or were supported by the two recently published GWAS. However, all the studies to date, including the two GWAS, have been relatively poorly powered to detect loci with small effect sizes, an issue exacerbated by the observation that the observed genetic associations show stronger effects in patients stratified by ANCA specificity rather than with AAV as a whole. It is possible therefore that some of the candidate gene associations described above will be supported by larger, more appropriately powered studies or through meta-analysis with the existing studies.

Pathogenetic mechanisms supported by the genetic evidence

The association of AAV with the MHC documented in the two GWAS and in the major candidate gene studies confirmed an autoimmune, possibly autoantigen driven mechanism, involved in the development of both PR3 and MPO AAV. In PR3-AAV a central role is suggested for the auto antigen PR3 supported by several lines of evidence published over the years including studies showing a higher level of membrane bound PR3 in GPA patients (74). Genetic factors might also impact on PR3 membrane expression or enzymatic activity but this still needs clarification and will be the subject of future studies. An important role is also proposed for α1-antitrypsin, this enzyme is the major inhibitor of PR3 activity potentially reducing peripheral tissue damage. A role of the null Z allele and risk of AAV, already proposed in a number of small studies (24, 35, 75), was confirmed by the EVGC GWAS; with the association at the *SERPINA1* locus documented in this study being attributable to the Z allele or a SNP in strong linkage disequilibrium with it (12). Therefore, predisposed subjects with a "favourable" PR3 protein and/or low α1-antitrypsin activity might be at increased risk of developing AAV in the presence of disease-associated environmental factors.

In terms of environmental factors, a central role is played by infections. Any infective process, and in particular chronic nasal infection with *Staphylococcus aureus*, may cause disease flares and represents one of the main causes of disease persistence and lack of response to treatment in localised forms of GPA (76). For this reason, the potential association of *TLR9*, which encodes a protein that promotes neutrophil activation, may be interesting from the pathogenetic point of view.

The documented *PTPN22* and *CTLA4* associations suggest a key role of both the B and T cell compartments in the pathogenesis of AAV illustrating the importance of anti CD20 and anti-T lymphocyte treatments for AAV (8, 69, 77).

Future perspective

The evidence of different genetic backgrounds in AAV defined by ANCA specificity points towards the need for larger GWAS in PR3 and MPO-positive patients separately. This approach will allow the identification of additional novel associations defined by auto-antibody specificity; meta-analysis of the data from the different GWAS should allow the identification of shared predispositions that, although likely to exist, may require big cohorts to reach genome-wide significance.

A GWAS in EGPA as well as ANCA-negative AAV is indicated although the rarity of these diseases may lead to problems building suitably powered cohorts. A further point of interest would be to identify possible overlap associations between MPO-positive patients with different clinical diagnoses (*i.e.* EGPA and MPA or GPA).

References

1. JENNETTE JC, FALK RJ, ANDRASSY K *et al*.: Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994; 37: 187-92.
2. SABLE-FOURTASSOU R, COHEN P, MAHR A *et al*.: Antineutrophil cytoplasmic antibodies and the Churg-Strauss syndrome. *Ann Intern Med* 2005; 143: 632-8.
3. MASI AT, HUNDER GG, LIE JT *et al*.: The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). *Arthritis Rheum* 1990; 33: 1094-100.
4. MAHR A, KATSAHIAN S, VARET H *et al*.: Revisiting the classification of clinical phenotypes of anti-neutrophil cytoplasmic antibody-associated vasculitis: a cluster analysis. *Ann Rheum Dis* 2013; 72: 1003-10.
5. WATTS R, LANE S, HANSLIK T *et al*.: Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. *Ann Rheum Dis* 2007; 66: 222-7.
6. DE GROOT K, RASMUSSEN N, BACON PA *et al*.: Randomized trial of cyclophosphamide versus methotrexate for induction of remission in early systemic antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2005; 52: 2461-9.
7. BASU N, MCCLEAN A, HARPER L *et al*.: The characterisation and determinants of quality of life in ANCA associated vasculitis. *Ann Rheum Dis* 2014; 73: 207-11.
8. ALBERICI F, JAYNE DR: Impact of rituximab trials on the treatment of ANCA-associated vasculitis. *Nephrol Dial Transplant* 2013.
9. KALLENBERG CG, HEERINGA P: Complement is crucial in the pathogenesis of ANCA-associated vasculitis. *Kidney Int* 2013; 83: 16-8.
10. TANNA A, SALAMA AD, BROOKES P, PUSEY CD: Familial granulomatosis with polyangiitis: three cases of this rare disorder in one Indoasian family carrying an identical HLA DPB1 allele. *BMJ Case Rep* 2012; 2012.
11. KNIGHT A, SANDIN S, ASKLING J: Risks and relative risks of Wegener's granulomatosis among close relatives of patients with the disease. *Arthritis Rheum* 2008; 58: 302-7.
12. LYONS PA, RAYNER TF, TRIVEDI S *et al*.: Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med* 2012; 367: 214-23.
13. XIE G, ROSHANDEL D, SHERVA R *et al*.: Association of granulomatosis with polyangiitis (Wegener's) with HLA-DPB1*04 and SEMA6A gene variants: evidence from genome-wide analysis. *Arthritis Rheum* 2013; 65: 2457-68.
14. JAGIELLO P, GENCIK M, ARNING L *et al*.: New genomic region for Wegener's granulomatosis as revealed by an extended association screen with 202 apoptosis-related genes. *Hum Genet* 2004; 114: 468-77.
15. FALK RJ, TERRELL RS, CHARLES LA, JENNETTE JC: Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. *Proc Natl Acad Sci U S A* 1990; 87: 4115-9.
16. RADFORD DJ, SAVAGE CO, NASH GB: Treatment of rolling neutrophils with anti-neutrophil cytoplasmic antibodies causes conversion to firm integrin-mediated adhesion. *Arthritis Rheum* 2000; 43: 1337-45.
17. COTCH MF, FAUCI AS, HOFFMAN GS: HLA typing in patients with Wegener granulomatosis. *Ann Intern Med* 1995; 122: 635.
18. HECKMANN M, HOLLE JU, ARNING L *et al*.: The Wegener's granulomatosis quantitative trait locus on chromosome 6p21.3 as characterised by tagSNP genotyping. *Ann Rheum Dis* 2008; 67: 972-9.
19. PAPIHA SS, MURTY GE, AD'HIA A, MAINS BT, VENNING M: Association of Wegener's granulomatosis with HLA antigens and other genetic markers. *Ann Rheum Dis* 1992; 51: 246-8.
20. STASSEN PM, COHEN-TERVAERT JW, LEMS SP, HEPKEMA BG, KALLENBERG CG, STEGEMAN CA: HLA-DR4, DR13(6) and the ancestral haplotype A1B8DR3 are associated with ANCA-associated vasculitis and Wegener's granulomatosis. *Rheumatology* (Oxford) 2009; 48: 622-5.
21. LUO H, CHEN M, YANG R, XU PC, ZHAO MH: The association of HLA-DRB1 alleles with antineutrophil cytoplasmic antibody-associated systemic vasculitis in Chinese patients. *Hum Immunol* 2011; 72: 422-5.
22. ELZOUKI AN, SEGELMARK M, WIESLANDER J, ERIKSSON S: Strong link between the alpha 1-antitrypsin PiZ allele and Wegener's granulomatosis. *J Intern Med* 1994; 236: 543-8.
23. BASLUND B, SZPIRT W, ERIKSSON S *et al*.: Complexes between proteinase 3, alpha 1-antitrypsin and proteinase 3 anti-neutrophil cytoplasm autoantibodies: a comparison between alpha 1-antitrypsin PiZ allele carriers and non-carriers with Wegener's granulomatosis. *Eur J Clin Invest* 1996; 26: 786-92.
24. BORGMANN S, ENDISCH G, URBAN S, SITTER T, FRICKE H: A linkage disequilibrium between genes at the serine protease inhibitor gene cluster on chromosome 14q32.1 is associated with Wegener's granulomatosis. *Clin Immunol* 2001; 98: 244-8.
25. MORRIS H, MORGAN MD, WOOD AM *et al*.: ANCA-associated vasculitis is linked to carriage of the Z allele of alpha(1) antitrypsin and its polymers. *Ann Rheum Dis* 2011; 70: 1851-6.
26. PERSSON U, TRUEDSSON L, WESTMAN KW, SEGELMARK M: C3 and C4 allotypes in anti-neutrophil cytoplasmic autoantibody (ANCA)-positive vasculitis. *Clin Exp Immunol* 1999; 116: 379-82.
27. WIECZOREK S, HOFFJAN S, CHAN A *et al*.: Novel association of the CD226 (DNAM-1) Gly307Ser polymorphism in Wegener's granulomatosis and confirmation for multiple sclerosis in German patients. *Genes Immun* 2009; 10: 591-5.
28. WIECZOREK S, HOLLE JU, MULLER S, FRICKE H, GROSS WL, EPPLEN JT: A functionally relevant IRF5 haplotype is associated with reduced risk to Wegener's granulomatosis. *J Mol Med* (Berl); 88: 413-21.
29. GISCOMBE R, WANG X, HUANG D, LEFVERT AK: Coding sequence 1 and promoter single nucleotide polymorphisms in the CTLA-4 gene in Wegener's granulomatosis. *J Rheumatol* 2002; 29: 950-3.
30. ZHOU Y, GISCOMBE R, HUANG D, LEFVERT AK: Novel genetic association of Wegener's granulomatosis with the interleukin 10 gene. *J Rheumatol* 2002; 29: 317-20.
31. BARTFAI Z, GAEDE KI, RUSSELL KA, MURAKOZY G, MULLER-QUERNHEIM J, SPECKS U: Different gender-associated genotype risks of Wegener's granulomatosis and microscopic polyangiitis. *Clin Immunol* 2003; 109: 330-7.
32. DIJSTELBLOEM HM, SCHEEPERS RH, OOST WW *et al*.: Fcgamma receptor polymorphisms in Wegener's granulomatosis: risk factors for disease relapse. *Arthritis Rheum* 1999; 42: 1823-7.
33. FANCIULLI M, NORSWORTHY PJ, PETRETTO E *et al*.: FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. *Nat Genet* 2007; 39: 721-3.
34. WIECZOREK S, HOLLE JU, BREMER JP *et al*.: Contrasting association of a non-synonymous leptin receptor gene polymorphism with Wegener's granulomatosis and Churg-Strauss syndrome. *Rheumatology* (Oxford) 2010; 49: 907-14.
35. GENCIK M, MELLER S, BORGMANN S, FRICKE H: Proteinase 3 gene polymorphisms and Wegener's granulomatosis. *Kidney Int* 2000; 58: 2473-7.
36. JAGIELLO P, ARIES P, ARNING L *et al*.: The PTPN22 620W allele is a risk factor for Wegener's granulomatosis. *Arthritis Rheum* 2005; 52: 4039-43.
37. MARTORANA D, MARITATI F, MALERBA G *et al*.: PTPN22 R620W polymorphism in the ANCA-associated vasculitides. *Rheumatology* (Oxford) 2012; 51: 805-12.
38. HUSMANN CA, HOLLE JU, MOOSIG F *et al*.: Genetics of toll like receptor 9 in ANCA associated vasculitides. *Ann Rheum Dis* 2013.
39. TSUCHIYA N, KOBAYASHI S, HASHIMOTO H, OZAKI S, TOKUNAGA K: Association of

HLA-DRB1*0901-DQB1*0303 haplotype with microscopic polyangiitis in Japanese. *Genes Immun* 2006; 7: 81-4.
40. KAWASAKI A, INOUE N, AJIMI C *et al*.: Association of IRF5 polymorphism with MPO-ANCA-positive vasculitis in a Japanese population. *Genes Immun* 2013; 14: 527-9.
41. MAMEGANO K, KUROKI K, MIYASHITA R *et al*.: Association of LILRA2 (ILT1, LIR7) splice site polymorphism with systemic lupus erythematosus and microscopic polyangiitis. *Genes Immun* 2008; 9: 214-23.
42. VAGLIO A, MARTORANA D, MAGGIORE U *et al*.: HLA-DRB4 as a genetic risk factor for Churg-Strauss syndrome. *Arthritis Rheum* 2007; 56: 3159-66.
43. WIECZOREK S, HELLMICH B, GROSS WL, EPPLEN JT: Associations of Churg-Strauss syndrome with the HLA-DRB1 locus, and relationship to the genetics of antineutrophil cytoplasmic antibody-associated vasculitides: comment on the article by Vaglio *et al*. *Arthritis Rheum* 2008; 58: 329-30.
44. WIECZOREK S, HELLMICH B, ARNING L *et al*.: Functionally relevant variations of the interleukin-10 gene associated with antineutrophil cytoplasmic antibody-negative Churg-Strauss syndrome, but not with Wegener's granulomatosis. *Arthritis Rheum* 2008; 58: 1839-48.
45. CHANG DY, LUO H, ZHOU XJ, CHEN M, ZHAO MH: Association of HLA genes with clinical outcomes of ANCA-associated vasculitis. *Clin J Am Soc Nephrol* 1996; 7: 1293-9.
46. SPENCER SJ, BURNS A, GASKIN G, PUSEY CD, REES AJ: HLA class II specificities in vasculitis with antibodies to neutrophil cytoplasmic antigens. *Kidney Int* 1992; 41: 1059-63.
47. CAO Y, SCHMITZ JL, YANG J *et al*.: DRB1*15 allele is a risk factor for PR3-ANCA disease in African Americans. *Clin J Am Soc Nephrol* 2011; 22: 1161-7.
48. LHOTTA K, VOGEL W, MEISL T *et al*.: Alpha 1-antitrypsin phenotypes in patients with anti-neutrophil cytoplasmic antibody-positive vasculitis. *Clin Sci* (Lond) 1994; 87: 693-5.
49. CALLEA F, GREGORINI G, SINICO A *et al*.: alpha 1-Antitrypsin (AAT) deficiency and ANCA-positive systemic vasculitis: genetic and clinical implications. *Eur J Clin Invest* 1997; 27: 696-702.
50. CHAND S, HOLLE JU, HILHORST M *et al*.: Caveolin-1 single nucleotide polymorphism in antineutrophil cytoplasmic antibody associated vasculitis. *PLoS One* 2013; 8: e69022.
51. GENCIK M, MELLER S, BORGMANN S *et al*.: The association of CD18 alleles with anti-myeloperoxidase subtypes of ANCA-associated systemic vasculitides. *Clin Immunol* 2000; 94: 9-12.
52. SLOT MC, SOKOLOWSKA MG, SAVELKOULS KG, JANSSEN RG, DAMOISEAUX JG, TERVAERT JW: Immunoregulatory gene polymorphisms are associated with ANCA-related vasculitis. *Clin Immunol* 2008; 128: 39-45.
53. CARR EJ, NIEDERER HA, WILLIAMS J *et al*.: Confirmation of the genetic association of CTLA4 and PTPN22 with ANCA-associated vasculitis. *BMC Med Genet* 2009; 10: 121.
54. ZHOU XJ, CHENG FJ, LV JC *et al*.: Higher DEFB4 genomic copy number in SLE and ANCA-associated small vasculitis. *Rheumatology* (Oxford) 2012; 51: 992-5.
55. WILLCOCKS LC, LYONS PA, CLATWORTHY MR *et al*.: Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. *J Exp Med* 2008; 205: 1573-82.
56. CARR EJ, CLATWORTHY MR, LOWE CE *et al*.: Contrasting genetic association of IL2RA with SLE and ANCA-associated vasculitis. *BMC Med Genet* 2009; 10: 22.
57. BASCHAL EE, ALY TA, BABU SR *et al*.: HLA-DPB1*0402 protects against type 1A diabetes autoimmunity in the highest risk DR3-DQB1*0201/DR4-DQB1*0302 DAISY population. *Diabetes* 2007; 56: 2405-9.
58. FURUKAWA H, KAWASAKI A, OKA S *et al*.: human leukocyte antigens and systemic lupus erythematosus: a protective role for the HLA-DR6 alleles DRB1*13:02 and *14:03. *PLoS One* 2014; 9: e87792.
59. NOBLE JA, ERLICH HA: Genetics of type 1 diabetes. *Cold Spring Harb Perspect Med* 2012; 2: a007732.
60. TSUCHIYA N, KOBAYASHI S, KAWASAKI A *et al*.: Genetic background of Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis: association of HLA-DRB1*0901 with microscopic polyangiitis. *J Rheumatol* 2003; 30: 1534-40.
61. CLOUTIER JF, VEILLETTE A: Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. *J Exp Med* 1999; 189: 111-21.
62. BOTTINI N, MUSUMECI L, ALONSO A *et al*.: A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet* 2004; 36: 337-8.
63. CRISWELL LA, PFEIFFER KA, LUM RF *et al*.: Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. *Am J Hum Genet* 2005; 76: 561-71.
64. TODD JA, WALKER NM, COOPER JD *et al*.: Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* 2007; 39: 857-64.
65. MAINE CJ, HAMILTON-WILLIAMS EE, CHEUNG J *et al*.: PTPN22 alters the development of regulatory T cells in the thymus. *J Immunol* 2012; 188: 5267-75.
66. HASEGAWA K, MARTIN F, HUANG G, TUMAS D, DIEHL L, CHAN AC: PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 2004; 303: 685-9.
67. LEE YH, CHOI SJ, JI JD, SONG GG: CTLA-4 and TNF-alpha promoter-308 A/G polymorphisms and ANCA-associated vasculitis susceptibility: a meta-analysis. *Mol Biol Rep* 2012; 39: 319-26.
68. KAMESH L, HEWARD JM, WILLIAMS JM *et al*.: CT60 and +49 polymorphisms of CTLA 4 are associated with ANCA-positive small vessel vasculitis. *Rheumatology* (Oxford) 2009; 48: 1502-5.
69. LANGFORD CA, MONACH PA, SPECKS U *et al*.: An open-label trial of abatacept (CTLA4-IG) in non-severe relapsing granulomatosis with polyangiitis (Wegener's). *Ann Rheum Dis* 2013.
70. HOLLE JU, WINDMOLLER M, LANGE C, GROSS WL, HERLYN K, CSERNOK E: Toll-like receptor TLR2 and TLR9 ligation triggers neutrophil activation in granulomatosis with polyangiitis. *Rheumatology* (Oxford) 2013; 52: 1183-9.
71. MURAKOZY G, GAEDE KI, RUPRECHT B *et al*.: Gene polymorphisms of immunoregulatory cytokines and angiotensin-converting enzyme in Wegener's granulomatosis. *J Mol Med* (Berl) 2001; 79: 665-70.
72. TSE WY, ABADEH S, JEFFERIS R, SAVAGE CO, ADU D: Neutrophil FcgammaRIIIb allelic polymorphism in anti-neutrophil cytoplasmic antibody (ANCA)-positive systemic vasculitis. *Clin Exp Immunol* 2000; 119: 574-7.
73. TSE WY, ABADEH S, MCTIERNAN A, JEFFERIS R, SAVAGE CO, ADU D: No association between neutrophil FcgammaRIIa allelic polymorphism and anti-neutrophil cytoplasmic antibody (ANCA)-positive systemic vasculitis. *Clin Exp Immunol* 1999; 117: 198-205.
74. WITKO-SARSAT V, LESAVRE P, LOPEZ S *et al*.: A large subset of neutrophils expressing membrane proteinase 3 is a risk factor for vasculitis and rheumatoid arthritis. *J Am Soc Nephrol* 1999; 10: 1224-33.
75. GRIFFITH ME, LOVEGROVE JU, GASKIN G, WHITEHOUSE DB, PUSEY CD: C-antineutrophil cytoplasmic antibody positivity in vasculitis patients is associated with the Z allele of alpha-1-antitrypsin, and P-antineutrophil cytoplasmic antibody positivity with the S allele. *Nephrol Dial Transplant* 1996; 11: 438-43.
76. POPA ER, STEGEMAN CA, ABDULAHAD WH *et al*.: Staphylococcal toxic-shock-syndrome-toxin-1 as a risk factor for disease relapse in Wegener's granulomatosis. *Rheumatology* (Oxford) 2007; 46: 1029-33.
77. WALSH M, CHAUDHRY A, JAYNE D: Long-term follow-up of relapsing/refractory anti-neutrophil cytoplasm antibody associated vasculitis treated with the lymphocyte depleting antibody alemtuzumab (CAMPATH-1H). *Ann Rheum Dis* 2008; 67: 1322-7.