On the pathogenesis and clinical outcome of ANCA-associated vasculitis

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"*Plus j'ai avancé en age, mieux j'ai compris ton amitié et la supériorité de ta raison*."
("*The longer I live, the better I understand the kindness of thy heart and the high quality of thy mind*.")

L. Pasteur (Dedication to his father in Fermentation of Dextro-Tartrate of Lime, 1879)

To my parents and grandparents

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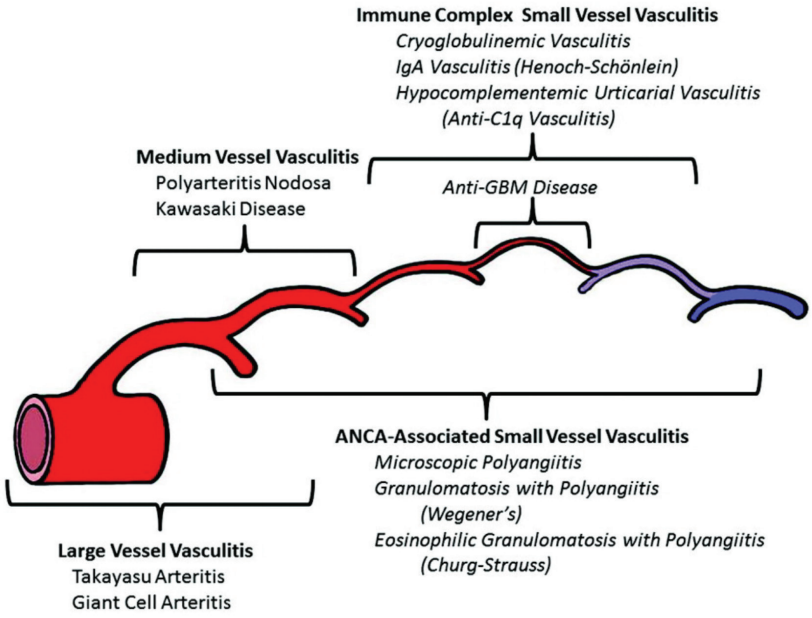
Chapter I

General introduction and outline of this thesis

Vasculitis means inflammation of blood vessels. The International Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC) provides definitions for the different vasculitides and subcategorises them into three major categories: large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis (**figure 1**).[1, 2]

Figure 1. The International Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC)



The International Chapel Hill Consensus Conference Nomenclature of Vasculitides (CHCC) subcategorises the different vasculitides into three major categories: large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis. The figure depicts (from left to right) aorta, large artery, medium artery, small artery/arteriole, capillary, venule, and vein. Anti-GBM = anti–glomerular basement membrane; ANCA = antineutrophil cytoplasmic antibody. Reproduced from Jennette et al.[2] with permission.

Large-vessel vasculitis predominantly affects the aorta and its major branches. Two important vasculitides within this category are Takayasu arteritis and giant cell arteritis. Medium-vessel vasculitis predominantly affects medium sized arteries. Kawasaki disease is an example of a medium-vessel vasculitis. Small-vessel vasculitis is further subcategorised into immune complex small-vessel vasculitis and antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis. Immune complex small-vessel vasculitis shows immunoglobulin (Ig) and complement deposits in the blood vessel walls. Two important vasculitides within this category are IgA vasculitis and anti–glomerular basement membrane

(anti-GBM) disease. ANCA-associated vasculitis is a necrotizing vasculitis with few or no immune deposits that is typically associated with ANCA-seropositivity by indirect immunofluorescence or enzyme-linked immunosorbent assay (ELISA).[3]

ANCA-associated vasculitis

This thesis focuses on different aspects of ANCA-associated vasculitis. ANCA first became widely recognized after a key publication in The Lancet in 1985 in which van der Woude *et al.* described circulating antibodies in patients with vasculitis that were in several ways similar to the granulocyte-specific antinuclear antibodies (GS-ANA) described in rheumatoid arthritis.[4] It should however be mentioned that Davies *et al.* had already described the presence of this class of antibodies in patients with pauci-immune glomerulonephritis in 1982.[5] Van der Woude *et al.* first named these antibodies ACPA (anticytoplasmic antibodies). This term was later replaced by ANCA, as the antibodies were found to be directed against neutrophil (and monocyte) constituents. The two most important ANCA antigens are proteinase 3 (PR3)[6-8] and myeloperoxidase (MPO)[9].

ANCA-associated vasculitis comprises the clinical diagnoses granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis[10-12]), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss Syndrome).[2]

GPA is a necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract.[13] Approximately 90-95% of patients with active, generalized GPA are ANCA-positive; a small subset of GPA patients is ANCA-negative.[14-17] Thus, the absence of ANCA does not exclude the diagnosis of GPA. About 70% of the ANCA-positive GPA patients are PR3-ANCA positive; the remainder are MPO-ANCA positive.[18]

MPA is characterized by a non-granulomatous necrotizing systemic vasculitis. Nearly 90% of patients with MPA are ANCA-positive.[14-17] About 60% of the ANCA-positive MPA patients are MPO-ANCA positive; the remainder are PR3-ANCA positive.[9, 18] Because PR3-ANCA and MPO-ANCA may occur in both GPA and MPA, these conditions cannot be distinguished on the basis of ANCA-serotype.

EGPA is an eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract. In contrast to GPA and MPA, EGPA is associated with asthma and eosinophilia. Although variable from study to study, approximately 50% of patients with EGPA are ANCA-positive.[19, 20] About 70-90% of the ANCA-positive EGPA patients are MPO-ANCA positive.[20, 21]

The clinical diagnoses GPA and MPA are often grouped together on the grounds of striking similarities in clinical presentation and comparable histologic

findings.[18, 22] Nevertheless, there are marked differences between these disease entities, fuelling an ongoing debate as to whether GPA and MPA are different entities within the same disease spectrum or represent distinct auto-immune diseases.[23-25] Although there are overlaps, a clinical diagnosis of GPA usually involves PR3-ANCA positivity whereas a clinical diagnosis of MPA usually involves MPO-ANCA positivity. In this respect, data from a large genome-wide association study (GWAS) are interesting.[26] These data indicate that within the spectrum of ANCA-associated vasculitis distinct subtypes may be recognized. ANCA-serotype was demonstrated to be the main indicator of these subtypes.[26]

To date, the concept of a single disease spectrum has led to the inclusion of GPA and MPA patients in the same clinical trials and the development of similar treatment strategies for these patients.[14-17, 27, 28] Evidence that the different entities in ANCA-associated vasculitis actually represent distinct auto-immune diseases may in the future lead to the development of more specific therapeutic strategies. The existence of different subtypes within the spectrum of ANCA-associated vasculitis and their clinical implications are further investigated in **Chapter 2** and **Chapter 3** of this thesis.

Aetiology and pathogenesis of ANCA-associated vasculitis

The pathogenesis of ANCA-associated vasculitis has not been completely elucidated. However, over the past decades, significant progress has been made in the understanding of this complex disease. Environmental exposures, genetic factors, influences of the immune system, and the intensity and duration of the injury are all hypothesized to be involved in the pathogenesis of ANCA-associated vasculitis. Interestingly, the variability in aetiological and synergistic factors that may lead to the development of ANCA-associated vasculitis is hypothesized to contribute to the clinicopathologic differences between patients.[29]

Onset and exacerbation of ANCA-associated vasculitis occur more often during winter and spring. This points towards the existence of pathogenic, infectious or other environmental, factors that are typically present during these seasons.[30] Numerous studies demonstrated that pro-inflammatory factors, for example induced by infections, act synergistically in ANCA-associated vasculitis onset and exacerbation.[31, 32] In particular, *Staphylococcus aureus* infection was linked to ANCA-associated vasculitis onset.[33-35] Moreover, nasal carriage of *Staphylococcus aureus* was demonstrated to be an important risk factor for relapse in patients with GPA,[36] and treatment with trimethoprim-sulfamethoxazole was shown to aid in the induction of remission[37-46] and the prevention of relapses[37]. These treatment effects could be ascribed to the immunosuppressant and/or anti-staphylococcal properties of trimethoprim-sulfamethoxazole.

Evidence that genetic factors contribute to the pathogenesis of ANCA-associated vasculitis comes from familial association studies,[47-51] differences in the prevalence of ANCA-associated vasculitis between ethnic groups,[52] and numerous candidate gene associations studies[53]. Quite recently, two GWAS performed by the Vasculitis Clinical Research Consortium and the European Vasculitis Genetic Consortium also identified genetic associations in ANCA-associated vasculitis.[26, 54] Both GWAS found a strong association with a single nucleotide polymorphism (SNP) in human leukocyte antigen (*HLA*)-*DPB1*. The European Vasculitis Genetic Consortium GWAS also found a strong association between PR3-ANCA vasculitis and *PRTN3* (the gene encoding PR3) and *SERPINA1* (the gene encoding α1-antitrypsin; a major inhibitor of PR3), and between MPO-ANCA vasculitis and *HLA-DQ*. Moreover, this GWAS demonstrated genetic distinctions between GPA and MPA patients and between PR3-ANCA positive and MPO-ANCA positive patients. The numerous candidate gene association studies and two GWAS have revealed a great number of genetic variants that possibly contribute to the pathogenesis of ANCA-associated vasculitis. In **Chapter 2** of this thesis, we conducted a meta-analysis to investigate the genetic variants that are most likely associated with ANCA-associated vasculitis. We included raw data from the European Vasculitis Genetic Consortium GWAS to increase the validity of the meta-analysis.

Are ANCA pathogenic?

In vitro and *in vivo* evidence support a pathogenic role for ANCA in the pathogenesis of ANCA-associated vasculitis.[55, 56] *In vitro* data include experiments demonstrating that ANCA IgG activates cytokine-primed neutrophils,[57-59] that ANCA-activated neutrophils induce endothelial cell injury,[60, 61] and that ANCA-activated neutrophils activate the alternative complement pathway[62].

The most convincing evidence that ANCA are pathogenic comes from animal models. Xiao *et al.* demonstrated that intravenous injection of purified murine anti-MPO IgG into wild type mice or immunodeficient Rag2-/- mice (these mice fail to generate mature T and B lymphocytes) induces proteinuria and haematuria in these mice. Moreover, the histopathologic lesions found in the kidneys of these mice were comparable to those in renal biopsies of patients with ANCA-associated glomerulonephritis.[56] Furthermore, Little *et al.* demonstrated that rats immunised with human MPO develop anti-human MPO-ANCA that binds these rats' neutrophils and induces pauci-immune crescentic glomerulonephritis and pulmonary haemorrhage with histologic evidence of lung vasculitis in these rats.[63] Thus far, it has not been possible to successfully reproduce these findings in a PR3-ANCA vasculitis animal model.[29, 64]

Clinical evidence for the pathogenicity of ANCA includes the occurrence of pulmonary-renal syndrome in a neonate shortly after birth from a mother with MPO-ANCA positive MPA, most likely caused by transplacental transfer of maternal MPO-ANCA.[65, 66] This clinical evidence is limited, however, in that it comprises only one case-report and until today no sequelae have been reported. Further clinical evidence for the pathogenicity of ANCA includes the induction of ANCA, particularly high titres of MPO-ANCA, by certain drugs (e.g. propylthiouracil and hydralazine[67]) and the subsequent onset of disease manifestations in humans. More clinical evidence is found in the beneficial effect of plasma exchange in the treatment of ANCA-associated vasculitis.[17]

One observation plaguing the contention that ANCA are pathogenic was the presence of ANCA in only 90-95% of ANCA-associated vasculitis patients.[14-17] However, the remaining 5-10% of patients that have long been assumed to be ANCA-negative based on the results of conventional clinical assays might be classified as ANCA-positive after all. Roth *et al.* demonstrated that, when using a highly sensitive epitope-excision method, ANCA-negative patients' purified IgG reacts with a specific MPO-epitope.[68] This MPO-ANCA epitope was blocked from reacting with ANCA IgG in serum because of competitive binding by ceruloplasmin, which is a natural inhibitor of MPO that is naturally present in serum.

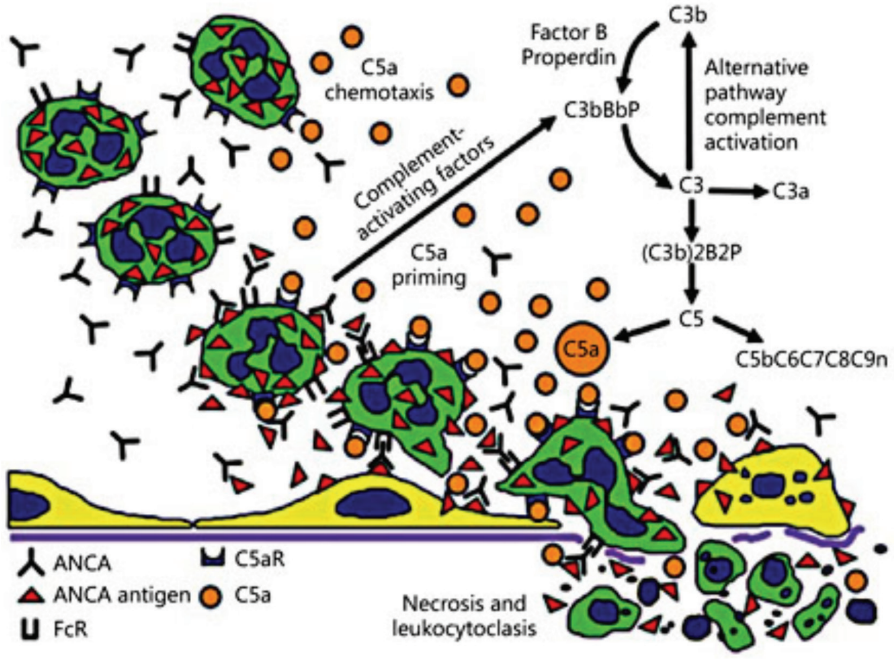
The pathogenicity of ANCA was also questioned since healthy individuals were also demonstrated to have ANCA.[69-72] Interestingly, Roth *et al.* demonstrated that compared with MPO-ANCA occurring in vasculitis, natural MPO-ANCA are present in lower titres, have lower avidity, have less subclass diversity, and are less capable of activating neutrophils *in vitro*.[68] Moreover, the epitope specificity of the repertoire of MPO-ANCA occurring in vasculitis patients was shown to differ from the epitope specificity of MPO-ANCA occurring in healthy individuals.[68] Thus, not all ANCA seem to be equal.[73]

Presumed pathogenic sequence for acute vascular injury in ANCA-associated vasculitis

The putative pathogenic sequence inducing acute vascular inflammation in ANCA-associated vasculitis is depicted in **figure 2**.[29] Starting from the upper left, resting neutrophils have ANCA autoantigens (e.g. PR3 and MPO) sequestered in their cytoplasmic granules. Exposure to priming factors, e.g. cytokines induced by infection or pathogenic factors released by complement activation, leads to the exposure of ANCA-antigens on the neutrophil's surface and in the microenvironment. Circulating ANCA bind to these antigens and activate them by Fcγ receptor engagement and F(ab$'$)2 binding. ANCA-activated neutrophils penetrate vessel walls and release factors that initiate necrosis and

apoptosis of the neutrophils and the environment. Meanwhile, the alternative complement pathway is activated and generates C5a, a chemoattractant for neutrophils that also primes the arriving neutrophils for activation by ANCA, generating a positive feedback effect.

Figure 2. The putative pathogenic sequence inducing acute vascular inflammation in antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis.



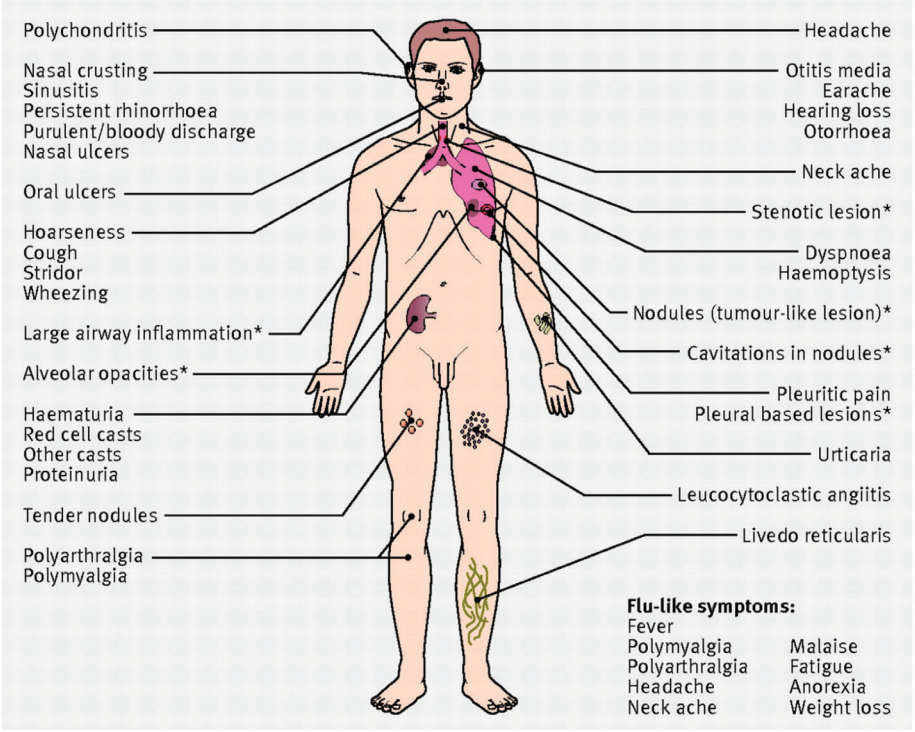
Starting from the upper left, resting neutrophils have ANCA-antigens (e.g. proteinase 3 (PR3) and myeloperoxidase (MPO)) in their cytoplasmic granules. Exposure to priming factors, e.g. cytokines induced by infection or pathogenic factors released by complement activation, leads to the exposure of the ANCA-antigens on the neutrophil's surface and in the microenvironment. Circulating ANCA bind to these antigens and activate the neutrophils. ANCA-activated neutrophils penetrate vessel walls and release factors that initiate necrosis and apoptosis of the neutrophils and the environment. Meanwhile, the alternative complement pathway is activated and generates C5a, a chemoattractant for neutrophils that also primes the arriving neutrophils for activation by ANCA, generating a positive feedback effect. Reproduced from Xiao et al.[29] with permission.

Disease manifestations

The peak age of ANCA-associated vasculitis onset is 65-74 years.[74] Men are more often affected than women, but when women are affected, they tend to have a younger age at disease onset than men.[75] Patients typically present with prodromal 'flu-like' symptoms that have been present for several weeks to months.[3] These symptoms include malaise, fever, headache, polyarthralgia, polymyalgia, and unintended weight loss.[13, 76] Presenting symptoms can be very similar to symptoms of non-vasculitic diseases such as infections, post-viral syndrome, and malignancies. It can therefore be challenging for clinicians to pinpoint the diagnosis early in the diagnostic process. A survey including 701 patients with ANCA-associated vasculitis demonstrated a lag of three to 12 months between disease onset and diagnosis, suggesting that diagnostic delay is a problem.[75] Correct diagnosis on the first visit to a physician was accomplished in only 7% of patients and 50% of patients had to visit at least four physicians before the correct diagnosis was made.

Figure 3 depicts the various manifestations of ANCA-associated vasculitis. Although disease symptoms in GPA and MPA overlap, the incidences of these symptoms can differ significantly between the two conditions. For instance, ear, nose, and throat (ENT) symptoms occur in 90% of GPA patients and in only 35% of MPA patients.[13, 76] Large observational studies demonstrated that the airways and lung parenchyma are commonly affected, as are the kidneys, although renal involvement can be asymptomatic until renal failure occurs.[76-78] Approximately half of patients develop skin manifestations.[76-78] Clearly, ANCA-associated vasculitis can become manifest in virtually all organs. Therefore, thorough physical examination is important to determine the full extent of the disease.[3]

Figure 3. The various manifestations of antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis.



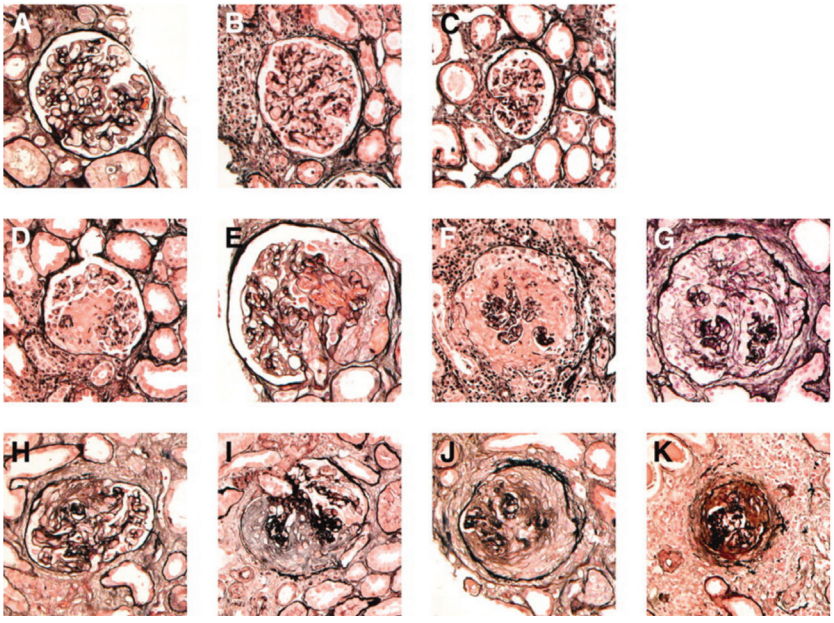
*These lesions can be seen on chest radiography and computed tomography. Reproduced from Berden et al.[3] with permission.

Chapters 3 and 4 of this thesis focus on renal involvement in ANCA-associated vasculitis. Renal involvement is common and is important with respect to patient morbidity and mortality.[22] The kidneys become involved in approximately 80% of GPA patients and 90% of MPA patients.[13] A key study published in 1958 demonstrated that the main cause of death in untreated ANCA-associated vasculitis patients is uraemia due to rapidly progressive renal failure.[79]

Rapidly progressive renal failure with an active sediment (i.e. red cell casts and/or proteinuria) in patients who are seropositive for ANCA is suggestive of ANCA-associated glomerulonephritis. The morphologic changes in the renal biopsy are the gold standard for establishing the diagnosis of ANCA-associated glomerulonephritis.[80, 81] In these biopsies, light microscopy shows necrotizing and crescentic glomerulonephritis.[82] Immunofluorescence microscopy shows little or no immunoglobulin or complement staining (the so-called pauci-immune staining pattern). By electron microscopy, subendothelial edema, microthrombosis, and degranulation of neutrophils are present, but immune deposits are absent.[83]

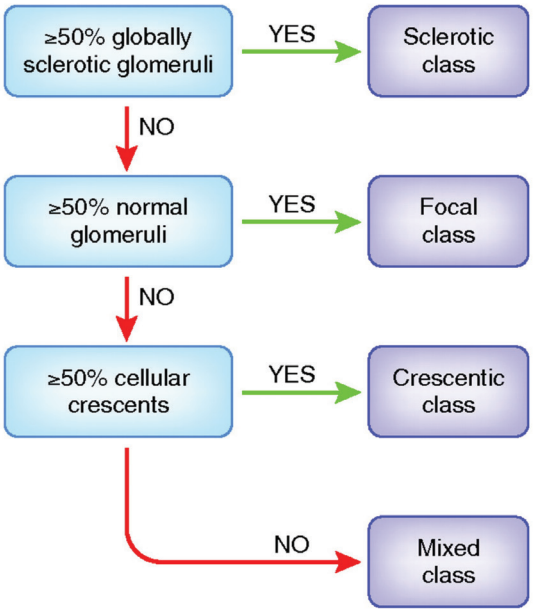
Several studies have demonstrated strong associations between histopathological parameters in the renal biopsy and renal outcome. The most consistent findings were associations between the percentage of normal glomeruli and favorable renal outcome and between the percentage of sclerotic glomeruli and poor renal outcome.[84-87] Moreover, the presence of active lesions such as cellular crescents was found to be positively associated with renal recovery under immunosuppressive treatment.[84, 85] In 2010, the histopathological classification of ANCA-associated glomerulonephritis was devised within the collaboration of the European Vasculitis Society (EUVAS) with the aim of further adding to the prognostication of patients with ANCA-associated glomerulonephritis.[88] This classification system is built around glomerular pathology and distinguishes four classes: focal class biopsies contain ≥50% normal glomeruli; crescentic class biopsies contain ≥50% glomeruli with cellular crescents; sclerotic class biopsies contain ≥50% sclerotic glomeruli; and biopsies without predominant lesions are assigned to the mixed class. Examples of glomerular lesions typically for each of the four classes are depicted in **figure 4**. The classification flowchart is depicted in **figure 5**.

Figure 4. Examples of glomerular lesions that can be observed in the four classes of the histopathological classification of antineutrophil cytoplasmic antibodies (ANCA)-associated glomerulonephritis.



A-C show examples of normal glomeruli; D-G show examples of cellular crescents; H-J show examples of fibrous crescents; and K shows an example of global glomerulosclerosis. Reproduced from Berden et al.[88] with permission.

Figure 5. The classification flowchart for the histopathological classification of ANCA-associated glomerulonephritis.



Reproduced from Berden et al.[88] with permission.

The study by Berden *et al.* wherein the classification system was proposed incorporated a validation study including 100 patients.[88] Multiple regression analysis showed that baseline renal function and the histopathological classification were the only independent predictors of renal outcome. The histopathological classification of ANCA-associated glomerulonephritis has been validated in various cohorts.[89-109] The outcomes of these validation studies, points of consideration, and future perspectives are discussed in **Chapter 4** of this thesis.

Treatment of ANCA-associated vasculitis

Early diagnosis of ANCA-associated vasculitis is of major importance as prompt instigation of treatment is essential to prevent progressive organ damage, particularly end-stage renal disease, and death.[79] In the last 20 years, a significant number of large, international trials were performed with the aim of improving the treatment modalities in ANCA-associated vasculitis.[14-17, 27, 28, 110-112]

Treatment of ANCA-associated vasculitis traditionally consists of two phases.[113] Phase I consists of aggressive remission-inducing immunosuppression classically consisting of cyclophosphamide and corticosteroids for the duration of three to six months. Phase II consists of remission maintenance treatment with,

amongst others, azathioprine or methotrexate while corticosteroids are tapered and if possible stopped.[114] The choice of treatment depends on disease severity and organ manifestations. Recently, rituximab was introduced for both induction and remission maintenance treatment in ANCA-associated vasculitis.[27, 28, 115]

Induction treatment

Induction treatment of ANCA-associated vasculitis consists of cyclophosphamide treatment until complete clinical remission is achieved, which is often after three to six months. The CYCLOPS trial demonstrated that, while achieving similar remission rates, intravenous pulsed cyclophosphamide treatment results in a lower cumulative cyclophosphamide dose than oral cyclophosphamide treatment.[15] However, long-term analysis of this cohort demonstrated a higher relapse risk in the intravenous group than in the oral group.[116] The increased frequency of relapses in the intravenous group was not associated with increased mortality or renal damage.

Two randomised controlled trials, RITUXVAS[27] and RAVE[28], investigated rituximab – a chimeric monoclonal antibody directed against pre-B cells and mature B lymphocytes – in the treatment of ANCA-associated vasculitis. RITUXVAS included only newly diagnosed patients, whereas in RAVE both new and relapsing patients were included. In both trials, rituximab was non-inferior to cyclophosphamide for remission induction. Moreover, both trials demonstrated no significant difference between the number of adverse events in the rituximab group and the cyclophosphamide group. However, concerns were raised about a possible higher malignancy rate in patients treated with rituximab.[117, 118] **Chapter 5** of this thesis reports the first 10-year follow-up study investigating malignancy risk in patients with ANCA-associated vasculitis treated with cyclophosphamide according to current treatment regimens. **Chapter 6** reports the first study to compare the long-term malignancy risks between rituximab-based treatment and cyclophosphamide-based treatment in ANCA-associated vasculitis.

The NORAM trial investigated methotrexate induction treatment in patients with ANCA-associated vasculitis with early systemic disease without significant renal involvement.[14] Methotrexate was demonstrated to have similar efficacy to cyclophosphamide for remission induction, but was associated with a higher relapse rate. Long-term follow-up of these patients demonstrated that methotrexate treatment is associated with less effective disease control and prolonged use of steroids.[119]

Evidence from a retrospective study[120] and a prospective pilot trial[121] indicates that mycophenolate mofetil is effective in the induction treatment of ANCA-associated vasculitis. An advantage of mycophenolate mofetil over cyclophosphamide includes a more favourable safety profile in terms of toxicity. The MYCYC trial compares mycophenolate mofetil to cyclophosphamide

for induction treatment in ANCA-associated vasculitis (ClinicalTrials.gov NCT00414128). The MYCYC trial results are expected in 2018.

Maintenance treatment

Maintenance treatment aims at the prevention of relapses. The landmark EUVAS trial CYCAZAREM demonstrated that cyclophosphamide exposure in patients with ANCA-associated vasculitis can be safely reduced by the substitution of cyclophosphamide by azathioprine shortly after remission achievement.[16] Relapse risk and severe adverse event occurrence were similar between the cyclophosphamide group and the azathioprine group. Currently, azathioprine, alongside with methotrexate, is the first choice for maintenance treatment in ANCA-associated vasculitis.[122]

Methotrexate can be used as an alternative to azathioprine for remission maintenance in patients with ANCA-associated vasculitis in whom renal function is not severely impaired. The WEGENT trial demonstrated similar remission maintenance and adverse events rates between methotrexate and azathioprine maintenance treatment.[114]

Rituximab was recently introduced for remission maintenance treatment in ANCA-associated vasculitis patients who relapsed on other maintenance therapies or who are at high risk of relapse. The MAINRITSAN trial demonstrated a superiority of rituximab over azathioprine as maintenance treatment after cyclophosphamide induction treatment.[115] Adverse events rates were similar in the rituximab group and the azathioprine group. The ongoing RITAZERAM trial compares rituximab to azathioprine as maintenance treatment in relapsing patients who achieved remission following rituximab induction treatment (ClinicalTrials.gov NCT01697267).

The IMPROVE trial demonstrated that mycophenolate mofetil is less effective than azathioprine in remission maintenance after remission induction with cyclophosphamide.[111] Therefore, mycophenolate mofetil maintenance treatment is only considered in ANCA-associated vasculitis patients in whom azathioprine and methotrexate are contraindicated.

Prognosis of patients with ANCA-associated vasculitis

As stated previously, the natural history of untreated ANCA-associated vasculitis is that of a rapidly progressive, usually fatal disease.[79] The introduction of immunosuppressive therapy in the 1960s has dramatically reduced the 1-year mortality rate of patients with ANCA-associated vasculitis from 82% to 10%.[32, 79, 123] Nevertheless, patients continue to have an increased mortality risk compared to the general population.[123]

A large, prospective 5-year follow-up study that included 535 patients demonstrated that ANCA-associated vasculitis patients have a 2.6 times increased mortality risk compared to the general population.[123] Infection (48%) and active vasculitis (19%) were the main causes of death during the first year after diagnosis. This finding emphasizes the importance of finding the right balance between disease control and immunosuppressive treatment. After the first year of diagnosis, patients continued to have a 1.3 times increased mortality risk.[123] Cardiovascular disease (26%), malignancy (22%), and infection (20%) accounted for the majority of these deaths.

About 14% of patients will experience at least one major cardiovascular event within 5 years after ANCA-associated vasculitis diagnosis.[124] Patients with GPA are reported to have a 3.6 times increased myocardial infarction risk compared to the general population.[125] In addition, when matched for renal function and other traditional risk factors, cardiovascular risk is still doubled in patients with ANCA-associated vasculitis.[126] PR3-ANCA positivity, older age, and diastolic hypertension were shown to be independent determinants of poor cardiovascular outcome.[124] Factors contributing to the increased cardiovascular risk in vasculitis include the chronic inflammatory state, endothelial dysfunction, renal dysfunction, and the use of corticosteroids, which accelerates the development of hypertension, dyslipidaemia, and diabetes.[127, 128]

Malignancies were demonstrated to be the second leading cause of death after the first year of ANCA-associated vasculitis diagnosis.[123] A number of studies, using data from retrospective monocentre cohorts,[76, 129-131] prospective multicentre clinical trials,[132, 133] and nationwide registry linkage,[134] demonstrated that patients with ANCA-associated vasculitis have an increased malignancy risk compared to the general population.[135] In particular, the risks of leukaemia, lymphoma, bladder cancer, and non-melanoma skin cancer (NMSC) were increased. Except for one 7-year follow-up study published in 1992,[76] average follow-up in these studies was at most five years[129-132]. Moreover, the observation period in most studies was 1960-1990. Thus, the patients included in these studies were treated with treatment regimens that are outdated and, consequently, the findings of these studies do not represent current malignancy risks.[76, 129, 131, 134] As explained previously, treatment regimens in ANCA-associated vasculitis have changed significantly in recent years based on efforts to reduce cumulative cyclophosphamide exposure.[14-17] Moreover, rituximab has emerged as a promising substitute for cyclophosphamide in the treatment of ANCA-associated vasculitis.[27, 28, 136, 137] **Chapter 5** of this thesis reports the first 10-year follow-up study that investigates malignancy risk in ANCA-associated vasculitis patients treated with cyclophosphamide according to current treatment regimens. **Chapter 6** reports the first study to compare the long-term malignancy risks between rituximab-based treatment and cyclophosphamide-based treatment in ANCA-associated vasculitis.

Several factors have been hypothesized to contribute to the increased malignancy risk in patients with ANCA-associated vasculitis. Firstly, immunosuppressive treatment may decrease the immune system's ability to recognize and eradicate malignant cells. The importance of a well-functioning immune system in the prevention of malignancies is well-demonstrated by the increased malignancy risk observed in HIV-positive patients.[138] Moreover, immunosuppressive therapy itself may have direct mutagenic properties, as, for example, demonstrated in cyclophosphamide-induced bladder cancer.[139-143] Furthermore, long-standing immune activation per se may be oncogenic; for example, long-standing immune activation is hypothesized to contribute to the increased lymphoma risk seen in a number of chronic autoimmune rheumatic conditions.[144]

The European Vasculitis Society and the European Vasculitis Genetics Consortium

Research in ANCA-associated vasculitis is hindered by the low incidence of this complex disease. Therefore, in 1994 the EUVAS was founded with the objective of uniting vasculitis researchers and clinicians and promoting the study of vasculitis. The apparent need for clinical trials was demonstrated by the wide heterogeneity in treatment, poor patient outcomes, and high levels of treatment-related toxicity.[122] Thus far, the EUVAS has conducted a significant number of clinical trials with the aim of optimizing the treatment of ANCA-associated vasculitis.[14-17, 27, 110, 111, 145] Long-term follow-up studies of the first four EUVAS trials (CYCAZAREM,[16] NORAM,[14] MEPEX,[17] and CYCLOPS[15]) have been published and longer follow-up is pending. The aim of these studies was to investigate several long-term outcomes in ANCA-associated vasculitis, including long-term patient survival,[123] disease relapse,[146] cardiovascular events,[124] malignancies,[132] disease-related damage,[147-149] and severe adverse events[150]. To harmonize the clinical trials, scoring systems were created for both presenting clinical manifestations[151-153] and renal histology[154]. Other studies conducted by the EUVAS include ANCA assay standardization studies[18, 155, 156] and renal histopathological studies[84, 85, 88, 157-162]. The study presented in **Chapter 3** of this thesis was performed within the collaboration of the EUVAS. The European Vasculitis Genetics Consortium has grown out of EUVAS activity and focuses on the genetics of ANCA-associated vasculitis. In 2010, the European Vasculitis Genetic Consortium published the first GWAS in ANCA-associated vasculitis.[26] The study presented in **Chapter 2** of this thesis was performed within the collaboration of the European Vasculitis Genetic Consortium.

Outline of this thesis

The role of genetic variants in ANCA-associated vasculitis is explored in **Chapter 2** of this thesis. The numerous candidate gene association studies and two GWAS have revealed a great number of genetic variants that could contribute to the pathogenesis of ANCA-associated vasculitis. In **Chapter 2** we report the results of a meta-analysis investigating the genetic variants that are most likely associated with ANCA-associated vasculitis. We included raw data from the European Vasculitis Genetic Consortium GWAS to increase the validity of the meta-analysis.[26] Moreover, in the light of the ongoing debate as to whether the different subtypes within ANCA-associated vasculitis represent different entities within the same disease spectrum or are distinct auto-immune diseases, we investigated whether these subtypes have distinct genetic backgrounds. In **Chapter 3** we investigated whether ENT involvement represents a separate phenotype in ANCA-associated vasculitis by exploring the relationship between ENT manifestations and renal outcome.

As discussed previously, renal disease is a common and severe manifestation of ANCA-associated vasculitis that can lead to end-stage renal disease and death. **Chapter 4** reviews the outcomes of studies validating the histopathological classification of ANCA-associated glomerulonephritis and discusses points of consideration and future perspectives.

The prognosis of patients with ANCA-associated vasculitis has improved dramatically in recent years, shifting attention towards the long-term complications these patients experience. In **Chapter 5** the malignancy risk in patients with ANCA-associated vasculitis treated with current immunosuppressive regimens was investigated and its relationship with cyclophosphamide treatment was explored. Recently, rituximab was introduced for both induction and remission maintenance treatment in ANCA-associated vasculitis with the promise of, amongst others, further reducing malignancy risk. **Chapter 6** reports the first study to date to compare the long-term malignancy risks between rituximab-based treatment and cyclophosphamide-based treatment in ANCA-associated vasculitis.

The findings of this thesis will be summarized and placed in a more general perspective in **Chapter 7**.

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Chapter II

Genetic variants in ANCA-associated vasculitis: a meta-analysis

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Abstract

Background

Genetic factors may influence the pathogenic pathways leading to antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). We performed a meta-analysis to determine the genetic variants most likely associated with AAV and investigated whether diagnostic and serological subtypes within AAV have distinct genetic backgrounds.

Methods

Studies investigating the association between genetic variants and AAV in humans were searched in PubMed, EMBASE and Web of Science. All variants investigated in at least two studies were selected. Subsequently, all studies assessing these variants were included in this meta-analysis. Additionally, data on these variants from the largest genome-wide association studies in AAV were included to increase the validity of this meta-analysis.

Results

The literature search yielded 5180 articles. 62 articles investigating 140 genetic variants were included, 33 of which were associated with AAV in a meta-analysis. These genetic variants were in or near the following genes: *CD226*, *CTLA-4*, *FCGR2A, HLA-B*, *HLA-DP*, *HLA-DQ*, *HLA-DR*, *HSD17B8*, *IRF5*, *PTPN22*, *RING1/RXRB*, *RXRB*, *STAT4, SERPINA1* and *TLR9*. Moreover, we identified genetic distinctions between granulomatosis with polyangiitis and microscopic polyangiitis and between proteinase 3 ANCA vasculitis and myeloperoxidase ANCA vasculitis. In 76% of the genetic variants, subdivision based on ANCA serotype resulted in higher ORs than subdivision based on clinical diagnosis.

Conclusions

This meta-analysis identified 33 genetic variants associated with AAV, supporting a role for alpha-1-antitrypsin, the major histocompatibility complex system, and several distinct inflammatory processes in AAV pathogenesis. Our results indicate that subdivision of AAV based on ANCA serotype has a stronger genetic basis than subdivision based on clinical diagnosis.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic autoimmune disease in which patients often have circulating proteinase 3 (PR3)-ANCA or myeloperoxidase (MPO)-ANCA.[1,2] The clinical syndromes within the spectrum of AAV are granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis.

The subtypes within AAV show significant differences in clinical and epidemiological characteristics,[3,4] leading to debate regarding whether these subtypes are part of a single disease spectrum or represent distinct diseases.[5–7] To date, the prevailing concept of a single disease spectrum has resulted in similar treatment strategies in clinical trials[8–10] and to suggestions that genetic studies should consider the subtypes together.[11] Evidence that these subtypes are pathogenically distinct may lead to the development of syndrome-specific therapeutic strategies.

Both environmental and genetic factors are thought to be involved in the pathogenesis of AAV.[12] Evidence for the role of genetic factors comes from differences in the prevalence of AAV between ethnic groups,[13] familial association studies[14] and genetic associations studies including the two genome-wide association studies (GWAS) performed in AAV.[15,16]

Attempts to replicate findings of genetic association studies performed in AAV have yielded inconsistent outcomes. Small sample sizes and false-positive results arising from the low prior probabilities of genetic associations may be responsible for these inconsistencies.[17] These factors are especially relevant in complex diseases such as AAV.[18]

The aim of this study was to determine the genetic variants associated with AAV. Therefore, we performed a meta-analysis to assess the pooled effect of all genetic variants that have been investigated in AAV in at least two studies. To increase the validity of this meta-analysis, we also included previously unpublished data from the largest GWAS performed in AAV.[15] Moreover, we conducted stratified analyses based on clinical diagnosis and ANCA serotype to investigate whether these different AAV subtypes have distinct genetic backgrounds.

Methods

Literature search and eligibility

A comprehensive search string was carried out in collaboration with a librarian. PubMed, EMBASE and Web of Science were searched until April 2014 for studies investigating genetic variants in patients with AAV. The search strategy consisted of multiple queries combining 'Anti-Neutrophil Cytoplasmic Antibody-Associated

Vasculitis', 'ANCA', 'vasculitis', 'Granulomatosis with Polyangiitis', 'Wegener's Granulomatosis', 'Microscopic polyangiitis', 'Eosinophilic granulomatosis with polyangiitis', 'Churg Strauss Syndrome', 'PR3', 'MPO', 'Polymorphisms' or 'Genes'. To minimise the chance of omitting references, a second broader search was performed for genetic variants in vasculitis in general rather than AAV alone (supplementary table S1). The specific genes and polymorphisms that resulted from the previous searches were added in a next search to minimise the chance of omitting references. To ensure maximum sensitivity, no limits, filters or language restrictions were placed on the searches.

Two observers (CR and DvH) independently reviewed the titles and abstracts of the citations retrieved by the search and read potentially relevant studies independently. Studies that compared genetic variants between patients with AAV and controls without AAV derived from the general population were eligible. Cases had to have an AAV by fulfilling either the Chapel Hill Consensus Conference criteria,[19] American College of Rheumatology criteria,[20] European Medicines Agency algorithm,[21] 1998 Japanese criteria proposed by the Research Committee on Intractable Vasculitides, the Ministry of Health, Labour and Welfare of Japan,[22] or clinical, histological and serological criteria. Definitions of cases and controls in each included study are depicted in supplementary table S2. All genetic variants investigated in at least two studies were included. Genetic variants investigated in multiple cohorts in one publication were also included. This was the case for genetic variants in *GHSR*,[23] *LEPR*[23] and *TLR9*.[24] For the included genetic variants, all genetic studies were identified to estimate the pooled effect of the genetic variant in a meta-analysis, irrespective of their p values. To increase the validity of this meta-analysis, we also included unpublished data on these genetic variants from the Lyons *et al*[15] GWAS. This was possible for the genetic variants in this meta-analysis that were not human leucocyte antigen (HLA) serotypes or tandem repeats and that were genotyped in the Lyons *et al* GWAS. Moreover, we included data from the stage 1 analysis including all single-nucleotide polymorphisms (SNPs) with a p value $<10^{-4}$ from the Xie *et al*[16] GWAS.

Data extraction

Minor allele frequencies of the included genetic variants were extracted from included studies. Studies investigating the same genetic variant published by the same author(s) were checked for overlapping patient groups, in which case only the study with the largest patient group was included. Studies that reported insufficient data to calculate an OR were excluded.

Statistical analysis

ORs and 95% CIs were calculated at the allele level. To account for potential heterogeneity, random-effects model was performed in all analyses that included

at least five studies.[25] Because the HLA serotypes are not completely independent from the HLA alleles, the following HLA variants were collapsed in the analyses: *HLA-DR1*, *HLA-DRB1*01* and *HLA-DRB1*0101*; *HLA-DR3* and *HLA- DRB1*03*; *HLA-DR7* and *HLA-DRB1*07*; *HLA-DR8* and *HLA-DRB1*08*; and *HLA-DR9*, *HLA-DRB1*09* and *HLA-DRB1*0901*. Collapsing other HLA variants was not possible because some studies investigated multiple, closely related, HLA variants in the same patients. If these HLA variants had been collapsed, the same patients would have been included in the same analysis multiple times, increasing their weight in the analysis and introducing a systematic error. To determine whether the disease subtypes within AAV represent parts of a single disease spectrum or distinct clinical entities, we performed pre-specified subgroup analysis stratifying patients according to clinical diagnosis (GPA/MPA) and ANCA serotype (PR3-ANCA/MPO-ANCA and cytoplasmic (c)-ANCA/perinuclear (p)-ANCA), if enough data were available. Moreover, we performed stratified analyses based on ethnicity for all genetic variants investigated in both Caucasian and Asian patients. We assumed difference in effect estimates likely to be present in case subgroups showed significant effects in opposite directions, that is, having a protective effect in one subgroup but leading to an increased risk of AAV in the other subgroup. Heterogeneity within studies was displayed by I^2, which reflects the percentage of total variation across studies beyond chance.[26] Egger and Harbord tests were used to assess publication bias.[27] All of the p values presented are nominal p values and are not corrected for multiple testing. Correction for multiple testing was not performed as the strong linkage disequilibrium between variants tested in the major histocompatibility complex (MHC) region makes calculating an appropriate multiple correction factor impractical. All analyses were performed using STATA V.12 (StataCorp. 2011; Stata Statistical Software: Release 12, College Station, Texas, USA).

Results

Initial search and results

Our literature search yielded 5180 articles, from which we identified 140 genetic variants published in 62 articles. All included studies were case–control studies, two of which were GWAS. These articles were published from 1978 through 2014, and the number of patients with AAV included ranged from 12 to 1445. The characteristics of all included studies are provided in supplementary table S2. Additionally, previously unpublished data from the Lyons *et al*[15] GWAS were available and included in the meta-analysis for 18 genetic variants: *CD226* rs763361, *CTLA-4* rs231775, *CTLA-4* rs3087243, *FCAR* rs16986050, *FCGR2A*rs1801274, *GHSR* rs509035, *HLA-DPB2* rs3130215, *IL1*β rs1143634, *IL6* rs1800795, *IL10* rs1800896, *IRF5* rs10954213, *PTPN22* rs2476601, *RING1/RXRB*

rs213213, *RXRB* rs6531, *RXRB* rs9277935, *STAT4* rs7574865, *SERPINA1* Z allele and *TNF*αrs1800629.

Thirty-three genetic variants were significantly associated with AAV after meta-analysis (table 1 and supplementary figure S1), and 107 genetic variants were not associated with AAV after meta-analysis (supplementary table S3). The ORs for the significant associations after meta-analysis ranged from 0.35 to 0.81 for protective genetic variants and from 1.13 to 2.94 for the genetic variants associated with an increased risk of AAV.

AAV is associated with the Z and S alleles of SERPINA1

Both the S allele and Z allele of *SERPINA1* were significantly associated with AAV, with pooled ORs of 1.30 (95% CI 1.03 to 1.63) and 2.94 (95% CI 2.22 to 3.88), respectively. Subgroup analysis showed that the association with the *SERPINA1* Z allele was present in both PR3-ANCA (pooled OR 2.58 (95% CI 1.57 to 4.25)) and MPO-ANCA (pooled OR 2.01 (95% CI 1.04 to 3.87)) positive patients and in both c-ANCA (pooled OR 3.53 (95% CI 2.28 to 5.49)) and p-ANCA (pooled OR 3.13 (95% CI 1.21 to 8.13)) positive patients (supplementary table S4 and figure S2).

AAV is associated with genetic variants in the MHC region

Seventeen genetic variants in *HLA-B*, *HLA-DP*, *HLA-DQ* and *HLA-DR* remained significantly associated with AAV after meta-analysis (table 1). *HLA-DPA1* rs9277341 had the strongest protective effect (pooled OR 0.35 (95% CI 0.30 to 0.40)), and *HLA-DPB1*0401*was the strongest contributor to an increased risk of AAV (pooled OR 1.99 (95% CI 1.44 to 2.74)). *RING1/RXRB* rs213213, *RXRB* rs6531 and *RXRB* rs9277935 were also significantly associated with AAV with pooled ORs of 1.71 (95% CI 1.57 to 1.86), 1.63 (95% CI 1.50 to 1.77) and 0.44 (95% CI 0.37 to 0.50), respectively.

AAV is associated with genetic variants involved in inflammatory processes

CTLA-4 rs231775 was associated with an increased risk of AAV (pooled OR of 1.16 (95% CI 1.06 to 1.28)) while *CTLA-4* rs3087243 and *CTLA-4* $(AT)_{86}$ had a protective effect (pooled ORs of 0.81 (95% CI 0.75 to 0.87) and 0.54 (95% CI 0.43 to 0.67), respectively). *PTPN22* rs2476601, *CD226* rs763361 and *IRF5* rs10954213 were also significantly associated with AAV, with pooled ORs of 1.39 (95% CI 1.24 to 1.56), 1.14 (95% CI 1.07 to 1.21) and 0.77 (95% CI 0.70 to 0.83), respectively. Moreover, *TLR9* rs352162 and rs352140 were significantly associated with AAV with pooled ORs of 1.58 (95% CI 1.43 to 1.75) and 1.13 (95% CI 1.02 to 1.25), respectively.

Table 1. Genetic variants significantly associated with AAV after meta-analysis

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry[a]
CD226 rs763361 (T)	3	2422/17898	1.14 (1.07 – 1.21)	<0.001	0	0.444	0.792
CTLA-4 $(AT)_{86}$	4	303/543	0.54 (0.43 – 0.67)	<0.001	89	<0.001	0.946
CTLA-4 rs231775 (G)	3	1002/6179	1.16 (1.06 – 1.28)	0.002	60	0.080	0.080
CTLA-4 rs3087243 (A)	3	2015/7855	0.81 (0.75 – 0.87)	<0.001	25	0.262	0.122
FCGR2A rs1801274 (C)	6	1239/6209	0.90 (0.82 – 0.99)	0.028	0	0.834	0.788
HLA-B5	2	335/6573	0.59 (0.38 – 0.92)	0.019	0	0.432	N/A
HLA-B8	6	475/7855	1.48 (1.04 – 2.11)	0.028	47	0.096	0.063
HLA-DPA1 rs9277341 (C)	2	1032/2200	0.35 (0.30 – 0.40)	<0.001	54	0.116	0.215
*HLA-DPB1*0301*	5	1154/1337	0.38 (0.21 – 0.69)	0.002	78	<0.001	0.938
*HLA-DPB1*0401*	5	1154/1337	1.99 (1.44 – 2.74)	<0.001	84	<0.001	0.738
HLA-DPB2 rs3130215 (A)	3	1417/7249	1.40 (1.29 – 1.52)	<0.001	99	<0.001	0.446
*HLA-DQB1*0303*	3	176/218	1.82 (1.09 – 3.03)	0.021	17	0.301	0.916
HLA-DR6	5	487/6222	0.50 (0.27 – 0.95)	0.033	55	0.062	0.997
*HLA-DRB1*1101*	2	268/465	1.89 (1.15 – 3.08)	0.011	0	0.487	N/A
*HLA-DRB1*1201*	2	216/465	0.37 (0.15 – 0.91)	0.031	0	0.491	N/A
*HLA-DRB1*13*	4	233/833	0.47 (0.32 – 0.70)	<0.001	0	0.504	0.884
*HLA-DRB1*14*	4	322/862	1.91 (1.07 – 3.42)	0.029	0	0.728	0.700
*HLA-DRB1*15*	3	236/633	1.86 (1.39 – 2.50)	<0.001	69	0.021	0.347
*HLA-DRB1*1501*	2	216/465	1.68 (1.20 – 2.34)	0.002	0	0.925	N/A
HLA-DRB3	4	260/1845	0.62 (0.49 – 0.79)	<0.001	68	0.024	0.689
HLA-DRB4	4	260/1845	1.69 (1.36 – 2.10)	<0.001	61	0.055	0.533

Table 1. Genetic variants significantly associated with AAV after meta-analysis (Continued)

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry[a]
HSD17B8 rs421446 (C)	2	738/1872	0.40 (0.34 – 0.48)	<0.001	0	0.620	N/A
IRF5 rs10954213 (G)	3	1535/6977	0.77 (0.70 – 0.83)	<0.001	99	<0.001	0.948
PTPN22 rs2476601 (A)	4	2099/8678	1.39 (1.24 – 1.56)	<0.001	0	0.693	0.500
RING1/RXRB rs213213 (A)	3	1414/7238	1.71 (1.57 – 1.86)	<0.001	73	0.026	0.187
RXRB rs6531 (C)	3	1557/6955	1.63 (1.50 – 1.77)	<0.001	96	<0.001	0.292
RXRB rs9277935 (T)	3	1417/7233	0.44 (0.37 – 0.50)	<0.001	73	0.025	0.393
SERPINA1 S allele	5	1474/5762	1.30 (1.03 – 1.63)	0.025	0	0.464	0.547
SERPINA1 Z allele	8	3662/8581	2.94 (2.22 – 3.88)	<0.001	41	0.092	0.078
STAT4 rs7574865 (T)	3	1520/6956	1.11 (1.01 – 1.22)	0.029	3	0.357	0.590
TLR9 rs352162 (T)	1	1289/1898	1.58 (1.43 – 1.75)	<0.001	96	<0.001	N/A
TLR9 rs352140 (T)	1	1289/1898	1.13 (1.02 – 1.25)	0.018	0	0.432	N/A
TLR9 rs352139 (T)	1	1289/1898	1.11 (1.00 – 1.23)	0.041	0	0.756	N/A

[a] Harbord test for funnel plot asymmetry was performed for all genetic variants, except for *CD226* rs763361, *CTLA-4* rs3087243, and *PTPN22* rs2476601. In these cases the Harbold test was not applicable and the Egger test was performed.

Genetic associations differ for the different diagnostic and serological subtypes of AAV

A significant association with GPA and /or MPA was present for 25 genetic variants, and a significant association with both GPA and MPA was present for ten genetic variants (supplementary table S4 and figure S2). In six of these ten genetic variants (60%), the associations were in opposite directions, that is, having a protective effect in one subgroup but leading to an increased risk of AAV in the other subgroup (figure 1). A significant association with PR3-ANCA and/or MPO-ANCA was present for 25 genetic variants, and a genetic association with both PR3-ANCA and MPO-ANCA was present for seven genetic variants. In four of these seven genetic variants (57%), the associations were in opposite directions, that is, having a protective effect in one subgroup but leading to an increased risk of AAV in the other subgroup (figure 1). Moreover, ORs were higher for ANCA serotype than for clinical diagnosis in 76% (16/21) of the genetic variants that were significantly associated with both clinical diagnosis and ANCA serotype.

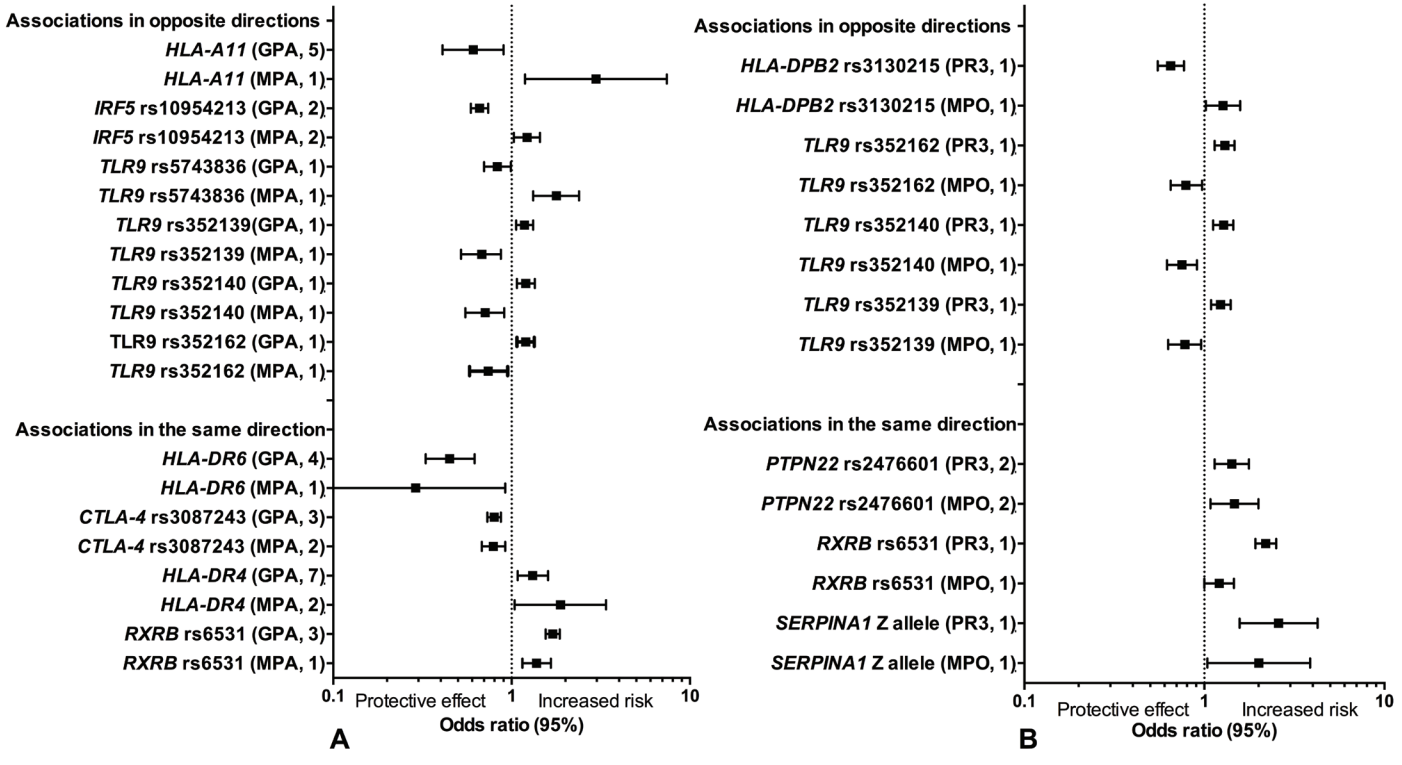
Genetic associations differ for AAV patients of Caucasian and Asian origin

The results of the stratified analyses performed for 42 variants investigated in both Caucasian and Asian patients are depicted in supplementary table S5. CTLA-4 $(AT)_{86}$, CTLA-4 $(AT)_{106}$ and *HLA-DR6* were significantly associated with AAV in the Caucasian patients but not in the Asian patients. Conversely, *HLA-B55* was significantly associated with AAV in the Asian patients but not in the caucasian patients. Of interest, *IRF5* rs10954213 (G) was significantly associated with AAV in both the Caucasian and Asian patients; however, it had a protective effect in the Caucasian patients while it increased the risk of AAV in the Asian patients. The results of these analyses should be interpreted with caution, because each analysis included only one study involving Asian patients.

Genetic variants identified by GWAS

To date, two GWAS have been performed in AAV. The first GWAS included patients with GPA and MPA and found *HLA-DP* rs3117242, *COL11A2* rs3130233, *COL11A2* rs3117016 and *SERPINA1* rs7151526 to be associated with AAV.[15] Moreover, *HLA-DP* rs3117242, *ARHGAP18* rs1705767 and *SERPINA1* rs7151526 were associated with PR3-ANCA vasculitis, and HLA-*DQ* rs5000634 was associated with MPO-ANCA vasculitis. The second GWAS included only patients with GPA and found *HLA-DPB1* rs9277554 and *HL-DPA1* rs9277341 to be associated with GPA.[16] *SEMA6A* rs26595 was associated with GPA at a genome-wide significance level when the results of the two cohorts that were included in this GWAS were combined. However, although *SEMA6A* rs26595 was not genotyped in the first GWAS,[15] data for a large number of proxy SNPs

Figure 1. Subgroup analysis based on clinical diagnosis (A) and antineutrophil cytoplasmic antibody (ANCA) serotype (B), with the clinical diagnosis (A) or ANCA serotype (B) and number of included publications depicted between the parentheses.



(A) In 6 of the 10 genetic variants (60%) in which there was an association with both granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA), the associations were in opposite directions. (B) In four of the seven genetic variants (57%) in which there was an association with both proteinase 3 (PR3)-ANCA vasculitis and myeloperoxidase (MPO)-ANCA vasculitis, the associations were in opposite directions.

across this locus were available, and these did not reach statistical significance (data not shown).

Discussion

This meta-analysis identified 33 genetic variants, in or near 15 genes, associated with AAV. Twenty of these 33 genetic variants were present in the MHC region. This study provides the first complete and comprehensive overview including all genetic variants investigated in AAV in at least two studies. Genetic variants in or near *CD226*, *CTLA-4*, *FCGR2A*, *HLA-B*, *HLA-DP*, *HLA-DQ*, *HLA-DR*, *HSD17B8*, *IRF5*, *PTPN22*, *RING1/RXRB*, *RXRB*, *STAT4*, *SERPINA1* and *TLR9* were associated with AAV in this meta-analysis. Of interest, *FCGR2A* and *STAT4* were not associated with AAV in the individual studies, but were significantly associated with AAV after meta-analysis. Moreover, we showed genetic distinctions between the clinical diagnoses GPA and MPA and between the ANCA serotypes PR3-ANCA and MPO-ANCA. Additionally, our results confirm that subdivision of AAV based on ANCA serotype has a stronger genetic basis than subdivision based on clinical diagnosis.

A number of the genetic variants associated with AAV in this meta-analysis have also been associated with other autoimmune diseases, such as *CTLA-4* rs3087243 in rheumatoid arthritis[28,29] and type 1 diabetes[30,31] and *PTPN22* rs2476601 in Crohn's disease,[32–34] Behçet's disease,[35] systemic lupus erythematosus[36–38] and giant cell arteritis.[39,40] These findings are in line with the fact that first-degree relatives of patients with AAV have an increased risk of other autoimmune diseases.[41, 42] Overlapping genetic variants may form the basis of a disturbed immune system, and together with environmental factors and other, more distinct genetic factors, form a 'bad hand of cards' that leads to the development of AAV.

The results of this meta-analysis support a role for the intricate relationship among alpha-1-antitrypsin and ANCA, the MHC system and other inflammatory processes in the pathogenesis of AAV. The association between *SERPINA1* and AAV supports the concept that ANCA are important in AAV pathogenesis. Alpha-1-antitrypsin is coded by *SERPINA1* and is a major inhibitor of PR3. It has been hypothesised that lower levels of alpha-1-antitrypsin, resulting from the presence of the Z and S alleles of *SERPINA1*, lead to increased levels of circulating PR3 and possibly trigger the synthesis of anti-PR3-ANCA.[43] This hypothesis implies that the association of AAV with alpha-1-antitrypsin deficiency is restricted to PR3-ANCA positive patients; however, in this meta-analysis, the association between the Z allele of *SERPINA1* was present in both PR3-ANCA-positive and MPO-ANCA-positive patients and in both c-ANCA-positive and p-ANCA-positive patients. Another hypothesis is that patients with AAV and alpha-1-antitrypsin

deficiency have a reduced ability to bind PR3 released by previously activated neutrophils, thus promoting PR3-mediated proteolytic vessel damage.

As noted, 20 genetic variants in the MHC region were associated with AAV in this meta-analysis. We, therefore, confirm an important role for the MHC region, but because of linkage disequilibrium, were unable to determine the nature of this association, that is, whether it represents single or multiple independent associations. Both GWAS showed that the SNP association signal in the MHC region was fully accounted for by *HLA-DPB1*, dramatically diminishing the associations of other SNPs in this region.[15, 16] The results of our meta-analysis also support a role for other inflammatory processes in the pathogenesis of AAV, with the associations with *CTLA-4* and *PTPN22* suggesting a role for a threshold of activation or suppression of T cells.

The different subtypes generally grouped under the umbrella term AAV have profound differences in ANCA specificities[3] and clinical outcomes.[4] The results of this meta-analysis indicate that these different AAV subtypes also have distinct genetic backgrounds, as previously shown in a GWAS.[15] Moreover, we found significant associations in opposite directions for the different AAV subgroups. Significant associations of the same SNP in opposite directions for different types of autoimmune diseases have been described before and could be indicative of different mechanisms of disease.[44] Larger studies are required to investigate this issue further in AAV.

The subdivision of AAV based on ANCA serotype had the stronger genetic basis in our meta-analysis; in 76% of the genetic variants, subdivision based on ANCA serotype resulted in higher ORs than subdivision based on clinical diagnosis. The results of these analyses should, however, be interpreted with caution because of the limited number of studies included in some of the analyses and need to be validated in other studies. Although until now the concept of a single disease spectrum has resulted in similar treatment strategies in patients with AAV, our limited results suggest that syndrome-specific therapeutics based on ANCA serotype strategies may be considered.

Our study has some limitations. First, in some of our analyses, the number of subjects or studies was limited; this limitation was especially the case in the subgroup analyses. Second, publication bias is an issue of concern in all meta-analyses. Authors might omit non-significant genetic associations and report only those associations that reach statistical significance. However, none of the tests performed to assess funnel plot asymmetry in this meta-analysis were significant. Furthermore, the studies included in this meta-analysis show heterogeneity with respect to clinical diagnosis, ANCA serotype, disease characteristics, ethnicity and study design. The clinical heterogeneity was accompanied by statistical heterogeneity for 16 of the 140 included genetic variants. However, there is no fully accepted statistical measure that precisely determines clinical heterogeneity.[27] To account for heterogeneity, random-effects models were

performed where possible.[25] Nevertheless, estimates reported in this study should be interpreted with caution, especially when statistical heterogeneity was present or when a small number of studies and/or relatively small groups of participants were included. Finally, it should be kept in mind that the genetic associations identified do not imply causality. While they provide insight into pathogenicity and suggest the involvement of certain pathways, these may not represent therapeutic targets.

In summary, this meta-analysis identified 33 genetic variants, in or near 15 genes, associated with AAV. Moreover, we showed genetic distinctions among the different AAV subtypes, supporting the concept that these subtypes may represent distinct autoimmune syndromes. These subtypes are most likely driven by ANCA serotype and not by clinical diagnosis.

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Chapter III

Renal function and ear, nose, throat involvement in anti-neutrophil cytoplasmic antibody-associated vasculitis: prospective data from the European Vasculitis Society clinical trials.

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Abstract

Objective

We investigated whether ENT involvement is associated with renal biopsy findings and renal function in patients with ANCA-associated vasculitis (AAV).

Methods

Newly diagnosed AAV patients derived from three international, multicentre trials were included. To investigate an association between ENT involvement and estimated glomerular filtration rate (eGFR) at diagnosis and 5-year follow-up, we performed multivariable regression analyses including clinical and histopathological parameters. To investigate whether our findings are specific to ENT involvement, we performed comparable analyses between eGFR and other early disease manifestations (arthralgia/arthritis, cutaneous and lung involvement).

Results

One hundred and eighty-five of the 414 patients had ENT involvement. The mean presenting eGFR of patients with and without ENT involvement was 39.16 and 23.88 ml/min/1.73m^2, respectively ($P<0.001$). Mean eGFR increased by 6.76 ml/min/1.73m^2 with each added ENT symptom ($P = 0.007$). Patients with ENT involvement had less interstitial fibrosis and tubular atrophy and a prognostically more favourable histopathological class on renal biopsy examination. Multivariable regression analyses correcting for clinical and histopathological parameters showed that ENT involvement is associated with both baseline and 5-year follow-up eGFR. There were no associations between baseline and 5-year follow-up eGFR and arthralgia/arthritis, cutaneous or lung involvement, suggesting that our findings are specific to ENT involvement.

Conclusion

The presence of ENT involvement in AAV patients is associated with prognostically favourable renal biopsy findings and better renal function. These results indicate that there may be different phenotypes of AAV defined by ENT involvement.

Introduction

Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are the major clinical subtypes of ANCA-associated vasculitis (AAV)[1]. Establishing an early diagnosis of AAV is complicated by the diversity and lack of specificity of symptoms AAV patients present with, introducing both patient and doctor delay[2]. To what extent clinical outcome of AAV depends on early recognition of the disease is largely unknown; however, it seems likely that early diagnosis and treatment can prevent progressive organ damage, particularly the occurrence of end-stage renal disease (ESRD).

The presence of ENT involvement may be an early sign of AAV[3]. ENT involvement occurs in ~53% of AAV patients [4] and is reflected in symptoms such as hearing loss, otalgia, (bloody) rhinorrhoea, otorrhoea, sinusitis, nasal crusting and recurrent otitis media[2]. The presence of ENT involvement is closely associated with the presence of PR3-ANCA and is observed much less often in MPO-ANCA-positive patients.

Approximately 80% of AAV patients will develop renal involvement during their disease course[5-7], which in a considerable proportion of patients will lead to ESRD and/or death[8-10]. Previously we found that patients with MPO-ANCA have more chronic lesions in their renal biopsy than patients with PR3-ANCA, which may reflect the association of a more smouldering disease with MPO-ANCA and a more active disease with PR3-ANCA[3].

Recent data from a genome-wide association study (GWAS) indicate that within AAV, distinct subtypes may be recognized[11]. Positivity for either MPO-ANCA or PR3-ANCA was found to be the most important indicator of these subtypes, supporting the hypothesis that PR3-ANCA vasculitis and MPO-ANCA vasculitis are distinct autoimmune syndromes.

The diagnosis of AAV is based on a combination of clinical symptoms, ANCA serology and histological findings. This combination of findings may also form the basis for a patient profile with respect to outcome and may be representative of certain distinct AAV phenotypes[12, 13]. One factor of interest is the presence or absence of ENT involvement, because the absence of ENT involvement has been shown to increase mortality risk in AAV patients[14-19]. In this study we focused on ENT involvement in AAV patients in relation to renal biopsy findings and renal outcome. In addition, we performed a subgroup analysis based on ANCA serotype, because of the well-known association of ENT disease and PR3-ANCA. We hypothesized that ENT involvement in AAV is associated with fewer chronic lesions in the renal biopsy and better renal outcome.

Methods

Study population and clinical parameters

Patients were recruited from 62 hospitals located in 15 countries. All patients were enrolled in three international, prospective multicentre trials conducted by the European Vasculitis Society (EUVAS): CYCAZAREM (Cyclophosphamide Versus Azathioprine During Remission For Generalised Vasculitis), CYCLOPS (Daily Oral Versus Pulse Cyclophosphamide During The Induction Phase For Generalised Vasculitis) and MEPEX (Plasma Exchange Versus Methylprednisolone For Severe Renal Vasculitis)[20-22]. Inclusion and exclusion criteria for all trials are described elsewhere[20-22]. Local research ethics committees approved the studies and all patients provided informed consent. All trials were performed in accordance with the Declaration of Helsinki. Disease definitions were adopted from the 1994 Chapel Hill Consensus Conference[23]and previous European Union studies[24, 25]. All patients had been newly diagnosed as having GPA or MPA as determined by local physicians. Only patients with generalized AAV and available BVAS data were included in this study[26]. The four-variable Modification of Diet in Renal Disease was used to determine the estimated glomerular filtration rate (eGFR) in all patients, including the patients with ESRD[27].

ENT involvement was assessed at trial entry and was defined as present if patients had at least one of the ENT items scored using the BVAS. These items included nasal obstruction, bloody nasal discharge, nasal crusts, sinus involvement, conductive hearing loss, sensorineural hearing loss, hoarseness/stridor, granulomatous sinusitis and subglottic inflammation[26]. When ENT involvement was suspected, this was confirmed by local ENT specialists.

To assess whether associations with renal function are related to ENT involvement or are confounded by reduced diagnostic delay in patients with ENT symptoms, we did comparative analyses investigating associations between eGFR and other organ manifestations that are likely to reduce diagnostic delay in a similar fashion, specifically looking at cutaneous involvement, lung involvement and arthralgia/arthritis as scored by the BVAS. Cutaneous involvement included cutaneous infarcts, purpura, other skin vasculitis, ulcers, gangrene and multidigit gangrene[26]. Lung involvement included persistent cough, dyspnoea or wheeze, haemoptysis/haemorrhage, massive haemoptysis, respiratory failure and nodules or cavities, pleural effusion/pleurosity or infiltrate on chest radiology[26].

Histopathological parameters

Paraffin sections of diagnostic renal biopsies stained with silver, periodic acid-Schiff, haematoxylin and eosin and trichrome were examined. Sections were reviewed by two of five participating pathologists (I.M.B., F.F., L.H.N., R.W. or J.A.B.). Both pathologists, blinded to patient data and the other observer's results, scored the biopsies separately and according to a previously standardized protocol

and the histopathological classification of ANCA-associated glomerulonephritis (AAGN)[28-30]. Discrepancies between observers were resolved during central reviews to achieve consensus for each biopsy.

A predefined set of histopathological parameters, including interstitial fibrosis and tubular atrophy (IFTA), interstitial infiltrate, tubulitis and histopathological class, were included in multivariable analyses. In analyses including the histopathological classification, only biopsies containing a minimum of seven whole glomeruli were included.

Statistical analysis

Student's *t*-test was used to investigate an association between baseline and 5-year follow-up eGFR and overall ENT involvement. Student's t-test was also used to investigate an association between baseline eGFR and individual BVAS ENT subitems. A chi-squared test was used to investigate the association between ANCA subtype and the distribution of ENT symptoms. A chi-squared test for trend (linear-by-linear association) was used to investigate the association between histological parameters and ENT involvement. Linear regression analysis was used to explore the relationship between the number of ENT symptoms and eGFR at presentation. A quadratic term analysis was not significant, therefore a linear model was considered appropriate for this analysis. Seven patients were positive for five ENT symptoms, but because including these patients violated the linearity of the model, these patients were excluded from the analysis.

Correlations of ENT involvement, age, PR3-ANCA, tubulitis, interstitial infiltrate, IFTA and histopathological class according to the AAGN classification with baseline and 5-year follow-up eGFR were assessed using Pearson's or Spearman's correlation test as appropriate. Along with ENT involvement, the following entry parameters were selected for inclusion in the multivariable model investigating baseline eGFR: age, PR3-ANCA, IFTA, tubulitis, interstitial infiltrate and histopathological class according to the AAGN classification. ENT involvement, age, PR3-ANCA, IFTA, tubulitis, interstitial infiltrate and protocolized treatment received during the trial period were included in a model investigating 5-year follow-up eGFR. Histopathological class was not included in this model, as including this parameter strongly reduced the number of patients that could be included in the analysis. Analysis of covariance (ANCOVA) investigating 5-year follow-up eGFR was performed by including baseline eGFR in the model. In prespecified sensitivity analyses, the same models were used to investigate the effect of ENT involvement on baseline and 5-year follow-up eGFR in patients only positive for PR3-ANCA. Multicollinearity statistics demonstrated no multicollinearity in the multivariable regression analyses performed, indicating that it was legitimate to perform these analyses. We did not include diagnosis (GPA or MPA) in the analyses, as diagnosis is often dependent on ENT involvement (patients with ENT involvement are often diagnosed as GPA

and patients without ENT involvement are often diagnosed as MPA). Correcting for diagnosis would mask the effect of ENT involvement.

Since renal biopsies were not available for all patients, including the predefined histopathological parameters limited the number of patients included in the analysis. This was particularly true for histopathological class since biopsies needed to contain at least seven whole glomeruli. To be able to include all patients we therefore created three models. The first model included only clinical data: ENT involvement, age and PR3-ANCA. In the second model we added the following histopathological parameters: tubulitis, interstitial infiltrate and IFTA. The third model included all of the above parameters as well as the histopathological class.

To investigate whether possible associations with renal function are specific to ENT involvement and not confounded by reduced diagnostic delay in patients with ENT involvement, we performed comparable analyses between baseline and 5-year follow-up eGFR and other early disease manifestations - specifically arthralgia/ arthritis, cutaneous involvement and lung involvement.

A *P*-value of <0.05 was considered significant. All analyses were performed using SPSS version 20.0 (IBM, Armonk, NY, USA).

Results

Patients

A total of 414 patients with generalized AAV recruited from the CYCAZAREM[20], CYCLOPS[22] and MEPEX[21] trials were included in this study. All 149 patients from the CYCLOPS trial were included. From the CYCAZAREM and MEPEX trials, 149 of the 155 and 116 of the 137 patients were included, respectively. Exclusion of 27 patients was based on incomplete data about the presence or absence of ENT involvement. Diagnostic renal biopsies were obtained from 199 patients, of which 152 contained a minimum of seven whole glomeruli. Two patients did not have renal involvement at trial entry. Both patients were included in the CYCAZAREM trial because of other disease manifestations that were considered to be life-threatening. Baseline eGFR of these patients was 102 and 118 ml/min/1.73m^2.

ENT involvement

Of the 414 patients included in the study, 185 (45%) presented with ENT involvement. Detailed clinical data at baseline of patients with and without ENT involvement are provided in Table 1. ENT involvement was associated with higher eGFR, younger age, diagnosis of GPA and PR3-ANCA positivity. ENT symptoms as scored using the BVAS are outlined in supplementary Table S1. Nasal obstruction, bloody nasal discharge and nasal crusting were the most common

symptoms. In patients with ENT involvement, the distribution of symptoms was similar in PR3-ANCAand MPO-ANCA-positive patients (supplementary Table S2).

Table 1. Baseline characteristics of patients with and without ENT involvement

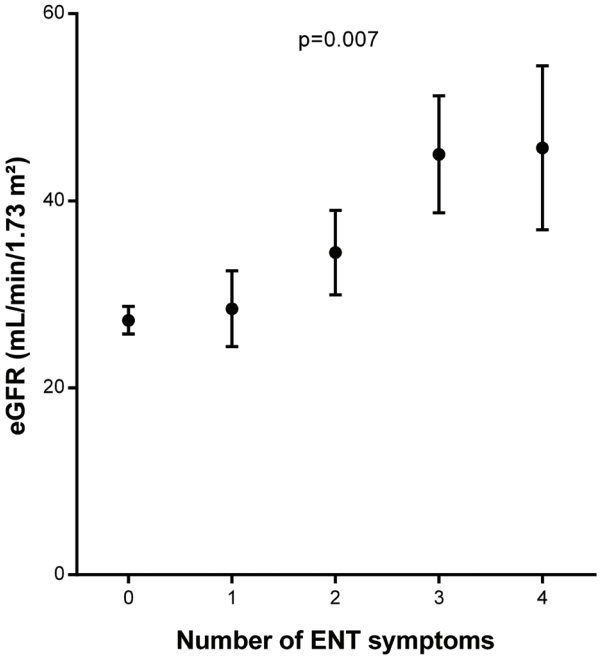
	ENT positive (n=185)	ENT negative (n=229)	P Value
eGFR, mL/min/1.73 m^2 (±SD)	39.16 (±33.11)	23.88 (±20.05)	<0.001
Gender, male / female	111 / 74	122 / 107	0.17
Age, years (SD)	56.49 (14.90)	60.89 (12.87)	0.001
Diagnosis, n (%)			<0.001
Granulomatosis with polyangiitis	142 (77)	41 (18)	
Microscopic polyangiitis	43 (23)	188 (82)	
ANCA-subtype, n (%)			<0.001
PR3-ANCA	124 (67)	77 (34)	
MPO-ANCA	46 (25)	138 (61)	
Double positive	9 (5)	3 (1)	
Negative	6 (3)	9 (4)	
Trial, n (%)			0.22
CYCAZAREM	75 (40)	74 (32)	
CYCLOPS	49 (27)	67 (29)	
MEPEX	61 (33)	88 (39)	

ANCA-serotype data was available for 412 of the 414 patients. Patients with and without ENT involvement are referred to as ENT positive and ENT negative, respectively. ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate; ANCA, anti-neutrophil cytoplasm antibody; PR3, proteinase 3; MPO, myeloperoxidase.

ENT involvement and baseline eGFR

The mean baseline eGFR of patients with and without ENT involvement was 39.16 (S.D. 33.11) and 23.88 (S.D. 20.05) ml/min/1.73m^2, respectively. The presence of overall ENT involvement was associated with higher eGFR [P<0.001 (95% CI 10.09, 20.46)]. Regarding specific ENT symptoms, the presence of nasal obstruction, bloody nasal discharge, nasal crusting, granulomatous sinusitis and conductive hearing loss was significantly associated with higher eGFR (supplementary Table S3). Moreover, the more ENT symptoms a patient had, the higher the eGFR was at presentation; average eGFR increased by 6.76 (95% CI 1.86-11.67) ml/min/1.73m^2 with each additional ENT symptom (P = 0.007; Fig. 1).

Figure 1. Increasing numbers of ENT symptoms are associated with increasing eGFR at diagnosis.



On average, eGFR increased 6.76 (95% CI 1.86 – 11.67) mL/min/1.73 m^2 with each additional ENT symptom (p=0.007). Data are presented as mean ± SEM. ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate.

ENT involvement and renal histology

The absence of IFTA in the renal biopsy was associated with ENT involvement (P<0.001; Table 2). Seventeen patients (20%) with ENT involvement had no IFTA in their renal biopsy, compared with four patients (4%) without ENT involvement. Nineteen patients (23%) with ENT involvement had severe IFTA in their renal biopsy, compared with 45 patients (39%) without ENT involvement. Furthermore, there was an association between histopathological class in the AAGN classification[30] and ENT involvement (P = 0.04; Table 2). Nineteen patients (30%) with ENT involvement had a focal class renal biopsy, compared with 16 patients (18%) without ENT involvement. Six patients (9%) with ENT involvement had a sclerotic class renal biopsy, compared with 13 patients (15%) without ENT involvement. Residuals analysis showed that the focal and sclerotic classes contributed most to the chi-square statistic.

Table 2. Histological variables in relation to ENT involvement

	ENT positive	ENT negative	P Value
IFTA, n (%)			< 0.001
None	17 (20)	4 (4)	
Focal interstitial fibrosis / small foci of tubular atrophy	48 (57)	66 (57)	
Diffuse interstitial fibrosis / extensive foci of tubular atrophy	19 (23)	45 (39)	
Histopathological classification of AAGN*, n (%)			0.04
Focal	19 (30)	16 (18)	
Crescentic	32 (50)	42 (48)	
Mixed	7 (11)	17 (19)	
Sclerotic	6 (9)	13 (15)	
Interstitial infiltrates**, n (%)			0.18
None	9 (11)	4 (3)	
Mild	32 (39)	48 (42)	
Moderate	32 (39)	45 (39)	
Severe	9 (11)	18 (16)	
Tubulitis, n (%)			
Absent	29 (34)	36 (31)	0.63
Present	55 (66)	79 (69)	

*Only biopsies with a minimum of seven whole glomeruli were included in this analysis (n=152).
**Data available for 197 patients. Renal biopsy specimens were available for 199 patients. Patients with and without ENT involvement are referred to as ENT positive and ENT negative, respectively. ENT, ear-, nose-, and throat; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody-associated glomerulonephritis.

ENT involvement is associated with higher eGFR independently of PR3-ANCA

Univariate analyses showed a correlation between baseline eGFR and ENT involvement, age, PR3-ANCA, tubulitis, interstitial infiltrate, IFTA and histopathological class (supplementary Table S4). ENT involvement was independently associated with higher baseline renal function in all three multivariable linear regression models (Table 3; supplementary Table S5). In addition to ENT involvement, age, tubulitis, interstitial infiltrate, IFTA and histopathological class were independently associated with eGFR. In this model including ENT involvement, PR3-ANCA is not associated with eGFR. To investigate whether a strong association between ENT involvement and PR3-ANCA is the reason why PR3-ANCA is not associated with eGFR in this model, we conducted the multivariable model excluding ENT involvement. In this analysis, PR3-ANCA was not associated with eGFR (data not shown). Moreover,

we also performed a stratified analysis in which we started with ENT disease and then investigated the effect of ANCA specificity on eGFR in patients both with and without ENT involvement. In these analyses, PR3-ANCA was again not associated with eGFR (data not shown). Both findings contradict that an association between ENT involvement and PR3-ANCA is the reason why PR3-ANCA is not significantly associated with eGFR.

A prespecified subgroup analysis that included only PR3-ANCA-positive patients revealed that ENT involvement was also independently associated with baseline eGFR in this subgroup of patients. In addition to ENT involvement, age, tubulitis, interstitial infiltrate and histopathological class were independently associated with baseline eGFR (Table 3).

ENT involvement is associated with higher eGFR independently of GPA diagnosis

Because of the strong association between ENT involvement and GPA diagnosis, it was not possible to include both parameters in the same multivariable linear regression model. To investigate the possibility of ENT involvement being a surrogate for the GPA phenotype instead of being independently associated with eGFR, we therefore conducted the multivariable regression analysis including only GPA patients. ENT involvement was independently associated with baseline eGFR in this analysis (supplementary Table S6), indicating that ENT involvement is not a surrogate for the GPA phenotype but is associated with eGFR independently of GPA diagnosis. In addition to ENT involvement, age, interstitial infiltrate, IFTA and histopathological class were independently associated with eGFR. PR3-ANCA was not associated with eGFR.

ENT involvement and renal function at 5-year follow-up

Data on renal function at 5-year follow-up were available for 81 and 72 patients with and without ENT involvement, respectively. Univariate analyses showed a correlation between 5-year follow-up eGFR and ENT involvement, age, interstitial infiltrate, IFTA and histopathological class (supplementary Table S4). As shown in Fig. 2, eGFR increased in patients both with and without ENT involvement during follow-up. Patients with ENT involvement had a better eGFR at 5-year follow-up compared with patients without ENT involvement. Mean eGFR at 5-year follow-up was 54.58 (S.D. 25.43) and 44.02 (S.D. 20.01) ml/min/1.73m^2 in patients with and without ENT involvement, respectively ($P = 0.005$). Multivariate analysis showed that ENT involvement, age and IFTA were independently associated with eGFR at 5-year follow-up (Table 3). PR3-ANCA was not associated with 5-year follow-up eGFR. When adding baseline eGFR to the multivariate model, ENT involvement was no longer associated with 5-year follow-up eGFR (supplementary Table S7). This finding implies that ENT involvement is not associated with an accelerated increase in eGFR, but that

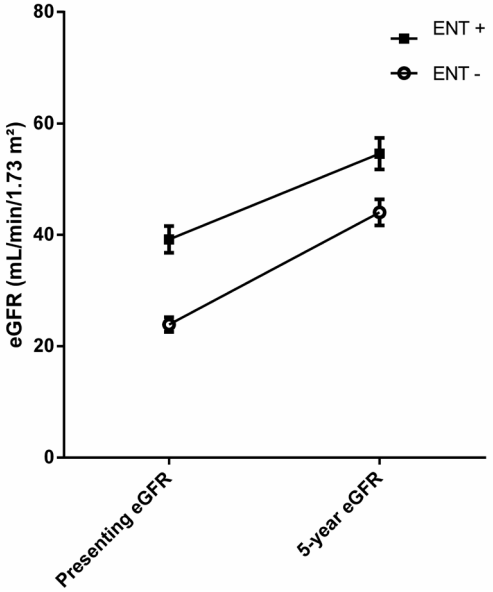
Table 3. Multivariable regression analyses investigating the relationship between ENT involvement and baseline and 5-year follow-up eGFR

	Model investigating baseline eGFR		**Model investigating 5-year follow-up eGFR[a]**		**Model investigating baseline eGFR in PR3-ANCA-positive patients**		**Model investigating 5-year follow-up eGFR in PR3-ANCA-positive patients[a]**	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
ENT involvement	9.14 (2.30 – 15.98)	0.009	9.86 (2.23 – 17.50)	0.01	12.92 (2.11 – 23.72)	0.02	16.40 (5.58 – 27.22)	0.004
Age	-0.60 (-0.83 – -0.36)	<0.001	-0.33 (-0.61 – -0.05)	0.02	-0.72 (-1.10 – -0.35)	<0.001	-0.17 (-0.54 – 0.21)	0.37
PR3-ANCA	-1.26 (-8.28 – 5.75)	0.72	-6.22 (-14.31 – 1.87)	0.13	N/A	N/A	N/A	N/A
Tubulitis	-17.82 (-25.78 – -9.86)	<0.001	-5.36 (-14.21 – 3.50)	0.23	-14.37 (-27.67 – -1.07)	0.04	-7.59 (-20.31 – 5.13)	0.24
Interstitial infiltrate	-6.86 (-11.20 – -2.52)	0.002	-3.54 (-10.15 – 3.07)	0.29	-8.91 (-16.52 – -1.31)	0.02	-5.14 (-14.44 – 4.17)	0.27
IFTA	-8.72 (-14.67 – -2.77)	0.004	-9.06 (-15.59 – -2.54)	0.007	-9.55 (-19.90 – 0.80)	0.07	-9.15 (-17.91 – -0.39)	0.04
AAGN classification	-6.20 (-9.84 – -2.57)	0.001	N/A	N/A	-8.41 (-14.97 – -1.85)	0.01	N/A	N/A

ENT involvement is associated with better eGFR at diagnosis and 5-year follow-up. A prespecified sensitivity analysis including only PR3-ANCA positive patients showed that this finding also held in this subgroup of patients. In all models, age is included per year unit. [a]Model is adjusted for within-trial therapy. 95% CI, 95% confidence interval; ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis; N/A, not applicable.

patients with ENT involvement start with a higher presenting eGFR and preserve this advantage during the 5-year follow-up. A prespecified subgroup analysis including only PR3-ANCA-positive patients revealed that ENT involvement is also associated with 5-year follow-up eGFR in this subgroup of patients (Table 3). Also in this subgroup of PR3-ANCA-positive patients, ENT involvement is no longer associated with 5-year follow-up eGFR when adding baseline eGFR to the model (supplementary Table S7).

Figure 2. Patients with ENT involvement have significantly better eGFR at diagnosis and 5-year follow-up.



Data are presented as mean ± SEM. ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate.

Cutaneous involvement, arthralgia/arthritis and lung involvement are not associated with baseline and 5-year follow-up eGFR

To investigate whether the higher eGFR in patients with ENT involvement is specific for ENT involvement or is attributable to less diagnostic delay in these patients, we analysed the relationship between eGFR and cutaneous involvement, arthralgia/arthritis and lung involvement, as these symptoms are likewise expected to reduce diagnostic delay. Of the 414 included patients, 98 (24%) had cutaneous involvement, 162 (39%) had arthralgia/arthritis and 207 (50%) had lung involvement. Multivariable linear regression analyses demonstrated no association between cutaneous involvement, arthralgia/arthritis or lung involvement and baseline eGFR (Table 4; supplementary Table S8). In all three

Table 4. Multivariable regression analyses investigating the relationships between baseline eGFR and other early disease manifestations

	Cutaneous model		**Arthralgia/arthritis model**		**Lung model**	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Cutaneous involvement	-2.98 (-11.58 – 5.62)	0.49	N/A	N/A	N/A	N/A
Arthralgia/arthritis	N/A	N/A	-4.85 (-11.40 – 1.71)	0.15	N/A	N/A
Lung involvement	N/A	N/A	N/A	N/A	-4.74 (-11.19 – 1.70)	0.15
Age	-0.63 (-0.87 – -0.38)	<0.001	-0.54 (-0.78 – -0.29)	<0.001	-0.61 (-0.85 – -0.37)	<0.001
PR3-ANCA	1.29 (-5.62 – 8.21)	0.71	0.41 (-6.52 – 7.33)	0.91	1.86 (-5.06 – 8.78)	0.60
Tubulitis	-16.54 (-24.62 – -8.47)	<0.001	-14.71 (-22.91 – -6.50)	0.01	-16.83 (-24.87 – -8.79)	<0.001
Interstitial infiltrate	-7.48 (-11.96 – -3.00)	0.001	-7.38 (-11.83 – -2.93)	0.001	-7.12 (-11.53 – -2.71)	0.002
IFTA	-9.57 (-15.62 – -3.51)	0.002	-9.92 (-16.01 – -3.83)	0.002	-9.97 (-15.97 – -3.97)	0.001
AAGN classification	-6.82 (-10.61 – -3.03)	0.001	-6.98 (-10.79 – -3.17)	<0.001	-6.79 (-10.49 – -3.09)	<0.001

To investigate whether our associations are specific to ENT involvement, analyses between baseline eGFR and other early disease manifestations were performed. There were no associations between baseline eGFR and cutaneous involvement, arthralgia/arthritis, or lung involvement. In all models, age is included per year unit. 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis; N/A, not applicable.

models, age, tubulitis, interstitial infiltrate, IFTA and histopathological class were associated with eGFR. Multivariable linear regression analyses also demonstrated no association between cutaneous involvement, lung involvement or arthralgia/arthritis and eGFR at 5-year follow-up (supplementary Table S9).

Discussion

Our study demonstrates that AAV patients with ENT involvement have a higher eGFR at diagnosis and 5-year follow-up than AAV patients without ENT involvement. Specifically, nasal obstruction, bloody nasal discharge, nasal crusting, granulomatous sinusitis and conductive hearing loss were associated with higher eGFR. Moreover, the number of ENT symptoms was related to renal function, with eGFR increasing with each added ENT symptom. Patients with ENT involvement had less IFTA and less chronic damage in their renal biopsies. We also found that renal histology of AAV patients with ENT involvement had features associated with better renal prognosis compared with renal histology of AAV patients without ENT involvement. In the multivariable analysis, PR3-ANCA was no longer associated with eGFR when including histological parameters in the model. This finding indicates an association between ANCA serotype and renal histological findings, as previously described[3]. Interestingly, we found that ENT involvement is associated with renal function independently of GPA diagnosis and PR3-ANCA.

Our findings can be explained by two possible mechanisms. It is possible that through ENT involvement, patients are diagnosed earlier with AAV. Therefore, in patients with ENT involvement, renal function may still be relatively preserved at presentation. Alternatively, the presence or absence of ENT involvement may represent different phenotypes of AAV. We will discuss a number of features that are either in favour or against these two notions.

Clinically overt ENT symptoms may lead to an earlier diagnosis of AAV by reducing patient and possibly doctor delay, as previously proposed[3]. This may explain the relatively preserved renal function in patients with ENT involvement. However, in our study, manifestations of the skin, lung and arthralgia/arthritis that would supposedly reduce patient and/or doctor delay were not associated with baseline and 5-year follow-up eGFR. Generalized symptoms (such as fever) have been shown to occur earlier in the disease than manifestations of the skin, lung and arthralgia/arthritis[31]. However, these symptoms were not associated with a higher eGFR in this cohort (data not shown). This lack of distinctiveness is probably due to the large proportion of patients with general symptoms at diagnosis (91% in our cohort).

Even though a number of studies have investigated diagnostic delay in AAV[31-33], to our knowledge, very few studies have investigated diagnostic delay for specific

organ involvement. Recently Poulton *et al.*[34] showed that AAV patients with ENT involvement were more likely to experience a delay in diagnosis because both physicians and patients focused on the more common causes of the ENT symptoms instead of regarding them as signs of vasculitis. Even though there was a risk of recall bias in this study, the authors tried to minimize this by interviewing patients within 5 years after diagnosis. Bligny *et al.*[18] showed that patients with ENT involvement have better survival than patients without ENT involvement. They argued that the better prognosis was likely related to different pathogenic routes and not to earlier diagnosis and treatment, as 13 of the 15 patients without ENT involvement had lung involvement, which can lead to earlier diagnosis. Interestingly, in AAV patients with ENT involvement, ENT symptoms mostly start within 12 months after diagnosis[35]. Considering these findings, the argument that ENT involvement is an easily recognizable sign of vasculitis that can lead to early recognition and better eGFR does not appear to hold.

A recently published GWAS provided support for the concept that PR3-ANCA vasculitis and MPO-ANCA vasculitis are distinct autoimmune syndromes[11]. Single nucleotide polymorphisms were shown to be associated more strongly with PR3-ANCA and MPO-ANCA than with the clinical syndromes MPA and GPA. In general, AAV patients with ENT involvement are likely to be PR3-ANCA positive and AAV patients without ENT involvement are likely to be MPO-ANCA positive[2]. In our multivariable analyses, ENT involvement overruled ANCA subtype in relation to the preservation of baseline and 5-year follow-up renal function, and in a prespecified subgroup analysis consisting of only PR3-ANCA-positive patients, ENT involvement was still associated with higher baseline and 5-year follow-up eGFR. Both findings indicate that ENT involvement is associated with a favourable renal profile independent of ANCA serotype and may be an important factor in the determination of different phenotypes of AAV.

One limitation of our study is that no data about the time from onset of symptoms to diagnosis of AAV were available. However, low reliability of a patient's interpretation of symptoms that may or may not have been attributable to AAV makes it hard and perhaps not desirable to use these data in an analysis. One strength of our study is the large number of patients we were able to include due to a large European collaboration. Moreover, renal biopsies were available for a large proportion of patients. Because our patients were recruited from three randomized controlled trials, clinical data were accurately documented prospectively.

Our study demonstrates that ENT involvement itself is an important clinical parameter of AAV, first because it is closely related to renal histology and outcome, and second because it may be a determinant of different phenotypes in AAV. Taking the findings of the GWAS into consideration, it is possible that within the subdivision of PR3-ANCA vasculitis and MPO-ANCA vasculitis,

AAV patients can be further categorized as ENT positive or ENT negative. Just as in SLE, where presenting symptoms in relation to clinical outcome have been compared to a hand of cards, in AAV, PR3-ANCA positivity together with the presence of ENT involvement may be representative of a relatively good hand of cards. Regarding future diagnostic and classification criteria for AAV, we suggest that the presence or absence of ENT should be taken into account as an important clinical parameter.

Key messages

- ENT involvement in ANCA-associated vasculitis patients is associated with higher baseline and 5-year follow-up estimated glomerular filtration rate.
- ENT involvement in ANCA-associated vasculitis patients is associated with prognostically favourable findings on renal biopsy examination.
- The presence or absence of ENT involvement may define different phenotypes of ANCA-associated vasculitis.

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Chapter IV

Histopathological classification of antineutrophil cytoplasmic antibody-associated glomerulonephritis: an update

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Curr Opin Nephrol Hypertens. 2014:224-31

Abstract

Purpose of review

This review discusses the findings of studies validating the histopathological classification of antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis, which was devised in 2010 by an international working group of pathologists and nephrologists in collaboration with the European Vasculitis Society.

Recent findings

So far, eight studies have validated the histopathological classification of ANCA-associated glomerulonephritis. The studies came from Japan, China, Australia, the United States, the Netherlands, and Turkey. These validation studies confirmed that the histopathological classification of ANCA-associated glomerulonephritis is of predictive value for renal outcome. This was especially the case for patients with either a focal or sclerotic-class renal biopsy, whereas the crescentic and mixed classes showed different results in the validation studies. These differences could be due to differences in patient populations or therapy, inter-rater reliability and lack of inclusion of tubulointerstitial lesions in the classification. Therapy is known to influence renal outcome, but due to the retrospective design of the to-date performed validation studies, this parameter could not be fully accounted for in these validation studies. Inter-rater reliability among three histopathologists was investigated in one study and was moderate.

Summary

The histopathological classification of ANCA-associated glomerulonephritis predicts renal outcome during follow-up, especially in patients with a focal or sclerotic-class renal biopsy. A large international validation study is currently being performed.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis is a systemic autoimmune disease affecting small and middle-sized blood vessels[1•]. Microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA) are the major clinical syndromes of ANCA-associated vasculitis, whereas renal limited vasculitis (RLV) and eosinophilic GPA (EGPA) occur less frequently[2]. Approximately 90% of ANCA-associated vasculitis patients have circulating antibodies against proteinase 3 (PR3)-ANCA or myeloperoxidase (MPO)-ANCA. These antibodies probably play a pathogenic role in the disease process and have recently been shown to represent genetically distinct subsets of patients with ANCA-associated vasculitis[3••, 4•, 5••, 6, 7••]. Renal involvement is a common and severe feature of ANCA-associated vasculitis, leading to end-stage renal failure (ESRF) and even death in a considerable number of patients[8-10].

ANCA-associated glomerulonephritis may show a variety of lesions, of which crescentic and focal necrotizing glomerulonephritis are the most prominent[11]. Clinicopathologic studies have demonstrated that the presence or absence of specific pathologic lesions in the renal biopsy is of important prognostic value for renal outcome, independently of baseline estimated glomerular filtration rate (eGFR). A high percentage of normal glomeruli is the strongest histological predictor of a favorable renal outcome; a high percentage of globally sclerotic glomeruli is associated with a worse renal outcome[12–14]. The percentage of cellular crescents is associated with recovery of renal function, irrespective of baseline eGFR[13]. Tubulointerstitial lesions, especially tubular atrophy, are associated with a worse renal outcome[12–14].

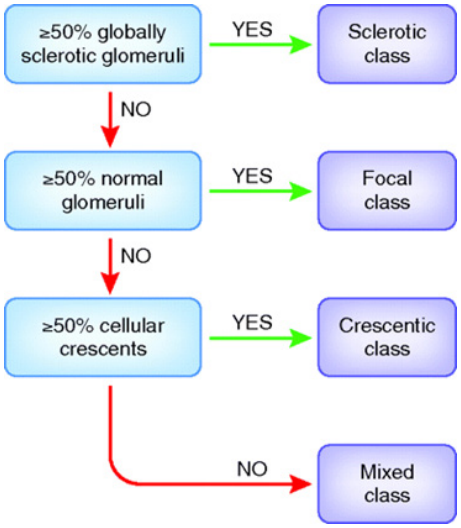
In 2010, the histopathological classification of ANCA-associated glomerulonephritis was devised by an international working group of pathologists and nephrologists in collaboration with the European Vasculitis Society (EUVAS)[15]. This review discusses the findings of studies validating the histopathological classification of ANCA-associated glomerulonephritis.

The histopathological classification of antineutrophil cytoplasmic antibody associated glomerulonephritis

The histopathological classification of ANCA-associated glomerulonephritis is built around glomerular pathology and encompasses four classes: focal, crescentic, mixed, and sclerotic-class renal biopsies[15]. The focal class is defined by the predominance of normal glomeruli (≥50%), the crescentic class by the predominance of cellular crescentic glomeruli (≥50%), and the sclerotic class by the predominance of globally sclerotic glomeruli (≥50%). The mixed class represents a heterogeneous glomerular phenotype in which no glomerular

feature predominates. A flowchart through which renal biopsies can be evaluated according to this classification system is depicted in Fig. 1. Tubulointerstitial lesions were not included in the classification system because these lesions did not improve the predictive value of the classification system in a first validation study incorporated in the publication by Berden *et al.*[15], and only increased its complexity.

Figure 1. Classification system flowchart



Biopsies are scored for glomerular lesions in the following order: globally sclerotic glomeruli, normal glomeruli and cellular crescentic glomeruli. Biopsies that do not fit into one of these classes will automatically be included in the mixed class. Reprinted, with permission from the *Journal of the American Society of Nephrology*.[15]

Baseline characteristics of the 100 patients of European descent included in the validation study incorporated in the publication by Berden *et al.* are depicted in Table 1. The phenotypical order of focal, crescentic, mixed, and sclerotic-class renal biopsies corresponded to the eGFR at baseline and at 1 and 5-year follow-up (Table 2). In addition, the histopathological classification and the baseline eGFR were the only independent predictors for eGFR at both 1 and 5 year follow-up in a multivariable analysis taking patient age, treatment, baseline eGFR, and the histopathological classification of ANCA-associated glomerulonephritis into account. The percentage of patients who developed ESRF increased with ascending class.

Table 1. Baseline characteristics of the patients included in the validation studies.

	Berden *et al.*[15]	**Iwakiri** *et al.*[16]	**Togashi** *et al.*[17]	**Muso** *et al.*[18]	**Chang** *et al.*[19]	**Hilhorst** *et al.*[20]	**Ford** *et al.*[21]	**Ellis** *et al.*[22]	**Unlu** *et al.*[23]
Number of patients	100	102	54	87	121	164	120	76	141
Age (median[a] or mean[b]; (SD[c] or range[d]))	62.6[a] (20.4 - 80.7[d])	66.3[b] (±11.3[c])	66.9[b] (36-85[d])	63.0[a] (17–85[d])	57.2[b] (15–81[d])	61.0[b] (±14.6[c])	66[b] (8-87[d])	58 (NR)	49.09[b] (7 – 80[d])
Male (n; (%))	54 (54)	54 (53)	28 (52)	37 (43)	64 (53)	113 (69)	72 (60)	43 (57)	80 (57 %)
Period	1995 - 2002	2000 - 2010	1990 - 2010	2001 - 2010	1997 - 2010	1979 - 2011	1993 - 2011	1995 - 2011	NR
Geographical area	Europe	Japan	Japan	Japan	China	Netherlands	Australia	US	Turkey
Number of glomeruli per biopsy (mediana or mean[b]; (rangec or SD[d]))	14.8[a] (10.0-49.0)[c]	19.0[b] (10-61)[c]	25.8[b] (10-64)[c]	26.5[a] (10-98)[c]	25.7[b] (±10.4)[d]	NR	22[a] (±14)[d]	NR	18.5[b] (7-60)[c]
Diagnosis						NR	NR		
GPA (n; (%))	39 (39)	3 (3)	28 (52)	0 (0)	49 (40.5)			43 (57)	55 (39)
MPA (n; (%))	61 (61)	97 (95)	25 (46)	87 (100)	68 (56.2)			31 (41)	20 (14)
EGPA (n; (%))	0 (0)	2 (2)	1 (2)	0 (0)	0 (0)			0 (0)	0 (0)
RLV (n; (%))	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.3)			3 (3)	39 (28)
Unknown (n; (%))	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	27 (19)
ANCA-subtype									
PR3-ANCA (n; (%))	45 (45)	5 (5)	0 (0)	0 (0)	13 (11)	83 (51)	28 (23)**	30 (39)**	60 (43)**
MPO-ANCA (n; (%))	47 (47)	86 (84)	54 (100)	76 (87)	108 (89)	81 (49)	75 (63)**	32 (42)**	61 (43)**
Double positive (n; (%))	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	17 (14)	0 (0)	5 (4)
Negative (n; (%))	2 (2)	11 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (18)	25 (18)
Missing (n; (%))	2 (3)	0 (0)	0 (0)	11 (13)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 1. Baseline characteristics of the patients included in the validation studies. (Continued)

	Berden *et al.*[15]	Iwakiri *et al.*[16]	Togashi *et al.*[17]	Muso *et al.*[18]	Chang *et al.*[19]	Hilhorst *et al.*[20]	Ford *et al.*[21]	Ellis *et al.*[22]	Unlu *et al.*[23]
Histopathological class									
Focal class (n; (%))	16 (16)	46 (45)	17 (31)	40 (46.0)	33 (27.3)	81 (49)	34 (28)	20 (26)	31 (22)
Crescentic class (n; (%))	55 (55)	32 (31)	8 (15)	7 (8.0)	53 (43.8)	43 (26)	33 (28)	18 (24)	69 (49)
Mixed class (n; (%))	16 (16)	18 (18)	19 (35)	26 (29.9)	24 (19.8)	39 (24)	33 (28)	27 (36)	29 (21)
Sclerotic class (n; (%))	13 (13)	6 (6)	10 (19)	14 (16.1)	11 (9.1)	1 (1)	20 (17)	11 (14)	12 (9)

NR, not reported; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; RLV, renal limited vasculitis; ANCA, Antineutrophil cytoplasmic antibodies; PR3, against proteinase 3; MPO, myeloperoxidase. amedian; bmean; crange; dSD; ** C-ANCA and p-ANCA were reported instead of PR3-ANCA and MPO-ANCA, respectively.

Table 2. Outcomes of the validation studies performed up to date.

	Berden *et al.*[15]	Iwakiri *et al.*[16]	Togashi *et al.*[17]	Muso *et al.*[18]	Chang *et al.*[19]	Hilhorst *et al.*[20]	Ford *et al.21*	Ellis *et al.*[22]	Unlu *et al.*[23]
Renal function at baseline	*Mean eGFR (±SD)*	*Median eGFR (IQR)*	*Mean eGFR (±SD)*	*Mean serum creatinine (±SD)*	*Mean eGFR (±SD)*	*Mean eGFR (±SD)*	*Median eGFR (range)*	*Mean eGFR ± SD*	*NR*
Focal class	56.4 (36.8)	38.1 (22.5–57.4)	49.1 (26.2)	1.51 ± 1.49	55.3 (39.9)	39.3 (29.4)	31 (3.4-128)	50.2 (63.4)	
Crescentic class	11.2 (10.9)	12.0 (7.1–19.7)	12.4 (5.1)	2.42 ± 1.67	37.8 (63.7)	16.8 (14.7)	8 (2.5-108)	16.4 (10.3)	
Mixed class	15.4 (16.2)	16.5 (8.7–31.6)	17.4 (9.6)	3.37 ± 3.17	17.9 (17.7)	24.3 (19.5)	21 (5-60)	26.5 (23.3)	
Sclerotic class	10.8 (9.5)	12.4 (9.8–27.4)	13.3 (4.9)	7.52 ± 4.92	8.3 (8.1)	14.6	10 (1.9-45)	26.5 (15.0)	
eGFR 1-year	*Mean (±SD)*	*Median (NR)*	*Mean (±SD)*	*NR*	*NR*	*Mean (±SD)*	*Median (range)*	*Mean (±SD)*	*NR*
Focal class	63.3 (23.7)	45.7	52.9 (11.4)			54.5 (20.9)	42 (67.2-104)	70.8 (29.6)	
Crescentic class	32.8 (20.8)	24.5	25.5 (6.3)			41.0 (21.1)	26 (4.6-95)	42.1 (22.4)	
Mixed class	24.5 (21.4)	26.0	31.3 (20.4)			36.7 (18.6)	34 (6.1-102)	37.8 (19.8)	
Sclerotic class	16.6 (15.9)	16.9	15.5 (4.1)			-	8 (3.3-41)	32.7 (15.3)	

Table 2. Outcomes of the validation studies performed up to date. (Continued)

	Berden *et al.*[15]	**Iwakiri** *et al.*[16]	**Togashi** *et al.*[17]	**Muso** *et al.*[18]	**Chang** *et al.*[19]	**Hilhorst** *et al.*[20]	**Ford** *et al.*[21]	**Ellis** *et al.*[22]	**Unlu** *et al.*[23]
eGFR at 2- or 5-year follow-up	*5-year*	*NR*	*2-year*	*NR*	*NR*	*2-year*	*NR*	*2-year*	*NR*
Focal class	65.6 (20.3)		60.9 (17.6)			53.5 (20.8)		76.7 (29.5)	
Crescentic class	39.5 (22.5)		33.9 (9.4)			38.8 (22.3)		41.7 (18.1)	
Mixed class	29.9 (16.7)		29.1 (9.2)			38.3 (16.0)		42.9 (21.6)	
Sclerotic class	20.4 (15.1)		7.4 (3.4)			-		37.8 (16.8)	
Renal survival at 1-year follow-up		*NR*				*NR*	*NR*	*NR*	*NR*
Focal class (%)	93		2	100	100				
Crescentic class (%)	84		22	86	73				
Mixed class (%)	69		11	96	83				
Sclerotic class (%)	50		33	35	29				
Renal survival at 2- or 5-year follow-up	*5-year*	*NR*	*NR*	*5-year*	*5-year*	*5-year*	*NR*	*NR*	*NR*
Focal class (%)	93			100	93	91			
Crescentic class (%)	76			86	60	64			
Mixed class (%)	61			96	72	69			
Sclerotic class (%)	50			29	29	-			
Development of ESRF or death	*ESRF*	*ESRF*	*ESRF*	*NR*	*ESRF*	*NR*	*ESRF or death*	*NR*	*ESRF*
Focal class (n; (%))	1 / 14 (7%)	2 / 46 (4.3)	2 / 46 (4)		3 / 33 (9)		11 / 34 (32)		4 / 31 (13)
Crescentic class (n; (%))	11 / 45 (45%)	9 / 32 (28)	9 /32 (28)		15 / 53 (28)		14 / 33 (42)		20 / 69 (29)
Mixed class (n; (%))	6 / 13 (46%)	8 / 18 (44)	8 / 18 (44)		4 / 24 (17)		13 / 33 (39)		10 / 29 (34)
Sclerotic class (n; (%))	7 / 10 (70%)	4 / 6 (67)	4 / 6 (67)		8 / 11 (73)		16 / 20 (80)		8 / 12 (67)

eGFR, estimated glomerular filtration rate; ESRF, end-stage renal failure; IQR, interquartile range; NR, not reported.

Validation of the histopathological classification of antineutrophil cytoplasmic antibody-associated glomerulonephritis

So far, eight studies from Japan, China, Australia, the United States, the Netherlands, and Turkey have validated the histopathological classification of ANCA-associated glomerulonephritis[16–23]. Five of these studies included more than 100 patients[16,19–21,23]. The distributions of diagnoses and ANCA serotype varied and are depicted in Table 1. We will review these validation studies below.

Validation of the classification system in the Asian population

Four validation studies came from Asia, three of which were from Japan and one from China[16–19]. All these studies had a predominance of MPO-ANCA-positive patients. The diagnosis of GPA or MPA was equally distributed in two studies[17,19]. Two other studies consisted of 95 and 100% of MPA patients, respectively[16,18]. At baseline, eGFR levels in all four studies showed a similar distribution over the four classes, with the best eGFR levels for patients with a renal biopsy classified as focal class and worst levels for patients with a renal biopsy classified as sclerotic class. In all these studies, patients with renal biopsies classified as crescentic and mixed class showed intermediate eGFR levels. The outcome with respect to renal function during follow-up in relation to the histopathological class varied somewhat amongst the studies as discussed below in detail. This may have been influenced by the different end-points of the studies.

Iwakiri *et al.*[16] reported a significant relationship between the four histopathological classes and eGFR at baseline and 1-year follow-up, which was greatly determined by a relatively good eGFR in patients with a focal-class renal biopsy (Tables 1 and 2). A renal survival curve over 120 months showed a distribution pattern similar to that of the validation study in the publication by Berden *et al.*, with patients with focal and sclerotic-class renal biopsies having the best and worst renal survival, respectively. There was no significant difference in the eGFR of patients with renal biopsies classified as crescentic or mixed class. The authors comment that discrimination between these two classes was hindered because, in their cohort, both the proportion of normal and sclerotic glomeruli was higher in the mixed class than in the crescentic-class renal biopsies.

Togashi *et al.*[17] reported that the phenotypical order of focal, crescentic, mixed and sclerotic class corresponded to the severity of renal function impairment at baseline and 1 and 5-year follow-up in a cohort of Japanese MPO-ANCA-positive patients (Tables 1 and 2). However, at all time points, the eGFR of patients with crescentic and mixed-class renal biopsies was similar. Overall, renal survival was relatively good with only five of the 54 patients developing ESRF during the 5-year follow-up. Of the patients developing ESRF, two patients had a crescentic and three patients had a sclerotic-class renal biopsy.

Muso *et al.*[18] focused on baseline eGFR and the development of ESRF in a Japanese cohort (Tables 1 and 2). The phenotypical order of focal, crescentic, mixed, and sclerotic-class renal biopsies corresponded to the order of severity of renal function impairment at baseline. At 5-year follow-up, the risk of ESRF development was not increased in patients with focal and mixed-class renal biopsies. Patients with a renal biopsy classified as sclerotic had a highly increased risk to develop ESRF. Patients with focal, mixed and crescentic-class renal biopsies had similar renal survival curves, particularly after 10 months of follow-up.

Chang *et al.*[19] validated the histopathological classification of ANCA-associated glomerulonephritis in a Chinese cohort (Table 1). The phenotypical order of focal, crescentic, mixed, and sclerotic-class renal biopsies corresponded to the order of severity of renal function impairment at baseline (Table 2). Renal survival was best for patients with a focal-class renal biopsy and worst for patients with a sclerotic-class renal biopsy. Comparing patients with a mixed and crescentic-class renal biopsy, 5-year renal survival was 72 and 60%, respectively. The authors stated that the patients with a mixed-class ANCA-associated glomerulonephritis in this cohort had relatively milder lesions than patients with a mixed-class ANCA-associated glomerulonephritis in the Berden *et al.* cohort, with more normal glomeruli, fewer sclerosed glomeruli and fewer glomeruli with fibrous crescents. In addition, tubulointerstitial lesions were not independent predictors of ESRF in a multivariable analysis taking the histopathological classification of ANCA-associated glomerulonephritis into account.

Summarizing the data from the Asian studies, we conclude that all studies show a clear tendency for eGFR at baseline being related to the phenotypical order of focal, crescentic, mixed, and sclerotic-class renal biopsies. Patients with a focal-class renal biopsy had the best eGFRs and patients with a sclerotic-class renal biopsy had the worst eGFRs. Follow-up data varied in time periods and whether ESRF or eGFR was chosen as the end-point. It may be tentatively concluded that there was no clear-cut difference in the outcome of patients with crescentic and mixed-class renal biopsies. In some studies, there was a tendency for patients in the mixed-class to have a slightly better renal outcome than patients in the crescentic class. The Asian studies differed from the studies in the Caucasian population which we discuss below, in terms of ANCA-serotype distribution, diagnosis distribution, and a patient population from the Asian race.

Validation of the classification system in the Caucasian population

The largest validation study reported so far came from a Dutch cohort consisting of 164 patients with an equal distribution of PR3-ANCA and MPO-ANCA patients (Table 1)[20]. There was only one patient with a renal biopsy classified as being in the sclerotic class, therefore this class could not be part of the analysis. The histopathological classification of ANCA-associated glomerulonephritis

predicted eGFR at both 1 and 2-year follow-up (Table 2). At both time points, eGFR differed significantly between patients with a focal and crescentic-class renal biopsy, and between patients with a focal and mixed-class renal biopsy, but not between patients with a crescentic and mixed-class renal biopsy. In addition, 5-year renal survival was significantly higher in patients with a focal-class renal biopsy than in patients with a crescentic or mixed-class renal biopsy, whereas renal survival in these two latter groups was similar. Subdividing the crescentic and mixed-class renal biopsies on the basis of more or less than 25% normal glomeruli in the renal biopsy showed that patients with more than 25% normal glomeruli had a significantly better renal survival.

A recently published study by Ford *et al.*[21] is the only study so far which investigated the inter-rater reliability of the classification system, showing variability among three histopathologists scoring 145 renal biopsies (k=0.46). Good agreement was found for classifying the sclerotic class (k=0.70), but only moderate agreement for classifying the focal, crescentic, and mixed classes (k=0.47, k=0.23, and k=0.51, respectively). There was no significant difference in ANCA subtype between patients in the different classification groups (Table 2). Most tubulointerstitial lesions were found in the sclerotic class. Renal function at presentation was best in patients with a focal or mixed-class renal biopsy, and worst in patients with a sclerotic or crescentic-class renal biopsy. At 1-year follow-up, eGFR declined in the phenotypical order of focal, crescentic, mixed, and sclerotic-class renal biopsies. Patients with a sclerotic-class renal biopsy had a significantly increased risk of ESRF development or death compared with patients with a focal, crescentic, or mixed-class renal biopsy. When taking ESRF development or death as outcome, there was no difference in outcome between patients with focal, crescentic or mixed-class renal biopsies. A drawback of this study is that no minimum amount of glomeruli was required in the renal biopsies.

Ellis *et al.*[22] performed a validation study in 76 patients from the United States. Diagnosis and ANCA serotype were equally distributed in this cohort (Table 1). Renal function at baseline was best in patients with a renal biopsy classified as focal and did not differ between patients with renal biopsies classified as crescentic, mixed, or sclerotic (Table 2). At both 1 and 2-year follow-up, renal function was best in patients with a focal-class renal biopsy, worst in patients with a sclerotic-class renal biopsy, and intermediate in patients with a crescentic or mixedclass renal biopsy. In contrast to the study by Ford *et al.*, this study showed that patients in the crescentic class were significantly more often c-ANCA-positive with a GPA phenotype. Renal survival at 1-year follow-up did not differ significantly between the classes, but the histopathological class was an independent predictor of eGFR at 1-year follow-up in linear regression analyses.

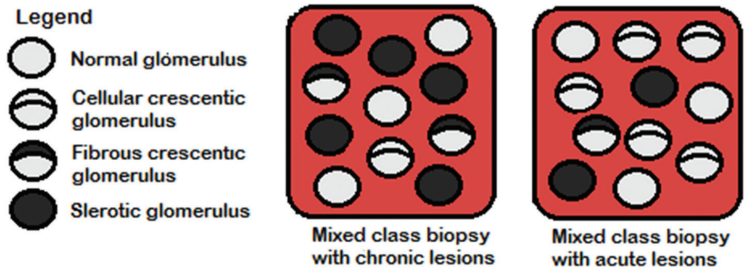
Unlu *et al.*[23] reported a study in Turkish patients that was not primarily focused on the validation of the histopathological classification for ANCA-associated glomerulonephritis, but evaluated the classification system with dialysis

as outcome in a subanalysis (Table 1). The classification system did predict for dialyses requirement in the log-rank test, but not in the Cox regression model (Table 2). Similar to the study performed by Ford *et al.*, in this study, no minimum amount of glomeruli was required in the renal biopsies.

Points of consideration and future perspectives

The studies validating the histopathological classification of ANCA-associated glomerulonephritis show that the classification system predicts clinical outcome, in particular, in patients with either a focal or sclerotic-class renal biopsy. There are conflicting outcomes with respect to the crescentic and the mixed-class renal biopsies. In a number of studies, the outcome of patients with a crescentic-class renal biopsy is similar to that of patients with a mixed-class renal biopsy. In other studies, patients with focal and crescentic-class renal biopsies seem to have similar outcomes. There is some evidence that the proportion of normal glomeruli is an important determinator of outcome and is taken as a parameter to subcategorize the classes. Whether additionally, the variation of study results is due to differences in patient populations, moderate inter-rater reliability, or other factors is currently unknown. Only one study investigated inter-rater reliability, showing moderate variability among histopathologists. In particular, the mixed-class renal biopsies may be 'suffering' from an 'identity crisis', as exemplified in Fig. 2. This figure shows that biopsies in this class may indeed show mixed findings as their denominator suggests, in which either an acute or chronic phenotype predominated. This may have important consequences for renal outcome if different phenotypes prevail in different studies. Renal outcome is also greatly influenced by the therapy a patient receives. However, due to the retrospective design of all to-date performed validation studies, it was not possible to fully account for this parameter in these studies. Another important issue is the influence of interstitial lesions on outcome, in addition to the classes which are primarily based on glomerular lesions. In a recent review by Haas *et al.*[24], it was questioned whether the lack of inclusion of tubulointerstitial changes would affect the value of the classification system. It was also questioned whether the classification system identified specific lesions most likely to respond to one or more immunosuppressive agents. These issues are being addressed in a large international validation study, which is currently being performed and incorporates histopathological, clinical, and therapeutical data.

Figure 2. Different phenotypes of mixed class ANCA-associated glomerulonephritis



Hypothetical example of how mixed class biopsies may represent quite different phenotypes of ANCA-associated glomerulonephritis in terms of activity and chronicity.

Conclusion

In general, validation studies supported the predictive value of the histopathological classification of ANCA-associated glomerulonephritis for renal outcome. This was especially the case in patients with a focal or sclerotic-class renal biopsy, whereas the crescentic and mixed classes showed different results in the validation studies. These differences could be due to differences in patient populations or therapy, moderate inter-rater reliability, and lack of inclusion of tubulointerstitial lesions in the classification system. To address these issues, a large international validation study is currently being performed.

Key points

- The histopathological classification of ANCA-associated glomerulonephritis predicts renal outcome during follow-up, especially in patients with either a focal or sclerotic-class renal biopsy.
- There are conflicting outcomes with respect to the crescentic and the mixed-class renal biopsies.
- This could be due to differences in patient populations or therapy, moderate inter-rater reliability, and lack of inclusion of tubulointerstitial lesions in the classification system.
- A large international validation study is currently being performed to address these issues.

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•• of outstanding interest.

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Chapter V

Incidence of malignancies in patients diagnosed with ANCA-associated vasculitis between 1991 and 2013

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Abstract

Objective

To investigate the incidence of malignancies during longitudinal follow-up of patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV), and to examine the effect of immunosuppressive therapy on malignancy risk in these patients.

Methods

The study population consisted of patients with histopathologically confirmed AAV, diagnosed between 1991 and 2013 at a large university hospital. The mean duration of followup was 10 years. Malignancy incidence was assessed using the Dutch National Pathology Database. Incidence rates from the Netherlands Cancer Registry were used to compare malignancy incidence in the AAV cohort to that in the general Dutch population.

Results

Thirty-six of 138 patients with AAV developed a total of 85 malignancies during a mean followup of 9.7 years. The sex-, age-, and calendar year-adjusted malignancy risk was 2.21-fold higher (95% confidence interval [95% CI] 1.64-2.92) than that in the general population. Non-melanoma skin cancers occurred most frequently (standardized incidence ratio 4.23 [95% CI 2.76-6.19]). The incidence rates of other malignancies were not significantly increased. Malignancy risk was associated with the duration of cyclophosphamide (CYC) therapy and, interestingly, was not increased in patients who had received CYC for <1 year.

Conclusion

Patients with AAV have a higher risk of malignancy than the general population, but this risk is accounted for solely by non-melanoma skin cancers. Over the years, the risk of other malignancies – specifically bladder and hematologic malignancies – has decreased in patients with AAV. This finding reflects ongoing efforts to reduce CYC exposure by developing new treatment regimens.

Introduction

The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs), including granulomatosis with polyangiitis (Wegener's) (GPA) and microscopic polyangiitis (MPA), are small-to-medium vessel vasculitides that affect multiple organs and are life-threatening when untreated[1]. The availability of immunosuppressive therapy has dramatically improved prognosis in patients with AAV. Before the introduction of immunosuppressive therapy for this disorder, the mortality rate within 1 year after diagnosis was 80%. Currently, the remission rate is 90%[2-4]. Moreover, the life expectancy of patients with AAV who survive 5 years after diagnosis approaches that of the general population[4, 5]. As a result, attention has shifted to the long-term complications experienced by patients with AAV. Malignancies have been shown to be the second most common cause of death >1 year after diagnosis[4]. In previous studies, the incidence of malignancies among patients with AAV has been shown to be increased compared to the general population[6-12]. This was particularly the case for bladder cancer, malignant lymphomas, leukemia, and non-melanoma skin cancers (NMSCs).

The results of earlier studies of malignancy risk in AAV must be interpreted in light of a number of factors. First, in previous studies, except for one published in 1992[6], followup was limited to ~5 years[7, 9, 10, 13]. Second, in some studies information on malignancy incidence was based on patient or physician questionnaires, which may have introduced reporting bias. Most importantly, the observation period in most studies dated from the 1960s to the 1990s[6-8, 13]. In recent years, immunosuppressive therapy regimens have changed, based on efforts to reduce exposure to steroids and cyclophosphamide (CYC)[14-16]. Therefore, previous data on malignancy incidence mostly reflect risks associated with therapy regimens that are no longer used.

In the current study, we investigated the incidence of malignancies in 138 patients with AAV treated with current immunosuppressive therapy regimens, who were followed up for a mean of 10 years. Specifically, we examined the effect of duration of CYC exposure on the risk of malignancy in these patients. A nationwide histology database was used to assess the incidence of malignancy in this cohort.

Patients and methods

Study population and data collection

This study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

We enrolled patients with histopathologically confirmed AAV diagnosed at Leiden University Medical Center between 1991 and 2013. Patients with coexisting autoimmune diseases were excluded. Definitions provided in the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides[17] were used. Of the 138 patients enrolled, 134 (97%) had undergone renal biopsy in which AAV was confirmed histopathologically (i.e., crescentic and/or necrotizing glomerulonephritis with few or no immune deposits detected by immunofluorescence and/or electron microscopy). In the 4 patients who did not undergo renal biopsy, the biopsies showing AAV originated from the lung (3 patients), the ear/nose/throat (ENT) (3 patients), and the skin (2 patients). All 4 of these patients had a clinical diagnosis of GPA, and all exhibited ANCA positivity. Twenty of the 138 patients in the study were negative for ANCA or had an unknown ANCA serotype; AAV in these patients was confirmed histologically by renal biopsy findings as defined above. Seven of these patients also had a second biopsy from another organ showing AAV (skin [4 patients], lung [2 patients], and ENT region [1 patient]).

Primary malignancies were identified via the Dutch National Pathology Database, a nationwide histopathology and cytopathology network and registry in The Netherlands. The database includes data from all pathology laboratories[18]. The cohort was linked to the Central Population Registry to obtain information on death and emigration. The observation time started on the date of AAV diagnosis and ended on the date of death, the date of last followup, or on May 1, 2013, whichever occurred first. We retrieved medical records to collect information on sex, date of birth, clinical diagnosis (GPA/MPA), ANCA serotype, renal function at diagnosis, renal transplantation, renal transplantation date (when applicable), and use of immunosuppressive medication (type and duration).

Standardized incidence ratio calculation

Malignancy occurrence in our cohort was compared to that in the general population by determining standardized incidence ratios (SIRs), calculated as the observed number of malignancies divided by the expected number of malignancies. To obtain the most accurate value, SIRs were calculated with matching for sex, age (5-year age groups), and calendar-year period (1-year time periods). The observed number of malignancies was the total number of malignancies that occurred in the cohort. If a patient developed multiple NMSCs, only the first NMSC was taken into account in the analyses. The expected number of malignancies was defined as the person-years at risk multiplied by the national cancer incidence rate data provided by the Netherlands Cancer Registry. Subgroup analyses were performed for the following variables: sex, age at study entry (dichotomous according to the median value), renal transplantation, clinical diagnosis, ANCA serotype, followup duration, and history of malignancy before AAV diagnosis. Moreover, we considered that the landmark European Vasculitis

Society (EUVAS) trial Cyclophosphamide versus Azathioprine as Remission Maintenance Therapy for ANCA-Associated Vasculitis (CYCAZAREM), published in 2003[19], led to a drastic reduction in CYC exposure in patients with AAV. Consequently, we performed separate subgroup analyses of patients diagnosed before 2003 and patients diagnosed in or after 2003, to investigate whether this change in immunosuppressive therapy regimen had an effect on malignancy risk.

Statistical analysis

Student's *t*-test and the chi-square test were used to assess the significance of differences in baseline characteristics of patients who did and those who did not develop malignancies. Survival rates were analyzed by log rank test. Malignancy-free survival time was calculated with the Kaplan-Meier method; the observation time started at AAV diagnosis and ended at the date of the first malignancy diagnosis, death, or May 1, 2013, whichever occurred first. SIRs and 95% confidence intervals (95% CIs) were calculated with an exact Poisson regression analysis, which allowed incorporation of patients with multiple malignancies, assuming a Poisson distribution of the observed number of cases[20-22]. For all subgroup analyses, exact Poisson regression models were performed to calculate the relative risk. *P* values less than 0.05 were considered significant.

Results

Patient characteristics

A total of 138 patients with histopathologically confirmed AAV were included in the study. Of the 117 patients (85%) with available clinical diagnoses, 79 (68%) had a diagnosis of GPA and 38 (32%) had a diagnosis of MPA; none had a diagnosis of eosinophilic granulomatosis with polyangiitis (Churg-Strauss). Of the 128 patients (93%) with available data on ANCA serotype, 55 (43%) were positive for proteinase 3 (PR3)-ANCA; 47 (37%) for myeloperoxidase (MPO)-ANCA, and 5 (4%) for both PR3-ANCA and MPO-ANCA; 11 patients (9%) were positive for ANCA but with no available data on whether they were PR3-ANCA or MPO-ANCA positive, and 10 patients (8%) were negative for ANCA. The mean $\pm$ SD age at AAV diagnosis was 59.3 $\pm$ 14.8 years, and did not differ significantly between patients who did and those who did not develop malignancy during followup. Fourteen patients had a history of malignancy occurrence prior to the AAV diagnosis. No malignancy diagnosed prior to the AAV diagnosis metastasized during the followup period. Additional demographic and clinical data are summarized in Table 1.

Table 1. Characteristics of the AAV patients included in the study

	Total sample	No malignancy occurrence	Malignancy occurrence	p-value
Mean age at diagnosis, years (SD)	59.3 (14.8)	58.9 (15.6)	60.7 (12.0)	0.533
Male, n (%)	87 (63)	62 (61)	25 (69)	0.355
Clinical diagnosis, n (%)				0.753
GPA	79 (57)	56 (55)	23 (64)	
MPA	38 (28)	28 (27)	10 (28)	
Unknown	21 (15)	18 (18)	3 (8)	
ANCA-serotype, n (%)				0.079
PR3-ANCA	55 (40)	35 (34)	20 (56)	
MPO-ANCA	47 (34)	39 (38)	8 (22)	
ANCA-negative	10 (7)	7 (7)	3 (8)	
PR3- and MPO-ANCA positive	5 (4)	5 (5)	0 (0)	
ANCA positive[1]	11 (8)	9 (9)	2 (6)	
Unknown	10 (7)	7 (7)	3 (8)	
Mean baseline serum creatinine, µl/l (SD)	337.0 (303.6)	332.5 (279.1)	348.0 (361.0)	0.813
Renal transplantation, n (%)	10 (7)	8 (8)	2 (6)	0.649
Previous history of malignancy, n (%)	14 (10)	11 (11)	3 (8)	0.675
Organ involvement, n (%)[2]				
Cutaneous	36 (31)	21 (25)	15 (46)	0.044
Eyes	35 (30)	21 (25)	14 (42)	0.075
Ear, nose, and throat	78 (67)	53 (63)	25 (76)	0.276
Chest	61 (52)	45 (54)	16 (49)	0.683
Cardiovascular	7 (6)	6 (7)	1 (3)	0.671
Gastrointestinal	16 (14)	13 (16)	3 (9)	0.551
Kidney	138 (100)	0 (0)	138 (100)	N/A
Nervous system	32 (27)	25 (30)	7 (21)	0.490
Deaths, n (%)	56 (41)	39 (38)	17 (47)	0.255[3]

The study population consisted of 138 patients with histologically confirmed antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (AAV). Data on clinical diagnosis and ANCA serotype were available for 117 patients and 128 patients, respectively. Except where indicated otherwise, values are the number (%). GPA = granulomatosis with polyangiitis (Wegener's); MPA = microscopic polyangiitis.

[1] Patients who were ANCA positive, but with no information in the medical records regarding whether they were positive for proteinase 3 (PR3)–ANCA or myeloperoxidase (MPO)–ANCA.

[2] All 138 patients had renal involvement; data on involvement of other organs were available for 117 patients.

[3] P = 0.044 versus patients with no malignancy occurrence.

Observed malignancies

Thirty-six patients developed malignancies (total of 85 malignancies) during the followup period (mean 9.7 years, median 8.0 years; 1,339 person-years). Of these malignancies, 61 were NMSCs, and they occurred in a total of 22 patients. The NMSCs included 42 basal cell carcinomas and 19 squamous cell carcinomas. In addition, 3 colon carcinomas, 3 breast carcinomas, 3 prostate carcinomas, 2 lung carcinomas, 2 soft tissue sarcomas, 2 unknown primary malignancies, and a variety of malignancies that occurred only once were observed (Table 2). Malignancy-free survival at 2, 5, and 10 years of followup was 99%, 84%, and 80%, respectively (Figure 1).

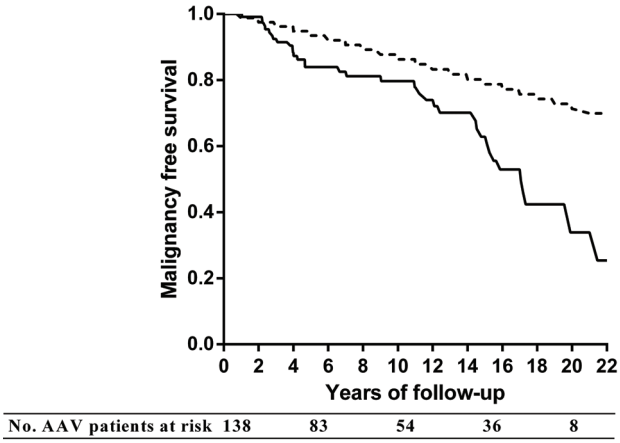

Figure 1. Years of malignancy-free survival in patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV) (solid line) compared to the age-and sex-matched general population (dashed line).

Comparison with the general population

The sex-, age-, and calendar year–adjusted malignancy risk among the AAV patients in this cohort was 2.21-fold higher than the risk in the general population (95% CI 1.64-2.92) (Table 2). This increased risk was attributable solely to an increased risk of developing NMSC (SIR 4.23 95% CI 2.76-6.19). We observed only 1 bladder carcinoma and 1 non-Hodgkin's lymphoma in the cohort. These incidences were not significantly increased compared to the general population, with SIRs of 1.43 (95% CI 0.04-7.96) and 1.37 (95% CI 0.03-7.63), respectively. The risk of developing any of the other reported malignancies was also not increased compared to that in the general population (Table 2).

Table 2. SIRs calculated for malignancies overall and per observed malignancy

Malignancy type	PY	N malignancies	SIR	95% CI	p-value
All malignancies	1339	85	2.21	1.64 – 2.92	<0.001
NMSC	1339	61	4.23	2.76 – 6.19	<0.001
Non-NMSC	1339	24	1.46	0.93 – 2.17	0.095
By malignancy					
Breast carcinoma	1339	3	2.04	0.42 – 5.96	0.367
Colon carcinoma	1339	3	1.85	0.38 – 5.41	0.444
Prostate carcinoma	795	3	0.99	0.20 – 2.89	1.000
Lung carcinoma	1339	2	0.75	0.23 – 3.30	0.993
Soft tissue sarcomas	1339	2	7.69	0.93 – 27.79	0.057
Unknown primary malignancy	1339	2	3.92	0.47 – 14.17	0.187
Brain carcinoma	1339	1	5.56	0.14 – 30.95	0.329
Parotis carcinoma	1339	1	33.33	0.84 – 185.72	0.059
Bladder carcinoma	1339	1	1.43	0.04 – 7.96	1.000
Uterus carcinomas	544	1	4.00	0.10 – 22.29	0.442
Non-Hodgkin lymphoma	1339	1	1.37	0.03 – 7.63	1.000
Renal cell carcinoma	1339	1	2.44	0.06 – 13.59	0.672
Chondrosarcoma	1339	1	28.57	0.72 – 159.19	0.068
Esophageal cancinoma	1339	1	2.70	0.07 – 15.06	0.602
Melanoma	1339	1	1.92	0.05 – 10.71	0.811

The analysis encompassed 1,339 person-years (except for the sex-specific malignancies prostate carcinoma [795 person-years] and uterus carcinoma [544 person-years]). Standardized incidence ratio (SIR) (ratio of observed to expected malignancies) was adjusted for sex, age (5-year age groups), and calendar time period (1-year time periods). 95% CI = 95% confidence interval; NMSC = non-melanoma skin cancer.

Malignancy risk by clinical diagnosis and ANCA subtype

We performed prespecified subgroup analyses with stratification by sex, age, followup duration, clinical diagnosis, ANCA subtype, history of malignancy before AAV diagnosis, diagnosis and treatment initiation before or after publication of the CYCAZAREM trial (2003), and renal transplantation (Table 3). The risk of malignancy became increased compared to the general population after the duration of AAV exceeded 5 years (Table 3).

Table 3. SIRs for malignancy in the AAV patients stratified into various subgroups

	N patients	N malignancies	SIR (95% CI)	SIR p-value	RR (95% CI)	RR p-value
Gender						
Male	87	61	2.33 (1.64 – 3.21)	<0.001		
Female	51	24	1.93 (1.03 – 3.31)	0.041	0.83 (0.40 – 1.60)	0.680
Age						
≥ 61 years	68	44	2.20 (1.47 – 3.18)	0.001		
< 61 years	70	41	2.22 (1.39 – 3.36)	<0.001	0.99 (0.55 – 1.82)	1.000
Clinical diagnosis						
MPA	38	23	1.89 (0.98 – 3.30)	0.058		
GPA	79	54	2.53 (1.76 – 3.52)	<0.001	1.34 (0.68 – 2.83)	0.478
ANCA-serotype						
MPO-ANCA	47	16	1.87 (0.93 – 3.34)	0.076		
PR3-ANCA	55	53	2.72 (1.80 – 3.93)	<0.001	1.46 (0.70 – 3.24)	0.371
Renal transplantation						
No	128	78	2.17 (1.59 – 2.91)	<0.001		
Yes	10	7	3.33 (1.08 – 7.78)	0.037	1.53 (0.48 – 3.85)	0.495
Follow-up						
0 – 5	55	6	1.60 (0.52 – 3.73)	0.414		
5 - 10 years	29	19	2.91 (1.45 – 5.21)	0.004	1.82 (0.58 – 6.67)	0.385
>10 years	54	60	2.17 (1.50 – 3.03)	<0.001	1.36 (0.53 – 4.44)	0.702
Year of diagnosis						
<2003	83	73	2.43 (1.76 – 3.26)	<0.001		
≥2003	55	12	1.34 (0.49 – 2.92)	0.586	1.81 (0.77 – 5.20)	0.220
Previous history of malignancy						
No	124	80	2.24 (1.64 – 2.98)	<0.001		
Yes	14	5	1.97 (0.54 – 5.05)	0.297	1.13 (0.41 – 4.34)	1.000

Standardized incidence ratio (SIR) (ratio of observed to expected malignancies) depicts malignancy risk compared to the general population. Relative risk (RR) depicts malignancy risk compared to the referent group. None of the RR values were statistically significant. 95% CI = 95% confidence interval (see Table 1 for other definitions).

Analysis of the clinical diagnosis and ANCA serotype subgroups showed a significantly increased malignancy risk in patients with GPA and/or with PR3-ANCA compared to the general population, but not in patients with MPA and/or MPO-ANCA. However, within the AAV cohort, the malignancy risk was not significantly different between those who had GPA and/or were positive for PR3-ANCA and those who had MPA and/or were positive for MPO-ANCA (Table 3).

Malignancy risk in patients with a history of malignancy before AAV diagnosis

Fourteen of the 138 patients had a history of malignancy before AAV diagnosis and 124 did not. The total number of malignancies that developed in these 2 groups during followup were 5 and 80, respectively. SIRs were similar between patients with and patients without a history of malignancy before the AAV diagnosis (Table 3).

Malignancy risk before and after publication of the CYCAZAREM trial in 2003

Eighty-three of the 138 AAV patients were diagnosed before 2003, and 55 in or after 2003 (Table 3). The mean ± SD duration of CYC therapy was 14.5 ± 17.4 months and 5.1 ± 4.1 months in patients diagnosed before 2003 and patients diagnosed in or after 2003, respectively ($P < 0.001$). The 83 patients diagnosed before 2003 developed a total of 73 malignancies, which resulted in a 2.43-fold increase in malignancy risk compared to the general population (95% CI 1.76-3.26). The 55 patients diagnosed in or after 2003 developed a total of 12 malignancies; the malignancy risk in these patients was not significantly increased compared to the general population (SIR 1.34 [95% CI 0.49-2.92]).

Malignancy risk in patients with renal transplants

Ten patients (7%) underwent renal transplantation during followup. The mean ± SD time from AAV diagnosis to renal transplantation was 6.7 ± 4.8 years. Two of the 10 patients with renal transplants developed a total of 7 malignancies, during a mean ± SD followup of 12.8 ± 4.8 years. These malignancies included 3 squamous cell carcinomas, 2 basal cell carcinomas, 1 prostate carcinoma, and 1 parotid carcinoma. Malignancy risk compared to the general population was significantly increased among patients with AAV who had undergone renal transplantation (SIR 3.33 [95% CI 1.08-7.78]) as well as those who had not undergone transplantation (SIR 2.17 [95% CI 1.59-2.91]). We did not observe a significant effect of transplantation on malignancy risk in these subgroups ($P = 0.495$); however, since only 10 patients in the cohort had undergone transplantation, this subanalysis was presumably not sufficiently powered to detect a significant difference.

Effects of immunosuppressive therapy on malignancy risk

Data on immunosuppressive therapy were available for 117 patients (85%) (Table 4). All 117 patients received prednisolone, 110 (94%) received CYC, 62 (53%) received azathioprine (AZA), and 24 (21%) received mycophenolate mofetil (MMF) at some time during their disease. Moreover, 4 patients (3%) received methotrexate, and 5 patients (4%) received rituximab. The mean ± SD duration of CYC therapy was 17.6 ± 17.9 months and 8.0 ± 11.8 months, respectively, in patients who did and those who did not develop a malignancy during followup ($P = 0.001$) (Table 4).

Table 4. Immunosuppressive therapy in the AAV patients

	Total sample (n=117)	No malignancy occurence (n=84)	Malignancy occurence (n=33)	p-value
Ever treatment, n (%)				
Prednisolone	117 (100)	84 (100)	33 (100)	N/A
Cyclophosphamide	110 (94)	80 (95)	30 (91)	0.374
Azathioprine	62 (53)	43 (51)	19 (58)	0.533
Mycophenolate mofetil	24 (21)	20 (24)	4 (12)	0.159
Mean duration, months (SD)				
Prednisolone	37.7 (42.3)	29.7 (39.2)	58.1 (43.7)	0.001
Cyclophosphamide	10.7 (14.4)	8.0 (11.8)	17.6 (17.9)	0.001
Azathioprine	18.5 (31.4)	14.2 (28.3)	29.5 (36.5)	0.017
Mycophenolate mofetil	7.6 (21.1)	7.5 (16.7)	7.8 (29.9)	0.943

Some of the patients with antineutrophil cytoplasmic antibody–associated vasculitis (AAV) received consecutive treatments with cyclophosphamide and/or azathioprine and/or mycophenolate mofetil (47 patients received cyclophosphamide and azathioprine, 8 patients received cyclophosphamide and mycophenolate mofetil, 1 patient received azathioprine and mycophenolate mofetil, and 13 patients received cyclophosphamide, azathioprine, and mycophenolate mofetil).

NA = not applicable (analyses on prednisolone were not possible as all patients were treated with this immunosuppressant).

The duration of CYC exposure was directly associated with malignancy risk (Table 5). The malignancy risk in patients with AAV who had received CYC for <1 year was not significantly different from that in the general population. Patients treated with CYC for 12–24 months and those treated for >24 months showed increased malignancy risks, with SIRs of 3.83 (95% CI 1.98–6.70) and 4.67 (95% CI 2.55–7.83), respectively.

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The risk of malignancy in patients treated with CYC and corticosteroids was not different from that in patients treated with AZA or MMF maintenance therapy following CYC treatment (SIR 2.62 [95% CI 1.53–4.19] and SIR 2.02 [95% CI 1.29–3.01], respectively). The average duration of CYC treatment was 14 months in patients treated with CYC and corticosteroids and 10 months in patients who received other immunosuppressive agents for maintenance therapy after CYC treatment ($P = 0.234$).

Table 5. SIRs calculated according to duration of cyclophosphamide therapy

Cyclophosphamide therapy duration	N patients	N malignancies	SIR (95% CI)	SIR p-value	RR (95% CI)	RR p-value
0 – 6 months	65	15	1.52 (0.78 – 2.64)	0.212		
6 – 12 months	21	14	1.82 (0.79 – 3.59)	0.156	1.20 (0.43 – 3.19)	0.847
12 – 24 months	16	30	3.83 (1.98 – 6.70)	<0.001	2.53 (1.04 – 6.17)	0.040
> 24 months	15	18	4.67 (2.55 – 7.83)	<0.001	3.08 (1.31 – 7.32)	0.008

Standardized incidence ratio (SIR) (ratio of observed to expected malignancies) depicts malignancy risk compared to the general population. Relative risk (RR) depicts malignancy risk compared to the referent group. 95% CI = 95% confidence interval.

Discussion

This is the first published study to investigate malignancy incidence in relation to current therapy regimens in a cohort of patients with AAV followed up for a long period (mean 10 years). During this period, the risk of malignancy was 2.21-fold higher in patients with AAV compared to the general population. This increased risk was attributable solely to the occurrence of NMSC. There was no increased risk for all other reported malignancies compared to the general population. In particular, there was no increased risk of bladder cancer, leukemia, or malignant lymphomas, in contrast to data presented in previous reports[6–11,13]. In our cohort, risk of malignancy was directly associated with the duration of CYC therapy. Interestingly, malignancy risk was not increased among patients who had been exposed to CYC for <1 year.

Previous studies on malignancy risk in AAV showed 2.4-33-fold increases in the risk of developing bladder cancer, 3.2-5.9-fold increases in the risk of developing leukemia, and 1.1-11-fold increases in the risk of developing malignant lymphomas[12]. Followup periods in those studies were ~5 years[7,9–11] or were

not reported[8, 13], except in the case of one study conducted from 1967 to 1990 with a median followup of 8 years[6]. As noted, patients in our AAV cohort had no increased risk of developing any of these malignancies after a mean followup period of 10 years. Only 1 patient in our cohort, who received CYC for 4 years, developed bladder carcinoma (9 years after AAV was diagnosed). In addition, there was 1 non-Hodgkin's lymphoma (which occurred 13 years after the AAV diagnosis). This patient was treated with CYC for 4.5 years and AZA for 3.8 years.

The discrepancies between our findings and those of previous studies likely reflect the changes in AAV treatment regimens over the years. Most previous studies were conducted from the 1960s to the 1990s[6-8, 13]. Since that time, large, international therapeutic trials have been conducted[14-16, 19], which resulted in dramatic reductions in exposure to CYC. A previous 5-year followup study of patients initially recruited in those trials demonstrated no increased risk of bladder or hematologic malignancies compared to the general population[10]. Our study validates those results and, moreover, shows that even after 10 years of followup, the risk of developing these malignancies is not increased.

The followup period of our study (1991–2013) overlaps publication of the reports of the therapeutic trials conducted by the EUVAS[14-16, 19]. In the landmark EUVAS CYCAZAREM study published in 2003, it was concluded that early withdrawal of CYC and substitution of AZA at the time of remission did not increase relapse rates in patients with AAV, and that the duration of exposure to CYC therefore could be safely reduced by switching to maintenance therapy at the time of remission achievement[19]. In our cohort, the mean duration of CYC therapy was ~3 times longer in patients diagnosed before 2003 compared to patients diagnosed in or after 2003. Interestingly, we found that the malignancy risk compared to the general population is significantly increased among AAV patients diagnosed before 2003, but not among those diagnosed later. This indicates that the reduction in CYC exposure over time has resulted in a decrease in malignancy risk among patients with AAV.

Apart from increased malignancy risk induced through immunosuppressive therapy regimens, it has been suggested that an increased risk of malignancy may arise from chronic stimulation of the immune system due to vasculitis[23]. Moreover, more severe disease with greater disease activity is generally associated with longer duration of immunosuppressive therapy; thus, it is difficult to separate disease-related and therapy-related effects on malignancy risk. Our finding of a high incidence of malignancies only after 5 years of followup (i.e., after longer exposure to immunosuppressive therapy) suggests that this therapy may have an important effect on the development of malignancies, because intrinsic disease-associated malignancies would be more likely to occur within the first 5 years. Moreover, we found that the malignancy risk in patients with AAV decreased after reduction of CYC exposure, which clearly indicates a dose-dependent effect.

Subgroup analyses demonstrated a significantly increased malignancy risk among patients with GPA and/or PR3-ANCA positivity, but not among patients with MPA and/or MPO-ANCA positivity. Possible explanations for this could be that patients with GPA and/or PR3-ANCA positivity had superior survival or a higher risk of relapses and need for more immunosuppressive treatment compared to patients with MPA and/or MPO-ANCA positivity. Further analyses demonstrated that survival was not different between patients with GPA and/or PR3-ANCA positivity and patients with MPA and/or MPO-ANCA positivity (data not shown). However, the duration of CYC treatment was significantly longer among patients with PR3-ANCA positivity than among those with MPO-ANCA positivity (mean 14 months and 8 months, respectively). This finding further supports the notion that immunosuppressive treatment exposure increases malignancy risk in patients with AAV.

Currently, immunosuppressive therapy for AAV seems to be complicated by the occurrence of NMSC. It has been suggested that AZA might accelerate NMSC development by sensitizing the skin cell genome to ultraviolet A radiation[24]. Previous studies have shown an association between AZA exposure and the occurrence of NMSC in patients with transplants, myasthenia, autoimmune inflammatory rheumatic diseases, and inflammatory bowel disease[25-29]. Our data imply that there is also an association between AZA and NMSC in patients with AAV (Table 4). In contrast, we found no difference in the occurrence of NMSC between patients treated with CYC alone and those treated with AZA following CYC therapy, despite similar durations of CYC exposure. However, our analysis may not have been sufficiently powered to detect a significant difference. Although mortality associated with NMSCs is low, their impact should not be underestimated because they can cause significant morbidity. Our results support the notion that patients with AAV who receive immunosuppressive therapy should undergo regular skin cancer screening and be advised to protect themselves against ultraviolet radiation exposure[30].

One limitation of this study is the fact that the clinical data were collected retrospectively. However, for most patients these data were accessible and recorded punctually. Moreover, the analysis was confined to a Dutch patient population, which should be taken into account in comparing our results with those from the previous multicenter European study[10]. Finally, the limited number of patients included in this study could have resulted in insufficient power to detect significant differences, particularly in the subgroup analyses. One of the strengths of the study is the long followup period. This is the first study on malignancy risk in patients with AAV with a mean followup duration as long as 10 years. Moreover, the diagnosis of AAV was confirmed histologically in all of our patients. Finally, no malignancy in our patient cohort could have been missed, due to the accurate data reporting through the Dutch National Pathology Database.

In conclusion, the results of this 10-year followup study indicate that patients with AAV have a higher risk of NMSC than the general population. There was no increased risk of bladder cancer, leukemia, or malignant lymphomas, as was previously reported in patients with AAV. With current treatment regimens, CYC treatment appears to be safe for up to 1 year without causing an increase in malignancy risk. Longer exposure to CYC increased the risk of malignancy occurrence. Our results demonstrate that with treatment regimens currently in use for AAV, the risk of developing bladder and hematologic malignancies is lower than with previous standard regimens, underscoring the success of international efforts to find less cytotoxic treatment regimens for the disease. However, other toxicities associated with current therapies, e.g., in terms of infertility and infection, remain a concern. Quite recently, rituximab was introduced as part of AAV treatment regimens, showing success and with the promise of further reducing malignancy risk in AAV patients[16, 31, 32]. Continued efforts aimed at developing additional safe and effective therapies for AAV are, nevertheless, still warranted.

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Chapter VI

Effect of rituximab on malignancy risk in patients with ANCA-associated vasculitis

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Abstract

Objectives

Patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) treated with cyclophosphamide have an increased malignancy risk compared with the general population. We investigated whether treatment with rituximab instead of cyclophosphamide has decreased the malignancy risk in patients with AAV.

Methods

The study included patients with AAV treated at a tertiary vasculitis referral centre between 2000 and 2014. The malignancy incidence in these patients was compared with the incidence in the general population by calculating standardised incidence ratios (SIRs), adjusted for sex, age and calendar year. Malignancy incidence was compared between rituximab-treated and cyclophosphamide-treated patients.

Results

Of the 323 included patients, 33 developed a total of 45 malignancies during a mean follow-up of 5.6 years. This represented a 1.89-fold increased (95% CI 1.38 to 2.53) malignancy risk, and a non-significantly increased risk if non-melanoma skin cancer was excluded (SIR, 1.09; 95% CI 0.67 to 1.69). The risk of non-melanoma skin cancer was 4.58-fold increased (95% CI 2.96 to 6.76). Cyclophosphamide-treated patients had an increased malignancy risk compared with the general population (SIR, 3.10; 95% CI 2.06 to 4.48). In contrast, rituximab-treated patients had a malignancy risk similar to the general population (SIR, 0.67; 95% CI 0.08 to 2.43). The malignancy risk in cyclophosphamide-treated patients was 4.61-fold higher (95% CI 1.16 to 39.98) than in rituximab-treated patients.

Conclusions

The malignancy risk in patients with AAV was lower in rituximab-treated patients than in cyclophosphamide-treated patients. Notably, rituximab treatment was not associated with an increased malignancy risk compared with the general population. Rituximab could therefore be a safe alternative to cyclophosphamide in the treatment of AAV.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic autoimmune disease that affects small-sized to medium-sized blood vessels in multiple organs. AAV comprises granulomatosis with polyangiitis (formerly Wegener's granulomatosis), microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome).[1] Autoantibodies against proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) assist in the diagnosis of AAV, but patients can also be negative for ANCA.[2] Although the introduction of cyclophosphamide therapy for AAV has improved patient survival considerably,[3, 4] the carcinogenic effects of cyclophosphamide put patients at increased risk of developing malignancies. Several studies have reported increased malignancy risks in patients with AAV who were treated with cyclophosphamide compared with the general population, especially for non-melanoma skin cancer, bladder cancer, malignant lymphoma and leukaemia.[5–12] Moreover, two studies found a dose–response association between cyclophosphamide and malignancy risk.[8, 13] These results are restricted to patients with granulomatosis with polyangiitis and microscopic polyangiitis, and the malignancy risk in patients with eosinophilic granulomatosis with polyangiitis has not been investigated in detail before.

International efforts have been devoted to find less cytotoxic regimens for the treatment of AAV. In particular, the cumulative cyclophosphamide doses have been lowered,[14, 15] and rituximab has emerged as a promising substitute for cyclophosphamide.[16, 17] The initial findings from randomised controlled trials showed similar treatment efficacy in patients treated with either cyclophosphamide or rituximab.[18–20] However, concerns were raised about a possible higher malignancy rate in patients treated with rituximab.[21 ,22] Notably, the trials focused on treatment efficacy; thus, their results regarding malignancy incidence should be interpreted in light of their small sample sizes and the short follow-up of a maximum of 24 months.

This study investigated the long-term malignancy risk in 323 patients with AAV. This is, to our knowledge, the first study to compare the long-term malignancy risks between patients treated with rituximab and patients treated with cyclophosphamide.

Methods

Study population

The study included patients with AAV (granulomatosis with polyangiitis, microscopic polyangiitis or eosinophilic granulomatosis with polyangiitis) who were treated at the Vasculitis and Lupus Clinic at Addenbrooke's Hospital,

Cambridge, UK, between 2000 and 2014. The diagnosis was established according to the European Medicines Agency algorithm.[23] Follow-up began on the date of diagnosis and ended on the date of death, the date the patient was lost to follow-up or on 1 July 2015, whichever occurred first. Follow-up surveillance was performed at Addenbrooke's Hospital. This study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

Clinical data

The following data were obtained from the medical records of the patients: demographic characteristics, diagnosis, date of diagnosis, ANCA serotype, organ involvement, therapy regimen, renal transplantation and the occurrence of malignancies. Patients with incomplete or missing medical records were excluded from further analyses. The cumulative doses of cyclophosphamide and rituximab during follow-up were determined. For subgroup analysis, patients were categorised according to their cyclophosphamide and/or rituximab exposure into the following categories: patients treated only with cyclophosphamide, patients treated only with rituximab, patients treated with both cyclophosphamide and rituximab, or patients who were not treated with either cyclophosphamide or rituximab. In all categories, the treatment may also have included other immunosuppressive agents, such as glucocorticoids, azathioprine, mycophenolate mofetil, methotrexate and/or tumour necrosis factor (TNF)-α inhibitors.

Standardised incidence ratio calculations

Standardised incidence ratios (SIRs) were calculated to compare the malignancy incidence between the study cohort and the general UK population, expressing the malignancy risk relative to the general population and matching for sex, age and calendar year. The SIR is the observed number of malignancies divided by the expected number of malignancies. The observed number of malignancies was the total number of primary invasive malignancies. The expected number of malignancies was the number of person-years at risk in our cohort multiplied by the malignancy incidence rates in the general UK population as obtained from the Office for National Statistics and matched for sex, 5-year age group and 1-year calendar time period.[24] Since the malignancy incidence rates were available until 2013, the malignancy incidence rate in 2013 was extrapolated to 2014 and 2015. The SIR was calculated for malignancies at all sites, for all malignancies except non-melanoma skin cancers and for each malignancy site as reported in the study population. SIRs were stratified by sex, age category at diagnosis (younger than the median age of 59 years vs 59 years or older), clinical diagnosis, ANCA serotype, renal transplantation and follow-up duration. Moreover, SIRs were compared in different treatment categories and according to the cumulative doses of cyclophosphamide and rituximab.

Statistical analyses

Student's t-test, the $\chi 2$ test, Fisher's exact test and the one-way analysis of variance (ANOVA) test were used to compare the characteristics of different subgroups (SPSS statistical software, V.23). SIR values were compared between subgroups by calculating relative risks (RRs). Exact Poisson regression analysis was used to calculate 95% CIs for the SIR and RR values assuming a Poisson distribution of the observed number of cases (SAS software, V.9.3; SAS Institute).[25–27] p Values less than 0.05 were considered significant in all analyses.

Results

Patient characteristics

The characteristics of the 323 patients with AAV included in this study are shown in table 1. The mean (SD) age at diagnosis was 56.4 (16.1) years, and the mean follow-up was 5.6 (3.2) years (1802 person-years). A total of 160 (49%) patients were diagnosed with microscopic polyangiitis; 109 patients (34%) were diagnosed with granulomatosis with polyangiitis; and 54 patients (17%) were diagnosed with eosinophilic granulomatosis with polyangiitis. Finally, 12 patients (4%) underwent renal transplantation, and 39 patients (12%) died during follow-up.

Malignancy occurrence

Of the 323 patients, 33 developed a total of 45 malignancies during follow-up. The sex, age and calendar year-adjusted malignancy risk was 1.89-fold higher in the patients with AAV than in the general population (95% CI 1.38 to 2.53) (table 2). There were 13 different malignancy types, with non-melanoma skin cancer occurring most frequently (10 basal cell carcinomas and 15 squamous cell carcinomas). The SIR for non-melanoma skin cancer was significantly increased (SIR, 4.58; 95% CI 2.96 to 6.76), while the risk for all malignancies excluding non-melanoma skin cancer was comparable to that of the general population (SIR, 1.09; 95% CI 0.67 to 1.69) (table 2).

Malignancy occurrence in the subgroups

The SIR for overall malignancy risk was stratified by gender, age, clinical diagnosis, ANCA serotype, renal transplantation and follow-up duration (supplementary table S1). Patients with eosinophilic granulomatosis with polyangiitis had the highest malignancy risk (SIR, 2.75; 95% CI 1.19 to 5.40), followed by those with granulomatosis with polyangiitis (SIR, 2.20; 95% CI 1.20 to 3.68) and those with microscopic polyangiitis (SIR, 1.59; 95% CI 1.01 to 2.38). Transplanted patients had a higher malignancy risk (SIR, 4.31; 95% CI 1.17 to 11.04) than patients who did not undergo renal transplantation (SIR, 1.79; 95% CI 1.29 to

Table 1. Characteristics of the patients with ANCA-associated vasculitis who were included in this study*

	All patients (N=323)	No malignancy occurrence (N=290)	Malignancy occurrence (N=33)	p Value†
Age (years) at diagnosis, mean (SD)	56.4 (16.1)	55.9 (16.3)	61.3 (12.7)	0.03
Follow-up (years), mean (SD)	5.6 (3.2)	5.5 (3.2)	6.3 (3.2)	0.20
Male, n (%)	149 (46)	135 (47)	14 (42)	0.65
Clinical diagnosis, n (%)				0.64
Microscopic polyangiitis	160 (49)	146 (50)	14 (42)	
Granulomatosis with polyangiitis	109 (34)	97 (33)	12 (36)	
Eosinophilic granulomatosis with polyangiitiis	54 (17)	47 (16)	7 (21)	
ANCA serotype, n (%)‡				0.89
MPO-ANCA	110 (34)	99 (34)	11 (33)	
PR3-ANCA	152 (47)	136 (47)	16 (49)	
Organ involvement, mean (SD)	2.3 (1.5)	2.3 (1.5)	2.2 (1.2)	0.85
Deaths, n (%)	39 (12)	30 (10)	9 (27)	0.01
Relapsing disease, n (%)	86 (28)	79 (28)	7 (22)	0.54
Renal transplantation, n (%)	12 (4)	11 (4)	1 (3)	1.00
Treatment, n (%)				
Glucocorticoids	318 (99)	286 (99)	32 (97)	0.33
Cyclophosphamide	233 (72)	207 (72)	26 (79)	0.38
Rituximab	155 (48)	144 (50)	11 (33)	0.07
Cyclophosphamide and rituximab	114 (35)	105 (36)	9 (27)	0.31
Azathioprine	218 (68)	196 (68)	22 (67)	0.89
Mycophenolate mofetil	154 (48)	141 (50)	13 (39)	0.31
Methotrexate	39 (12)	35 (12)	4 (12)	1.00
TNF-α inhibitors	19 (6)	15 (5)	4 (12)§	0.12

*Values are reported as means (SD) or as numbers (%).

†p Values were calculated using Student's t-test, $\chi 2$ test or Fisher's exact test.

‡ANCA serotype data were not available for 61 patients.

§Four of the 19 patients (21%) who received TNF-α inhibitors developed, in total, two basal cell carcinomas, one breast carcinoma and one prostate carcinoma. All four patients were also treated with cyclophosphamide, and one was treated with rituximab. Malignancy risk was similar in patients treated with and without a TNF-α inhibitor.

ANCA, antineutrophil cytoplasmic antibody; MPO-ANCA, myeloperoxidase-ANCA; PR3-ANCA, proteinase 3 ANCA; TNF, tumour necrosis factor.

2.43). The treatment duration and cumulative doses of cyclophosphamide and rituximab in subgroups are shown in supplementary table S2.

Table 2. SIR for malignancies overall and per observed malignancy site*

Malignancy or malignancy site	N observed malignancies	N expected malignancies	SIR (95% CI)†	p Value†
All sites	45	23.80	1.89 (1.38 to 2.53)	<0.001
Non-melanoma skin cancer	25	5.46	4.58 (2.96 to 6.76)	<0.001
All malignancies excluding non-melanoma skin cancer	20	18.33	1.09 (0.67 to 1.69)	0.76
Lung	4	2.61	1.53 (0.42 to 3.92)	0.53
Breast	3	2.82	1.06 (0.22 to 3.11)	1.00
Colon or rectum	3	1.98	1.52 (0.31 to 4.44)	0.63
Prostate	2	2.74	0.73 (0.09 to 2.64)	0.97
Bladder	1	0.65	1.53 (0.04 to 8.57)	0.96
Pancreas	1	0.52	1.94 (0.05 to 10.81)	0.81
Testis	1	0.04	24.66 (0.62 to 137.41)	0.08
Ovary	1	0.39	2.54 (0.06 to 14.14)	0.65
Melanoma	1	0.66	1.52 (0.04 to 8.49)	0.96
Tongue	1	0.07	13.70 (0.35 to 76.34)	0.14
Central nervous system	1	0.25	3.94 (0.10 to 21.95)	0.45
Kidney	1	0.49	2.03 (0.05 to 11.32)	0.78

*SIR is the ratio of the observed to expected malignancies adjusted for sex, age (per 5-year age group) and calendar time period (per 1-year calendar time period).
†Calculated by exact Poisson regression analysis.
SIR, standardised incidence ratio.

Effects of cyclophosphamide and rituximab on malignancy risk

Patients treated only with cyclophosphamide had a 3.10-fold higher (95% CI 2.06 to 4.48) malignancy risk than the general population (table 3), and a 1.14-fold higher (95% CI 0.49 to 2.25) malignancy risk if non-melanoma skin cancer was excluded. Patients treated only with rituximab had no increased malignancy risk compared with the general population (SIR, 0.67; 95% CI 0.08 to 2.43), which was similar if non-melanoma skin cancer was excluded (SIR, 0.88; 95% CI 0.11 to 3.19). The malignancy risk in patients treated only with cyclophosphamide was 4.61-fold higher (95% CI 1.16 to 39.98) than in patients treated only with rituximab and was 3.05-fold higher (95% CI 1.40 to 7.35) than in patients treated with both cyclophosphamide and rituximab (table 4). The mean cumulative cyclophosphamide dose was lower in patients treated only with cyclophosphamide

Table 3. SIR stratified according to treatment category*

Treatment†	N patients	SIR (95% CI)‡	SIR p value‡	Cyclophosphamide cumulative dose (g), mean (SD)§	Follow-up (years), mean (SD)‖	Organ involvement, mean¶
Only cyclophosphamide	119	3.10 (2.06 to 4.48)	<0.001	7.26 (4.94)	4.92 (3.10)	2.11 (1.49)
Only rituximab	41	0.67 (0.08 to 2.43)	0.86	0.00	6.34 (3.56)	2.35 (1.09)
Both	114	1.01 (0.46 to 1.93)	1.00	11.05 (11.63)	6.60 (2.84)	2.56 (1.63)
None	48	2.10 (0.77 to 4.56)	0.14	0.00	4.20 (2.94)	1.96 (1.44)

*Values are reported as means (SD) unless otherwise indicated. The SIR is the ratio of the observed to expected malignancies adjusted for sex, age (per 5-year age group) and calendar time period (per 1-year calendar time period).

†The 'only cyclophosphamide' group was treated with cyclophosphamide but not with rituximab. The 'only rituximab' group was treated with rituximab but not with cyclophosphamide. 'Both' indicates a group that received cyclophosphamide and rituximab. 'None' indicates a patient group that neither received cyclophosphamide nor rituximab, but instead had various heterogeneous treatments including glucocorticoids, azathioprine, mycophenolate mofetil and methotrexate. Other immunosuppressive drugs were also administered in all of the groups.

‡Calculated by exact Poisson regression analysis.

§The mean cumulative cyclophosphamide dose differed between the 'only cyclophosphamide' and 'both' groups (Student's t-test, p=0.002).

¶The mean follow-up duration differed between groups (ANOVA, p<0.001). The mean follow-up duration also differed when the 'only rituximab' and 'both group' were compared with the 'only cyclophosphamide' and 'none' group (Student's t-test, p<0.001).

**The mean organ involvement did not differ between groups (ANOVA, p=0.07).

ANOVA, analysis of variance; SIR, standardised incidence ratio.

than in patients treated with both cyclophosphamide and rituximab (7.3 g vs 11.1 g; p=0.002). The duration of follow-up was longer for patients who received rituximab than for patients who did not receive rituximab (p<0.001). In terms of mean organ involvement, the disease extent did not differ between the treatment groups (p=0.07) (table 3). Patients treated with cyclophosphamide received azathioprine maintenance therapy more frequently than those treated with rituximab (81% vs 42%; p<0.001). The SIR of malignancy for patients receiving a combination of cyclophosphamide and azathioprine was 3.20 (95% CI 2.05 to 4.76; p<0.001), whereas patients receiving a combination of rituximab and azathioprine expressed a comparable malignancy risk to that of the general population (SIR, 1.52; 95% CI 0.18 to 5.50; p=0.38).

Table 4. Relative risks (RR) according to treatment category

Treatment*	RR (95% CI)†	p Value†
Only cyclophosphamide versus only rituximab	4.61 (1.16 to 39.98)	0.03
Only cyclophosphamide versus both	3.05 (1.40 to 7.35)	0.003
Only cyclophosphamide versus none	1.48 (0.60 to 4.36)	0.52

*The 'only cyclophosphamide' group was treated with cyclophosphamide but not with rituximab. The 'only rituximab' group was treated with rituximab but not with cyclophosphamide. 'Both' indicates a group that received cyclophosphamide and rituximab. 'None' indicates a group that did not receive cyclophosphamide or rituximab. Other immunosuppressive drugs were also administered in all of the groups.

†RR represents the risk of malignancy compared with the reference group. Calculated by exact Poisson regression analysis.

Effects of cumulative cyclophosphamide and rituximab doses on malignancy risk

The mean (SD) cumulative cyclophosphamide and rituximab doses were 9.1 (9.0) g and 5.9 (3.4) g, respectively. The highest cyclophosphamide dose was 108 g, given intermittently for 7.6 years, during a follow-up period of 8.1 years, in which the patient experienced no relapses. The highest rituximab dose was 18 g, given intermittently over 6.1 years, during a follow-up period of 9.1 years, in which one relapse occurred. A positive dose–response relationship was found between cyclophosphamide therapy and the overall malignancy risk (table 5), and between cyclophosphamide therapy and the risk of non-melanoma skin cancer (supplementary table S3). The opposite relationship was found for patients treated with rituximab: the higher the cumulative rituximab dose, the lower the overall malignancy risk (table 5), and the lower the risk of non-melanoma skin cancer (supplementary table S3). Patients who did not receive rituximab had a 2.86-fold higher (95% CI 1.98 to 3.99) malignancy risk than the general population. No increased risk was observed when patients had a cumulative rituximab dose below

6.0 g (SIR, 1.41; 95% CI 0.57 to 2.90). A total of 83 patients received more than 6.0 g rituximab, and these patients had a non-significantly lower malignancy risk than the general population (SIR, 0.45; 95% CI 0.09 to 1.32) and a 6.32-fold lower (95% CI 1.99 to 32.15) malignancy risk than patients who did not receive rituximab (table 5). The cumulative cyclophosphamide and rituximab doses individually received by the patients who developed a malignancy during follow-up are shown in supplementary table S4.

Table 5. SIR stratified according to cumulative cyclophosphamide and rituximab doses*

Cumulative dose (g)	N patients	N observed malig-nancies	SIR (95% CI)†	SIR p value†	RR (95% CI)†	RR p value†
Cyclophos-phamide						
0	89	8	1.37 (0.59–2.70)	0.47	1 (reference)	
0.1–20	207	31	1.91 (1.30–2.71)	0.001	1.39 (0.63–3.50)	0.52
20–108	16	5	5.06 (1.64–11.82)	0.007	3.69 (0.95–12.78)	0.06
Rituximab						
0	167	34	2.86 (1.98–3.99)	<0.001	1 (reference)	
0.1–6	70	7	1.41 (0.57–2.90)	0.47	0.49 (0.18–1.13)	0.11
6–18	83	3	0.45 (0.09–1.32)	0.10	0.16 (0.03–0.50)	<0.001

*SIR is the ratio of the observed to expected malignancies adjusted for sex, age (per 5-year age group) and calendar time period (per 1-year calendar time period). SIR represents the malignancy risk compared with the general population, and the RR represents the malignancy risk compared with the reference group.

†Calculated by exact Poisson regression analysis.

RR, relative risk; SIR, standardised incidence ratio.

Discussion

This study compared the malignancy risks in patients with AAV treated with rituximab versus cyclophosphamide. Strikingly, patients treated with cyclophosphamide had a 4.61-fold higher risk than those treated with rituximab. In patients treated with cyclophosphamide, the malignancy risk was 3.10-fold higher than in the general population; in contrast, patients treated with rituximab did not show an increased risk compared with the general population. Patients treated with both rituximab and cyclophosphamide (N=114) had a lower malignancy risk than those treated with only cyclophosphamide, even though the mean cyclophosphamide dose was lower in the latter group. In addition,

there was a non-significant trend towards an inverse dose–response relationship between the cumulative rituximab dose and malignancy risk: the more rituximab a patient received, the lower the malignancy risk, with the risk actually falling below the risk in the general population if more than a cumulative dose of 6.0 g was given. The relative risk for developing a malignancy was more than six times lower in patients who had received a cumulative dose of rituximab of more than 6.0 g than in patients who had not received rituximab at all.

Interestingly, our findings – although the number of patients was relatively low – may point towards the possibility that rituximab has a protective role in the development of malignancies. This hypothesis is underlined by data showing a trend of an inverse dose–response relationship, and by the difference in malignancy development of the combined treatment group (i.e., patients receiving both cyclophosphamide and rituximab). Depletion of B cells due to rituximab may increase antitumour immunity, as was demonstrated in mouse models in which B-cell-deficient mice are resistant to the development of certain malignancies.[28, 29] The enhanced antitumour immune response in these mice is probably caused by decreased IL-10 production by B cells, leading to enhancement of the antitumour effects of cytotoxic T cells.[28] There is emerging evidence that regulatory B cells are the main mediators of this mechanism.[30] In humans, the hypothesis that rituximab enhances the antitumour immune response is supported by the trend towards a lower risk of developing a second primary malignancy in patients with non-Hodgkin's lymphoma treated with rituximab-containing chemotherapy compared with patients treated with chemotherapy that does not include rituximab.[31, 32] However, clarification of the effects of B-cell depletion on antitumour immunity in humans requires further investigation.

The increased risks of bladder and haematological malignancies that have been previously reported for patients treated with cyclophosphamide did not materialise in this study, possibly reflecting the ongoing efforts to reduce cumulative cyclophosphamide doses.[11] In accordance with two recent studies, only the risk of non-melanoma skin cancer was increased in the current study.[9, 11] To prevent the development of these lesions, all patients were given written information concerning the risks of non-melanoma skin cancer. Moreover, they were advised to avoid ultraviolet radiation, to use sunscreens and to promptly report skin lesions. Of the patients who developed non-melanoma skin cancer despite these preventative measures, the majority had received azathioprine as maintenance therapy before the occurrence of this malignancy. Therefore, the previously reported association between non-melanoma skin cancer and azathioprine exposure is confirmed in our study.[33–37] However, in our study, only the combination of cyclophosphamide and azathioprine treatment was associated with an increased malignancy risk. In contrast, patients treated with rituximab and azathioprine had a malignancy risk similar to the general population. Lowering cyclophosphamide and azathioprine exposure will most likely decrease

the malignancy risk. For patients with AAV who receive azathioprine, especially those who received cyclophosphamide as induction therapy, regular skin cancer screening should be started to control and prevent the development of non-melanoma skin cancers. Moreover, patients should be advised as to how to protect themselves against ultraviolet radiation.[38]

Previous studies that investigated the malignancy risk in patients with AAV were restricted to patients with granulomatosis with polyangiitis and microscopic polyangiitis, and did not include patients with eosinophilic granulomatosis with polyangiitis. Eosinophilic granulomatosis with polyangiitis has a lower incidence than granulomatosis with polyangiitis and microscopic polyangiitis, and it is treated similarly.[39] The 54 patients with eosinophilic granulomatosis with polyangiitis who were included in this study had a 2.75-fold increased malignancy risk compared with the general population. We therefore recommend that clinicians monitor patients with eosinophilic granulomatosis with polyangiitis for malignancies as carefully as patients with granulomatosis with polyangiitis and microscopic polyangiitis.

One limitation of this study is its retrospective design. However, it excluded patients with unclear or missing data. A second limitation is the relative short follow-up, with a mean of 5.6 years. Longer follow-up studies are now required to validate our findings. A third limitation of this study is the relatively small number of patients, particularly in the subgroup analyses. This could explain the non-significance of the inverse dose–response relationship between rituximab and malignancy risk. This relationship merits further investigation in larger studies. Finally, the study involved just one medical centre, so the findings may not be generalisable to other settings. One strength of this study is the large study population, in which, for the first time, the malignancy risk was evaluated in patients treated with rituximab during long-term follow-up. This is also the first study to analyse the malignancy risk in patients with eosinophilic granulomatosis with polyangiitis. Another strength of our study is the calculation of cumulative cyclophosphamide and rituximab doses. Finally, the calculation of sex, age and calendar-year period-matched SIRs ensured reliable comparisons between our cohort and the general population.

In conclusion, we demonstrated that patients with AAV who are treated with rituximab have a decreased burden of malignancy, which surpasses expectations from clinical trials data.[18, 19] Moreover, our results suggest that rituximab may protect against the occurrence of malignancies, a possibility that should be explored in further detail using larger cohort populations. Patients with AAV treated with rituximab had a strikingly lower malignancy risk than those treated with cyclophosphamide and no increased malignancy risk compared with the general population. Therefore, the rituximab dose currently used in clinical practice could be a safe alternative to cyclophosphamide in the treatment of AAV.

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Chapter VII

Summary and general discussion

More than 150 years after the publication of the first case-report of a patient with, what is now considered to be, antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, considerable progress has been made in the understanding of the pathogenesis, the diagnostic approach, and the treatment of this disease.[1] Research in ANCA-associated vasculitis has come a long way. However, the heart of the matter is still unsolved, as the exact nature of this complex disease and, consequently, the most appropriate disease classification system and optimal treatment strategies are still not unravelled. In this thesis, several aspects of ANCA-associated vasculitis concerning genetics, clinical and histopathological classification, and long-term outcome were investigated.

Genetics and disease classification

Despite the considerable progress made in recent decades, the pathophysiology of ANCA-associated vasculitis is still not fully unravelled. Amongst others, genetic factors are believed to contribute to the pathogenesis of this complex disease.[2] In **Chapter 2** of this thesis we conducted a meta-analysis to investigate the genetic variants that are most likely associated with ANCA-associated vasculitis. To increase the validity of our meta-analysis, we included raw data from a large genome-wide association study (GWAS).[3] This study provides the first complete and comprehensive overview of all genetic variants investigated in ANCA-associated vasculitis in at least two independent studies.

Thirty-three genetic variants, located in or near 15 genes, were found to be associated with ANCA-associated vasculitis. The genetic variants were located in or near the following genes: *CD226, CTLA-4, FCGR2A, HLA-B, HLA-DP, HLA-DQ, HLA-DR, HSD17B8, IRF5, PTPN22, RING1/RXRB, RXRB, STAT4, SERPINA1,* and *TLR9.* The results of this meta-analysis confirm that genetic factors contribute to the pathogenesis of ANCA-associated vasculitis. In particular, they support a role for the major histocompatibility complex (MHC), the innate and adaptive immune system, and several inflammatory processes in the pathogenesis of this complex disease; the identified genetic factors could potentially result in altered HLA mediated antigen presentation, inadequate T- and B-lymphocyte activation, and abnormal target antigen structure and/or function. The mechanisms whereby and the extent to which these genetic variants directly cause disease requires further investigation.

Most importantly, the results of our meta-analysis provide clear evidence of a genetic susceptibility that differed between granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) patients and between proteinase 3 (PR3)-ANCA positive and myeloperoxidase (MPO)-ANCA positive patients. Moreover, the associations were primary aligned with ANCA-serotype rather than with the clinically defined syndromes: in 76% of the genetic variants,

subdivision based on ANCA-serotype resulted in higher odds ratios than subdivision based on clinical diagnosis.

Considerable debate surrounds the classification of ANCA-associated vasculitis. GPA and MPA have overlapping clinical features, and while patients with typical characteristics are easy to classify, many patients are allocated inconsistently even by experienced clinicians. Moreover, even though the Chapel Hill Consensus Conference (CHCC) and European Medicines Agency (EMA) classification systems use identical names for the ANCA-associated vasculitis disease categories, a study performed by Lionaki *et al.* demonstrated substantial discrepancies between the two classification systems in the allocation of patients to these categories: 78% of patients classified as having MPA using the CHCC system were considered to have GPA by the EMA system.[4] The authors argued that this resulted in part from differences in disease definitions. For instance, the EMA system over-represents the diagnostic category of GPA because, according to the American College of Rheumatology criteria, essentially any upper respiratory involvement is considered to be GPA.[5] Overall, the inconsistencies between clinicians and between the classification systems and the lack of diagnostic criteria make the use of the terms GPA and MPA less accurate and less predictive of outcome.

Interestingly, a number of clinical studies demonstrated that disease course, treatment response, and patient outcome align more closely with ANCA-serotype than with clinical diagnosis. Lionaki *et al.* demonstrated that ANCA-serotype is closely associated with clinical features and the relative frequency of organ system involvement.[4] ANCA-serotype was shown to be more strongly associated with relapse than clinical diagnosis, and patients with PR3-ANCA positive GPA were shown to have a significantly different disease course than patients with MPO-ANCA positive GPA.[4,6] Unizony *et al.* investigated treatment response in patients enrolled in the Rituximab in ANCA-associated Vasculitis (RAVE) trial according to both clinical diagnosis (GPA versus MPA) and ANCA-serotype (PR3-ANCA versus MPO-ANCA).[7] Treatment response did not differ between GPA and MPA patients. In contrast, treatment response did differ between PR3-ANCA positive and MPO-ANCA positive patients: PR3-ANCA positive patients were shown to respond better to rituximab than to the combination of cyclophosphamide and azathioprine. These findings have led to the following question: is it the name of the disease or is it the serotype that is more important with respect to disease classification and individual patient prognosis?

Our genetic findings taken together with the clinical findings discussed previously underscore the need to account for ANCA-serotype in the classification of ANCA-associated vasculitis. Arguments to incorporate ANCA-serotype in the classification of ANCA-associated vasculitis include:

- Distinct genetic subsets correlate more strongly with ANCA-serotype than with clinical classification
- ANCA-serotype correlates better with clinical features than clinical classification
- ANCA-serotype is more reproducible and reliable than clinical classification
- ANCA-serotype better reflects the disease mechanism

However, there are problems with using only ANCA-serotype to classify patients. For example, using current assays, 5-10% of ANCA-associated vasculitis patients are ANCA-negative.[8-13] Moreover, better understanding of the granuloma formation in ANCA-associated vasculitis may reveal therapies that specifically target this component of the disease, which requires acknowledging this phenotype. Therefore, for now, a classification system based primarily on ANCA-serotype with subsequent subdivision based on clinical diagnosis – e.g. PR3-ANCA positive GPA – may be the best way to characterize patients until we truly understand the similarities and differences in the pathogenesis of the ANCA-associated vasculitides. This will provide practical classification criteria better aligned to patient phenotype, treatment responses, and outcomes, and will allow logical design and testing of future therapies.

In **Chapter 3**, we focused on ear, nose, and throat (ENT) involvement and ANCA-serotype with respect to renal histology at diagnosis and renal outcome. Previously, Hauer *et al.* demonstrated that MPO-ANCA positive vasculitis patients have more chronic lesions in their renal biopsies than PR3-ANCA positive vasculitis patients. The authors hypothesized that this finding may reflect an association of MPO-ANCA with a clinical phenotype of smouldering disease.[14] This could be indicative of different disease mechanisms in PR3-ANCA vasculitis and MPO-ANCA vasculitis, in line with the results presented in **Chapter 2**. However, PR3-ANCA positivity is also closely correlated with ENT disease.[15, 16] Thus, another hypothesis for the fewer chronic lesions in the renal biopsies of PR3-ANCA positive patients is that the clinically overt ENT manifestations in these patients lead to earlier diagnosis of ANCA-associated vasculitis by reducing patient's and/or doctor's delay.

In our study, patients with ENT involvement had fewer chronic lesions in their renal biopsies and better renal function at diagnosis and 5-year follow-up than patients without ENT involvement. Our results suggest that this is not caused by earlier diagnosis due to the clinically overt ENT symptoms: in the same patients, other manifestations that would also have reduced patient and/or doctor's delay, such as lung involvement, cutaneous manifestations, and

arthralgia/arthritis, were not associated with renal histology or renal outcome. To exemplify this point further; a study by Bligny *et al.* reported higher survival in ANCA-associated vasculitis patients with ENT involvement than patients without ENT involvement.[17] The authors argued that this is not likely caused by earlier diagnosis and treatment, as 87% of patients without ENT involvement had lung involvement, which, theoretically, also would have led to earlier diagnosis. Interestingly, a study by Poulton *et al.* even demonstrated an increased diagnostic delay in ANCA-associated vasculitis patients with ENT involvement.[18] The authors argued that both physicians and patients focused on the more common causes of the ENT symptoms instead of regarding them as signs of vasculitis. As could be expected, patients with skin manifestations often had a prompt diagnosis of ANCA-associated vasculitis.[18]

In our study, ENT involvement overruled ANCA-serotype in the multivariable analysis and was still associated with better renal outcome in a sub-analysis including only PR3-ANCA positive patients. Thus, independent of ANCA-serotype, ENT involvement itself seems to be associated with better renal histology and outcome. The findings of this study can be viewed in the context of the two distinct pathological features in GPA, namely: vasculitis, which is predominantly localized in the kidneys, and granulomatosis, which predominantly involves the airways.[19] The clinical heterogeneity of GPA reflects a spectrum of manifestations ranging from predominantly vasculitic disease at one end to predominantly granulomatous disease at the other. Thus, in our study, the patients with ENT involvement could have had a predominantly granulomatous disease, while the patients without ENT involvement had a predominantly vasculitic disease. In this light, the better renal prognosis in the patients with ENT involvement could be representative of the more benign renal course of 'granulomatous GPA' compared to 'vasculitic GPA'.

The pathological pathways leading to vasculitis or granuloma formation are hypothesized to be different. Immunological studies indicate that these two distinct pathological features correspond to different T cell immune responses; i.e. a T helper cell 1 (Th1)-type response in granulomatous disease and a Th2-type response in vasculitic disease.[20-22] Further studies are needed to adequately assess the potential prognostic value of ENT involvement, which, in turn, might provide further insight into the pathogenesis of GPA.

Histopathological classification of ANCA-associated glomerulonephritis

The histopathological classification of ANCA-associated glomerulonephritis was devised by an international working group of renal pathologists and nephrologists with the aim of further adding to the prognostication of patients with ANCA-

associated glomerulonephritis.[23] Thus far, 21 validation studies, consisting of cohorts from Asia, North-America, Australia, and Europe, have investigated the prognostic value of the classification system.[24-44] **Chapter 4** reviews the findings of these validation studies and puts them into a broader perspective. In general, the studies confirmed the predictive value of the classification system for renal outcome in the focal and sclerotic classes. However, several studies showed conflicting results with respect to the crescentic and mixed classes.[24-37]

A number of factors may have contributed to the inconsistent results between the validation studies. As indicated by their denominator, biopsies in the mixed class may indeed show mixed lesions in which either an acute or chronic phenotype can predominate. Therefore, differences in the compositions of the mixed classes in the various validation studies may have contributed to the different outcomes reported for this class. It could be argued that, in its current form, this class is too heterogeneous to have a straightforward predictive value in the classification system. A second explanation for the inconsistent results between the validation studies might be a problem of definitions; the crescentic class was originally defined as the predominance of cellular crescents, however, this definition may have lacked important details leading to inter-observer discrepancies among pathologists.[23, 29] Differences in patient population and treatment most probably also contributed to the variable results between the validation studies. Unfortunately, due to their retrospective design, it was not possible to fully account for treatment in most of the validation studies. A number of validation studies suggested that inclusion of tubulointerstitial parameters could increase the predictive value of the classification system. In contrast, Berden *et al.* argued that inclusion of tubulointerstitial parameters does not add meaningfully to the predictive value of the classification system and only increases its complexity.[23]

Currently ongoing studies that aim at the optimization of the histopathological classification of ANCA-associated glomerulonephritis are discussed in the 'Future perspectives' section of this chapter.

Treatment and outcome

Over the last decades, the introduction of immunosuppressive treatment has improved the prognosis of patients with ANCA-associated vasculitis dramatically. Maintenance of remission, on the other hand, still represents a challenge, necessitating prolonged immunosuppressive treatment.[45] Despite the clear success of conventional immunosuppressive therapies, their long-term side effects jeopardize outcomes. One of the long-term side effects of immunosuppressive treatment is the occurrence of malignancies.[46]

Chapter 5 reports the results of the first 10-year follow-up study investigating malignancy risk in patients with ANCA-associated vasculitis treated with current

treatment regimens. In these patients, overall malignancy risk was 2.21 times increased compared to the general population. This increased malignancy risk was attributable solely to the increased risk of non-melanoma skin cancer (NMSC). Importantly, in contrast to previous studies,[47] in our study the risks of bladder cancer, lymphomas, and leukaemia were not increased. These findings most likely reflect the decrease in cyclophosphamide exposure in the treatment of ANCA-associated vasculitis in the last decade.[8-10, 13]

The close relationship between disease activity and immunosuppressive treatment exposure makes it difficult to distinguish disease-related from treatment-related effects on malignancy risk. Nonetheless, a subgroup analysis – based on year of diagnosis and subsequent treatment – demonstrated that malignancy risk was no longer significantly increased after the major reduction in cyclophosphamide exposure that resulted from the publication of the CYCAZAREM trial.[10] Moreover, malignancy risk was directly correlated to the duration of cyclophosphamide treatment.

In summary, the data reported in **Chapter 5** demonstrate the effectiveness of international efforts to reduce toxicity in the treatment of ANCA-associated vasculitis. Despite the advances achieved, the road towards treatment modalities with less adverse effects in terms of, amongst others, infection risk, infertility, and malignancy occurrence in patients with prolonged cyclophosphamide exposure, is still long. Recently, rituximab was successfully introduced in the treatment of ANCA-associated vasculitis with the aim of, amongst others, further reducing these adverse treatment effects.[13, 48, 49]

Chapter 6 reports the first study that compares the long-term malignancy risks between rituximab-based and cyclophosphamide-based treatment in ANCA-associated vasculitis. Strikingly, malignancy risk was 4.61 fold increased in patients treated with cyclophosphamide compared to patients treated with rituximab. Malignancy risk was 3.10 fold increased in patients treated with cyclophosphamide compared to the general population. This increased malignancy risk was again solely attributable to the occurrence of NMSC. In contrast, malignancy risk was not increased in patients treated with rituximab. Interestingly, patients treated with both rituximab and cyclophosphamide had a lower malignancy risk than patients treated with cyclophosphamide alone, despite the mean cumulative cyclophosphamide dose being lower in the latter group. In addition, there was a trend towards an inverse dose-response relationship between the cumulative rituximab dose and malignancy risk, i.e., the more rituximab a patient received, the lower the malignancy risk. Notably, patients who cumulatively received more than 6.0g rituximab had a lower malignancy risk than the general population. This effect was observed for both haematological and non-haematological malignancies.

These findings hint towards the possibility that rituximab has an inhibitory effect on (haematological and non-haematological) malignancy occurrence. B cell

depletion – the mechanism of action of rituximab – was shown to increase anti-tumour immunity in murine models.[50, 51] This enhanced anti-tumour immune response is hypothesized to be caused by decreased IL-10 production by B regulatory cells, leading to enhancement of the anti-tumour effects of cytotoxic T cells.[50] In humans, the hypothesis that rituximab enhances the anti-tumour immune response is supported by the trend towards a lower risk of developing a second primary (non-haematological) malignancy in patients with non-Hodgkin lymphoma treated with rituximab-containing chemotherapy, compared to patients treated with conventional chemotherapy not including rituximab.[52, 53] Clearly, elucidation of the effects of B-cell depletion on anti-tumour immunity requires further investigation.

In conclusion, the results of the study reported in **Chapter 6** demonstrate that patients with ANCA-associated vasculitis treated with rituximab have a decreased burden of malignancy, surpassing expectations from clinical trial data.[54, 55] Moreover, our results suggest that rituximab may have an inhibitory effect on malignancy occurrence. Therefore, regarding the occurrence of malignancies, rituximab seems a superior alternative to cyclophosphamide in the treatment of ANCA-associated vasculitis.

Future perspectives

Despite the major advances made in the understanding of the pathogenesis, the diagnostic approach, and the treatment of ANCA-associated vasculitis, many questions are still unanswered and new questions arise every day. The results of the meta-analysis presented in this thesis confirm that genetic factors contribute to the pathogenesis of ANCA-associated vasculitis. However, functional studies remain to be performed to establish the precise role of these genetic variants and their effects on the disease process. Future studies in genetics and epigenetics will likely not only lead to a deeper understanding of the pathogenesis of ANCA-associated vasculitis, but perhaps also to a personalised medicine approach to clinical management and therapeutic target selection. In systemic lupus erythematosus, researchers have developed a personalized transcriptional immunomonitoring approach that enables patient stratification based on molecular networks best correlating with disease progression.[56] The development of such a personalized transcriptional immunomonitoring approach in ANCA-associated vasculitis would create some order in this complex heterogeneous disease, bringing patient-tailored treatment one step closer.

The genetic differences between PR3-ANCA vasculitis and MPO-ANCA vasculitis indicate that future genetic and clinical studies should be sufficiently powered to allow for independent analysis of PR3-ANCA positive and MPO-ANCA positive patients. Currently, a GWAS including GPA and MPA patients

is being conducted that is sufficiently powered to investigate genetic associations in the PR3-ANCA and MPO-ANCA subgroups independently.[57] Moreover, an ongoing GWAS is investigating whether there are genetic differences between MPO-ANCA positive and MPO-ANCA negative patients with eosinophilic granulomatosis with polyangiitis (EGPA).[57] Preliminary results of this GWAS point towards profound differences in the pathogenesis of these two EGPA subtypes and suggest a number of therapeutic approaches that might be effective in EGPA.

Current treatment strategies in ANCA-associated vasculitis do not take into account specific patient and disease characteristics. However, genetic and clinical data suggest that treatment should be tailored to specific parameters. Possible parameters that, now or in the future, could be taken into consideration for refining treatment, in the form of patient-tailored therapy, are: genetic markers, ANCA-serotype, severity scores, and scores and/or markers predicting relapse. A step in this direction is set by the identification of altered gene expressions that can predict treatment response in MPA patients.[58] The MAINRITSAN 2 trial (ClinicalTrials.gov NCT01731561) is currently investigating treatment response in rituximab treatment according to a fixed administration protocol versus individually tailored rituximab treatment based on ANCA-status and peripheral blood CD19 lymphocyte reappearance. In refractory rheumatoid arthritis, similar parameters (rheumatoid factor, anti-CCP antibodies, and serum IgG concentration) were shown to predict treatment response to rituximab.[59]

Presently, we are stepping into a new therapeutic era with targeted therapies – e.g. monoclonal antibodies such as rituximab – that could further improve the treatment of ANCA-associated vasculitis. This is a field for ongoing and future trials that investigate the optimal dosing and duration of rituximab both as induction and maintenance treatment. The currently ongoing RITAZAREM trial (ClinicalTrials.gov NCT01697267) will provide the largest trial dataset for the use of rituximab as remission-induction treatment for ANCA-associated vasculitis. This trial compares rituximab and azathioprine maintenance treatment following rituximab induction-treatment, and explores whether prolonged B-cell depletion leads to sustained treatment-free remission after treatment discontinuation. Although the first studies, as demonstrated in this thesis, indicate that rituximab treatment has resulted in less morbidity in the long run, more long-term follow-up studies with longer follow-up are warranted.

Moreover, it becomes more and more apparent that the burden of corticosteroids is responsible for a substantial part of the treatment-related adverse events in ANCA-associated vasculitis. Several ongoing studies are investigating different dosing regimens of steroids regarding efficacy and safety. The currently ongoing PEXIVAS trial (ClinicalTrials.gov NCT00987389) is the largest trial conducted in ANCA-associated vasculitis thus far and has recruited more than 700 patients from over 100 centres over the world. This 2-by-2 trial addresses the

effect of a reduced cumulative dosing regimen of glucocorticoids and the effect of adjuvant plasma exchange in patients with severe ANCA-associated vasculitis.

Regarding histology, future studies might answer the question whether renal biopsy findings will be directly useful to guide therapeutic decision-making. A large international validation study of the histopathological classification of ANCA-associated glomerulonephritis in which histopathological, clinical, and therapeutic data are being incorporated is currently being conducted. Moreover, the biopsies included in the 21 previously performed validation studies are now being collected with the aim of re-evaluating them by a group of experienced pathologists. The aim of these studies is to evaluate the predictive value of the classification system, formulate clearer and more concise definitions, and possibly also revise the histopathological classification of ANCA-associated glomerulonephritis.

Overall, the vasculitis patient and research communities can be optimistic about the future of research in ANCA-associated vasculitis and the potential for this work to unravel the puzzle of this complex disease and, most importantly, to improve the lives of patients with ANCA-associated vasculitis.

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Chapter VIII

Samenvatting in het Nederlands

Vasculitis betekent letterlijk: ontsteking van bloedvaten. Ontstekingen van bloedvaten kunnen overal in het lichaam voorkomen. In de meeste gevallen leiden zij, afhankelijk van de ernst van de ontstekingen, in meer of mindere mate tot functieverlies van de betrokken organen. Dit proefschrift gaat specifiek over vasculitis die gekenmerkt wordt door de aanwezigheid van anti-neutrofiele cytoplasmatische antilichamen (ANCA) in het bloed van patiënten. ANCA zijn afweerstoffen die gericht zijn tegen componenten in het celvocht (cytoplasma) van bepaalde typen witte bloedcellen (neutrofiele granulocyten en monocyten). De klassieke eiwitten waar ANCA zich tegen richten zijn proteïnase 3 (PR3) en myeloperoxidase (MPO).

ANCA-geassocieerde vasculitis omvat de volgende ziektebeelden:

- granulomatose met polyangiitis (GPA, voorheen de ziekte van Wegener genoemd);
- microscopische polyangiitis (MPA);
- eosinofiele granulomatose met polyangiitis (EGPA, voorheen Churg-Strauss-syndroom genoemd).

De meeste patiënten met GPA hebben PR3-ANCA in het bloed en de meeste patiënten met MPA hebben MPO-ANCA in het bloed. Ongeveer de helft van de EGPA patiënten heeft MPO-ANCA in het bloed, de andere helft heeft geen ANCA in het bloed. ANCA lijken een rol te spelen bij het ontstaan van ANCA-geassocieerde vasculitis; onder bepaalde omstandigheden activeren ANCA witte bloedcellen. Deze geactiveerde witte bloedcellen veroorzaken de bloedvatontstekingen.

Patiënten met ANCA-geassocieerde vasculitis hebben vaak 'algemene' klachten zoals malaisegevoel en gewichtsverlies. Daarnaast zijn vaak het keel-, neus- en oorgebied (KNO-gebied), de longen en de nieren van deze patiënten aangedaan. De ziekte kan zich ook manifesteren in de gewrichten, het zenuwstelsel en het maagdarmstelsel. Bij het uitblijven van een behandeling overlijden de meeste patiënten met ANCA-geassocieerde vasculitis kort na het stellen van de diagnose. Zonder behandeling is één jaar na de diagnose nog slechts één op de vijf patiënten in leven. De behandeling van ANCA-geassocieerde vasculitis bestaat uit ontstekingsremmende en afweeronderdrukkende medicijnen. Hoewel deze behandeling op korte termijn erg effectief is, keert de ziekte bij ongeveer de helft van de patiënten na verloop van tijd terug. Bovendien hebben deze medicijnen ernstige bijwerkingen waar patiënten zelfs aan kunnen overlijden.

Ongeveer 150 jaar geleden werd in de wetenschappelijke literatuur de eerste patiënt met ANCA-geassocieerde vasculitis beschreven. Sindsdien is de kennis omtrent het ziekteproces en de behandeling van dit complexe ziektebeeld aanzienlijk toegenomen. Vraagstukken omtrent de oorzaak van ANCA-geassocieerde vasculitis, de meest geschikte ziekteclassificatiesystemen en de meest optimale behandelstrategieën vormen de focus van de huidige studies naar

ANCA-geassocieerde vasculitis. De studies die in dit proefschrift zijn beschreven onderzoeken:
- de genetische achtergrond van ANCA-geassocieerde vasculitis;
- de klinische en histopathologische classificaties van ANCA-geassocieerde vasculitis;
- het ontstaan van maligniteiten (kankergezwellen) als gevolg van de behandeling van ANCA-geassocieerde vasculitis.

Genetica en ziekteclassificatie

Het ziekteproces waardoor ANCA-geassocieerde vasculitis ontstaat, is complex en nog niet volledig ontrafeld. Wetenschappers veronderstellen dat onder meer genetische factoren een rol spelen bij het ontstaan van ANCA-geassocieerde vasculitis. In **hoofdstuk 2** van dit proefschrift wordt de genetische achtergrond van ANCA-geassocieerde vasculitis onderzocht. In de afgelopen jaren hebben verschillende onderzoeksgroepen over de wereld een groot aantal studies uitgevoerd naar de genetische achtergrond van ANCA-geassocieerde vasculitis. Veel van deze studies zijn echter uitgevoerd met relatief kleine aantallen patiënten en controles. Hierdoor zijn de resultaten van deze studies soms lastig te interpreteren en/of onbetrouwbaar. **Hoofdstuk 2** beschrijft een meta-analyse van de genetische associatiestudies die zijn uitgevoerd bij patiënten met ANCA-geassocieerde vasculitis. Een meta-analyse is een statistische methode om resultaten van verschillende studies samen te voegen om zo tot een meer robuust resultaat te komen. Om de validiteit van de meta-analyse te vergroten zijn tevens ruwe data uit een grote Europese genoomwijde associatiestudie (GWAS) geïncludeerd. GWAS is een relatief nieuwe methode voor wetenschappers om genen te identificeren die betrokken zijn bij een bepaalde ziekte. Door middel van deze methode onderzoeken wetenschappers het genoom op kleine variaties, genaamd single nucleotide polymorphisms (SNPs), die vaker voorkomen bij mensen met een bepaalde ziekte dan bij mensen zonder deze ziekte.

De genetische varianten die in de meta-analyse significant geassocieerd waren met ANCA-geassocieerde vasculitis liggen in of dicht bij de volgende genen: *CD226*, *CTLA-4*, *FCGR2A*, *HLA-B*, *HLA-DP*, *HLA-DQ*, *HLA-DR*, *HSD17B8*, *IRF5*, *PTPN22*, *RING1/RXRB*, *RXRB*, *STAT4*, *SERPINA1* en *TLR9*. De resultaten van deze meta-analyse bevestigen dat genetische factoren bijdragen aan het ontstaan van ANCA-geassocieerde vasculitis. In het bijzonder ondersteunen zij een rol voor het major histocompatibility complex (MHC), het aangeboren en adaptieve immuunsysteem en verschillende ontstekingsprocessen in het ontstaan van deze complexe ziekte. Verder onderzoek naar het werkingsmechanisme van bovenstaande genen zal hopelijk niet alleen leiden tot nieuwe inzichten in de

ontstaanswijze, maar ook tot nieuwe aangrijpingspunten voor de therapie van ANCA-geassocieerde vasculitis.

De meta-analyse gepresenteerd in **hoofdstuk 2** toont genetische verschillen tussen patiënten met GPA en patiënten met MPA. Eveneens toont de meta-analyse genetische verschillen tussen patiënten met PR3-ANCA in het bloed en patiënten met MPO-ANCA in het bloed. De genetische verschillen tussen de ANCA-serotypes (PR3-ANCA en MPO-ANCA) waren groter dan de genetische verschillen tussen de klinische diagnosen (GPA en MPA). Deze bevinding impliceert dat PR3-ANCA-vasculitis en MPO-ANCA-vasculitis genetisch verschillende ziektebeelden zijn. Op grond van de sterke overeenkomsten in hun klinische en histologische manifestaties worden GPA en MPA tot op heden beschouwd als verschillende entiteiten binnen hetzelfde ziektebeeld. Niettemin wordt steeds meer duidelijk dat er opvallende verschillen tussen deze ziektebeelden zijn. Zo hebben GPA / PR3-ANCA-positieve patiënten een groter risico op een terugkeer van de ziekte dan MPA / MPO-ANCA-positieve patiënten. Daarnaast is behandeling met het relatief nieuwe geneesmiddel rituximab effectiever bij patiënten met PR3-ANCA dan bij patiënten met MPO-ANCA. Deze verschillen hebben geleid tot de vraag of de verschillende entiteiten binnen ANCA-geassocieerde vasculitis verschillende uitingen van eenzelfde ziektebeeld zijn of dat zij juist verschillende ziektebeelden representeren.

De sterke genetische verschillen tussen PR3-ANCA- en MPO-ANCA-positieve patiënten tezamen met de klinische verschillen tussen deze patiënten wijzen erop dat PR3-ANCA-vasculitis en MPO-ANCA-vasculitis verschillende ziektebeelden zijn. Dit onderstreept de noodzaak voor de incorporatie van het ANCA-serotype in de classificatie van ANCA-geassocieerde vasculitis. Deze nieuwe classificatie zal in de toekomst hopelijk leiden tot de ontwikkeling van meer specifieke therapeutische strategieën.

Hoofdstuk 3 van dit proefschrift richt zich op het voorkomen van KNO-betrokkenheid en het ANCA-serotype in relatie tot renale betrokkenheid bij ANCA-geassocieerde vasculitis. Eerder onderzoek heeft aangetoond dat patiënten met MPO-ANCA-vasculitis meer chronische laesies in het nierbiopt hebben dan patiënten met PR3-ANCA-vasculitis. Dit kan enerzijds – in lijn met de bevindingen uit **hoofdstuk 2** van dit proefschrift – duiden op verschillende ziektemechanismen in PR3-ANCA-vasculitis en MPO-ANCA-vasculitis. Anderzijds komen KNO-manifestaties – onder andere recidiverende bloedneuzen en gehoorverlies – vaker voor bij ANCA-geassocieerde vasculitispatiënten die PR3-ANCA-positief zijn. Het is mogelijk dat de PR3-ANCA-positieve patiënten door de klinisch aanwezige KNO-manifestaties vroeger gediagnosticeerd en behandeld worden dan MPO-ANCA-positieve patiënten. Dit zou een verklaring kunnen zijn voor de prognostisch gunstigere nierbiopten van de PR3-ANCA-positieve patiënten.

Vierhonderdenveertien patiënten met ANCA-geassocieerde vasculitis afkomstig uit 62 ziekenhuizen in 15 landen namen deel aan de studie gepresenteerd in **hoofdstuk 3** van dit proefschrift. In vergelijking met patiënten zonder KNO-manifestaties hadden patiënten mét KNO-manifestaties minder chronische laesies in de nierbiopten en een betere nierfunctie tijdens de diagnose en 5 jaar na de diagnose. Andere manifestaties die ook tot een snellere diagnose kunnen leiden, zoals gewrichtsklachten, vasculitis van de huid en vasculitis van de longen (zich onder andere uitend door het opgeven van bloed), waren niet geassocieerd met de nierfunctie tijdens de diagnose en 5 jaar na de diagnose. Hieruit volgt dat de betere renale uitkomsten bij patiënten met KNO-betrokkenheid waarschijnlijk niet worden veroorzaakt doordat deze patiënten vroeger gediagnosticeerd en behandeld worden. KNO-betrokkenheid was nog steeds geassocieerd met de nierfunctie in een subgroep-analyse waarin alleen PR3-ANCA positieve patiënten waren geïncludeerd. KNO-betrokkenheid lijkt dus een belangrijke klinische parameter te zijn die, onafhankelijk van ANCA-serotype, geassocieerd is met een betere nierfunctie.

De histopathologische classificatie van ANCA-geassocieerde glomerulonefritis

Bij circa 80 tot 90 procent van de patiënten met ANCA-geassocieerde vasculitis zijn de nieren betrokken bij het ziekteproces. Dat juist de nieren zo vaak aangetast worden door de vasculitis is niet verwonderlijk. Het filtreersysteem van de nieren bestaat namelijk uit kluwens van kleine bloedvaatjes, de glomeruli. Bij de patiënten waarbij de nieren betrokken zijn bij het ziekteproces ontsteken deze bloedvaatjes. Hierdoor kunnen de nieren op den duur schadelijke afvalstoffen niet meer uitscheiden en hopen deze stoffen zich in het lichaam op. Bij ernstige nierfunctiestoornissen is nierfunctievervangende therapie in de vorm van dialyse of niertransplantatie op den duur noodzakelijk.

In 2010 werd de histopathologische classificatie van ANCA-geassocieerde glomerulonefritis (ziektemanifestaties van vasculitis in de nier) ontwikkeld door een internationale werkgroep van nefropathologen en nefrologen. De histopathologische classificatie heeft als voornaamste doel het bijdragen aan een betere inschatting van de prognose van patiënten met ANCA-geassocieerde glomerulonefritis. Het classificatiesysteem is opgebouwd rondom glomerulaire pathologie en onderscheidt vier categorieën:

- De focale categorie; in deze categorie zijn minimaal 50 procent van de glomeruli niet aangetast door vasculitis.
- De crescentische categorie; in deze categorie tonen minimaal 50 procent van de glomeruli cellulaire crescents.

- De gemengde categorie; in deze categorie is geen van de karakteristieke afwijkingen dominant.
- De sclerotische categorie; in deze categorie zijn minimaal 50 procent van de glomeruli globaal gescleroseerd (verlittekend).

Een validatiestudie toonde aan dat deze categorieën in de genoemde volgorde geassocieerd zijn met oplopende ernst van nierfunctieverlies op de lange termijn.

Tot op heden is de histopathologische classificatie van ANCA-geassocieerde glomerulonefritis gevalideerd in 21 studies afkomstig uit Azië, Noord-Amerika, Australië en Europa. In **hoofdstuk 4** worden de bevindingen uit deze validatiestudies bediscussieerd en in breder perspectief geplaatst. De resultaten van deze studies bevestigen de voorspellende waarde van de focale en sclerotische categorieën voor de nierfunctie op de lange termijn. De verschillende validatiestudies toonden echter bij de crescentische en gemengde categorieën tegenstrijdige resultaten. In sommige studies hadden de patiënten met een nierbiopt in de crescentische categorie betere renale uitkomsten dan patiënten met een nierbiopt in de gemengde categorie. In andere studies was dit andersom en hadden patiënten met een nierbiopt in de gemengde categorie een betere nierfunctie. De gemengde categorie in haar huidige vorm lijkt dan ook te divers van aard te zijn om een voorspellende waarde voor de nierfunctie op de lange termijn te hebben.

Behandeling en langetermijnuitkomst

Zoals hiervoor besproken is behandeling van ANCA-geassocieerde vasculitis van levensbelang. Zonder behandeling is één jaar na de diagnose slechts één op de vijf patiënten in leven. Bij de behandeling van ANCA-geassocieerde vasculitis wordt onderscheid gemaakt tussen de initiële therapie om de vasculitis te onderdrukken (inductietherapie) en de therapie om de vasculitis onderdrukt te houden (onderhoudstherapie). De inductietherapie bestaat klassiek uit het afweeronderdrukkend medicijn cyclofosfamide in combinatie met prednison (een corticosteroïde). De onderhoudstherapie bestaat uit azathioprine of methotrexaat waarbij de prednison wordt afgebouwd en waar mogelijk wordt gestopt. Azathioprine en methotrexaat zijn afweeronderdrukkende medicijnen die milder zijn dan cyclofosfamide. Met deze behandeling is 90 procent van de patiënten met ANCA-geassocieerde vasculitis één jaar na het stellen van de diagnose nog in leven.

De behandeling van ANCA-geassocieerde vasculitis is erg effectief maar gaat helaas ook gepaard met ernstige complicaties. Een mogelijke complicatie van het langdurig gebruik van afweeronderdrukkende medicijnen is het optreden van maligniteiten. In **hoofdstuk 5** van dit proefschrift werd het risico op

maligniteiten onderzocht bij patiënten met ANCA-geassocieerde vasculitis die zijn behandeld met cyclofosfamide. De patiënten met ANCA-geassocieerde vasculitis hadden een tweemaal hoger risico op het ontwikkelen van maligniteiten ten opzichte van leeftijdsgenoten uit de algemene bevolking. Het risico op maligniteiten hield direct verband met de cumulatieve dosis cyclofosfamide die een patiënt kreeg; hoe meer cyclofosfamide, des te hoger het risico op het ontwikkelen van een maligniteit. Alleen het risico op het ontwikkelen van basaalcelcarcinomen en plaveiselcelcarcinomen van de huid was verhoogd. In tegenstelling tot oudere studies was in de studie gepresenteerd in **hoofdstuk 5** het risico op blaaskanker, leukemie en lymfomen niet verhoogd. Deze vooruitgang is waarschijnlijk het resultaat van internationale inspanningen (onder andere door middel van het uitvoeren van grote internationale klinische trials) om de behandeling van ANCA-geassocieerde vasculitis effectiever en veiliger te maken. Dit werd onder andere verwezenlijkt door een sterke verlaging van de totale dosis cyclofosfamide bij de behandeling van ANCA-geassocieerde vasculitis.

Cyclofosfamide blijft echter nog altijd ongunstige bijwerkingen hebben, met risico's op onder andere infecties, onvruchtbaarheid en, bij patiënten met veel cyclofosfamidegebruik, maligniteiten. In de wetenschappelijke wereld wordt dan ook voortdurend gezocht naar nieuwere en veiligere therapieën. Onlangs is rituximab met succes geïntroduceerd in de behandeling van ANCA-geassocieerde vasculitis. Twee klinische trials (RITUXVAS en RAVE) demonstreerden dat rituximab effectief is in de behandeling van ANCA-geassocieerde vasculitis en relatief weinig bijwerkingen heeft. Deze studies leidden echter wel tot zorgen over een mogelijk verhoogd risico op maligniteiten bij de ANCA-geassocieerde vasculitispatiënten die met rituximab werden behandeld.

Hoofdstuk 6 beschrijft de eerste langetermijnstudie waarin het risico op maligniteiten wordt onderzocht bij patiënten met ANCA-geassocieerde vasculitis die worden behandeld met rituximab. Patiënten die werden behandeld met cyclofosfamide hadden een ruim vier en een half maal hoger risico op het ontwikkelen van maligniteiten ten opzichte van de patiënten die werden behandeld met rituximab. Het risico op maligniteiten was bij de patiënten die werden behandeld met cyclofosfamide ruim 3 maal hoger ten opzichte van leeftijdsgenoten uit de algemene bevolking. Daarentegen hadden de patiënten die werden behandeld met rituximab geen verhoogd risico op het ontwikkelen van maligniteiten ten opzichte van leeftijdsgenoten uit de algemene bevolking. De cumulatieve dosis cyclofosfamide hield – net zoals in de studie beschreven in **hoofdstuk 5** – ook in deze studie direct verband met het risico op het ontwikkelen van maligniteiten. Concluderend laten de resultaten van de studie gepresenteerd in **hoofdstuk 6** zien dat, wat betreft het risico op maligniteiten, rituximab een veiliger alternatief is dan cyclofosfamide in de behandeling van ANCA-geassocieerde vasculitis.

Toekomstperspectieven

De resultaten uit dit proefschrift tonen aan dat genetische factoren betrokken zijn bij het ontstaan van ANCA-geassocieerde vasculitis. Vervolgstudies zullen de precieze rol van deze genetische varianten in het ziekteproces moeten vaststellen. Deze (epi)genetische studies zullen hopelijk niet alleen leiden tot diepere inzichten in het ontstaan van ANCA-geassocieerde vasculitis, maar ook tot therapie gericht op specifieke karakteristieken van de individuele patiënt (*personalized medicine*).

Daarnaast tonen de resultaten uit dit proefschrift ook aan dat PR3-ANCA vasculitis en MPO-ANCA vasculitis verschillende genetische achtergronden hebben. Deze bevinding onderstreept de noodzaak voor een nieuw classificatiesysteem van ANCA-geassocieerde vasculitis. Dit nieuwe classificatiesysteem behoort primair gebaseerd te zijn op ANCA-serotype in plaats van klinische diagnose. In dit classificatiesysteem zal dus gesproken worden van PR3-ANCA vasculitis en MPO-ANCA vasculitis in plaats van GPA en MPA. De genetische verschillen tussen patiënten met PR3-ANCA en MPO-ANCA impliceren ook dat toekomstige genetische en klinische studies zodanig opgezet moeten worden dat afzonderlijke analyse van deze patiëntengroepen zinvol is.

Bij de behandeling van ANCA-geassocieerde vasculitis wordt momenteel een deel van de specifieke patiënt- en ziektekenmerken buiten beschouwing gelaten. De resultaten van genetische en klinische studies impliceren echter dat het afstemmen van de behandeling op specifieke patiënt- en ziekteparameters tot betere behandelresultaten zal leiden. Mogelijke parameters die, nu of in de toekomst, in aanmerking komen voor het realiseren van 'therapie op maat' zijn: genetische markers, ANCA-serotype (PR3-ANCA of MPO-ANCA), scores die de ziekte-ernst weergeven en markers die een terugval voorspellen.

De resultaten uit dit proefschrift suggereren dat rituximab veelbelovend is in de behandeling van ANCA-geassocieerde vasculitis. De langetermijneffecten van rituximab bij patiënten met ANCA-geassocieerde vasculitis zijn echter onbekend en zullen door middel van vervolgstudies onderzocht moeten worden. Daarnaast wordt steeds duidelijker dat corticosteroïden verantwoordelijk zijn voor een substantieel deel van de, aan therapie gerelateerde, complicaties die patiënten met ANCA-geassocieerde vasculitis ervaren. Toekomstige studies zullen dan ook verschillende doseringsschema's met betrekking tot de werkzaamheid en veiligheid van corticosteroïden moeten onderzoeken. Tot slot zullen toekomstige studies ook moeten uitwijzen of bevindingen in het nierbiopt van patiënten met ANCA-geassocieerde vasculitis direct bruikbaar zijn om de therapeutische besluitvorming te sturen.

Appendices

Supplementary data
Authors and affiliations
Curriculum vitae
List of publications
Acknowledgements

Supplementary data

I. Supplementary data Chapter II

Genetic variants in ANCA-associated vasculitis: a meta-analysis

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Supplementary table 1. Literature search strategies

Database	Search Strategy
PubMed	*Two strategies:* 1. AAV and genes ("Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis"[Mesh] OR "ANCA-associated vasculitis"[all fields] OR "ANCA associated vasculitis"[all fields] OR "Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis"[all fields] OR "Pauci-Immune Vasculitis"[all fields] OR "Pauci Immune Vasculitis"[all fields] OR "Pauci-Immune Vasculitides"[all fields] OR "ANCA-Associated Vasculitides"[all fields] OR "ANCA Associated Vasculitides"[all fields] OR "ANCA-Associated Vasculitide"[all fields] OR ("ANCA"[all fields] AND vasculit*[all fields]) OR ("Anti-Neutrophil"[all fields] AND "Cytoplasmic"[all fields] AND "Antibody-Associated"[all fields] AND vasculit*[all fields]) OR "Churg-Strauss Syndrome"[all fields] OR "Churg Strauss Syndrome"[all fields] OR "Allergic Granulomatous Angiitis"[all fields] OR "Allergic Granulomatous Angiitides"[all fields] OR "Allergic Angiitis"[all fields] OR "Churg-Strauss Vasculitis"[all fields] OR "eosinophilic granulomatosis with polyangiitis"[all fields] OR ("eosinophilic"[all fields] AND "granulomatosis"[all fields] AND "polyangiitis"[all fields]) OR "Microscopic Polyangiitis"[all fields] OR "Microscopic Polyangiitides"[all fields] OR "Wegener Granulomatosis"[all fields] OR "Wegener's Granulomatosis"[all fields] OR "Wegeners Granulomatosis"[all fields] OR "granulomatosis with polyangiitis"[all fields] OR ("granulomatosis"[all fields] AND "polyangiitis"[all fields]) OR "anca"[tw] OR (("pr3"[all fields] OR "pr-3"[all fields] OR "mpo"[all fields]) AND ("Vasculitis"[mesh] OR vasculit*[all fields] OR "anca"[tw])) OR "Antibodies, Antineutrophil Cytoplasmic"[Mesh] OR "Antineutrophil Cytoplasmic Antibody"[all fields] OR "Anti-Neutrophil Cytoplasmic Antibody"[all fields] OR "Anti Neutrophil Cytoplasmic Antibody"[all fields] OR "Anti-Neutrophil Cytoplasmic Antibodies"[all fields] OR "Anti Neutrophil Cytoplasmic Antibodies"[all fields] OR "Antineutrophil Cytoplasmic Antibodies"[all fields]) AND ("genes"[mesh] OR "genes"[all fields] OR "gene"[all fields] OR "genetic"[all fields] OR "genetics"[Subheading] OR "genetics"[all fields] OR "genetics"[mesh] OR "polymorphism, genetic"[mesh] OR "polymorphism"[all fields] OR "polymorphisms"[all fields] OR "dna"[mesh] OR "dna"[all fields] OR "genome"[mesh] OR "genome"[all fields] OR "genomes"[all fields] OR "genomics"[mesh] OR "genomics"[all fields] OR "genomic"[all fields] OR "Genetic Phenomena"[mesh] OR "Genetic Structures"[Mesh])

Supplementary table 1. Literature search strategies (Continued)

Database	Search Strategy
PubMed	2. Vasculitis and genes (excluding AAV) ((“Vasculitis”[majr] OR vasculit*[ti]) AND (“genes”[majr] OR “genes”[ti] OR “gene”[ti] OR “genetic”[ti] OR “genetics”[ti] OR “genetics”[majr] OR “polymorphism, genetic”[majr] OR “polymorphism”[ti] OR “polymorphisms”[ti] OR “dna”[majr] OR “dna”[ti] OR “genome”[majr] OR “genome”[ti] OR “genomes”[ti] OR “genomics”[majr] OR “genomics”[ti] OR “genomic”[ti] OR “Genetic Phenomena”[majr] OR “Genetic Structures”[majr])) NOT ((“Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis”[Mesh] OR “ANCA-associated vasculitis”[all fields] OR “ANCA associated vasculitis”[all fields] OR “Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis”[all fields] OR “Pauci-Immune Vasculitis”[all fields] OR “Pauci Immune Vasculitis”[all fields] OR “Pauci-Immune Vasculitides”[all fields] OR “ANCA-Associated Vasculitides”[all fields] OR “ANCA Associated Vasculitides”[all fields] OR “ANCA-Associated Vasculitide”[all fields] OR (“ANCA”[all fields] AND vasculit*[all fields]) OR (“Anti-Neutrophil”[all fields] AND “Cytoplasmic”[all fields] AND “Antibody-Associated”[all fields] AND vasculit*[all fields]) OR “Churg-Strauss Syndrome”[all fields] OR “Churg Strauss Syndrome”[all fields] OR “Allergic Granulomatous Angiitis”[all fields] OR “Allergic Granulomatous Angiitides”[all fields] OR “Allergic Angiitis”[all fields] OR “Churg-Strauss Vasculitis”[all fields] OR “eosinophilic granulomatosis with polyangiitis”[all fields] OR (“eosinophilic”[all fields] AND “granulomatosis”[all fields] AND “polyangiitis”[all fields]) OR “Microscopic Polyangiitis”[all fields] OR “Microscopic Polyangiitides”[all fields] OR “Wegener Granulomatosis”[all fields] OR “Wegener’s Granulomatosis”[all fields] OR “Wegeners Granulomatosis”[all fields] OR “granulomatosis with polyangiitis”[all fields] OR (“granulomatosis”[all fields] AND “polyangiitis”[all fields]) OR “anca”[tw] OR ((“pr3”[all fields] OR “pr-3”[all fields] OR “mpo”[all fields]) AND (“Vasculitis”[mesh] OR vasculit*[all fields] OR “anca”[tw])) OR “Antibodies, Antineutrophil Cytoplasmic”[Mesh] OR “Antineutrophil Cytoplasmic Antibody”[all fields] OR “Anti-Neutrophil Cytoplasmic Antibody”[all fields] OR “Anti Neutrophil Cytoplasmic Antibody”[all fields] OR “Anti-Neutrophil Cytoplasmic Antibodies”[all fields] OR “Anti Neutrophil Cytoplasmic Antibodies”[all fields] OR “Antineutrophil Cytoplasmic Antibodies”[all fields]) AND (“genes”[mesh] OR “genes”[all fields] OR “gene”[all fields] OR “genetic”[all fields] OR “genetics”[Subheading] OR “genetics”[all fields] OR “genetics”[mesh] OR “polymorphism, genetic”[mesh] OR “polymorphism”[all fields] OR “polymorphisms”[all fields] OR “dna”[mesh] OR “dna”[all fields] OR “genome”[mesh] OR “genome”[all fields] OR “genomes”[all fields] OR “genomics”[mesh] OR “genomics”[all fields] OR “genomic”[all fields] OR “Genetic Phenomena”[mesh] OR “Genetic Structures”[Mesh]))

Supplementary table 1. Literature search strategies (Continued)

Database	Search Strategy
Embase	*Two strategies:* 1. AAV and genes (ANCA associated vasculitis/ OR "ANCA-associated vasculitis".mp OR "ANCA associated vasculitis".mp OR "Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis".mp OR "Pauci-Immune Vasculitis".mp OR "Pauci Immune Vasculitis".mp OR "Pauci-Immune Vasculitides".mp OR "ANCA-Associated Vasculitides".mp OR "ANCA Associated Vasculitides".mp OR "ANCA-Associated Vasculitide".mp OR ("ANCA".mp AND vasculit*.mp) OR ("Anti-Neutrophil".mp AND "Cytoplasmic".mp AND "Antibody-Associated".mp AND vasculit*.mp) OR "Churg-Strauss Syndrome".mp OR "Churg Strauss Syndrome".mp OR "Allergic Granulomatous Angiitis".mp OR "Allergic Granulomatous Angiitides".mp OR "Allergic Angiitis".mp OR "Churg-Strauss Vasculitis".mp OR "eosinophilic granulomatosis with polyangiitis".mp OR ("eosinophilic".mp AND "granulomatosis".mp AND "polyangiitis".mp) OR "Microscopic Polyangiitis".mp OR "Microscopic Polyangiitides".mp OR "Wegener Granulomatosis".mp OR "Wegener's Granulomatosis".mp OR "Wegeners Granulomatosis".mp OR "granulomatosis with polyangiitis".mp OR ("granulomatosis".mp AND "polyangiitis".mp) OR "anca".mp OR (("pr3".mp OR "pr-3".mp OR "mpo".mp) AND (exp Vasculitis/ OR vasculit*.mp OR "anca".mp)) OR neutrophil cytoplasmic antibody/ OR "Antineutrophil Cytoplasmic Antibody".mp OR "Anti-Neutrophil Cytoplasmic Antibody".mp OR "Anti Neutrophil Cytoplasmic Antibody".mp OR "Anti-Neutrophil Cytoplasmic Antibodies".mp OR "Anti Neutrophil Cytoplasmic Antibodies".mp OR "Antineutrophil Cytoplasmic Antibodies".mp) AND (exp Gene/ OR gene.mp OR genom*.mp OR genes.mp OR exp genetic polymorphism/ OR polymorphism*.mp OR dna.mp OR exp dna/ OR exp *Heredity/)

Supplementary table 1. Literature search strategies (Continued)

Database	Search Strategy
Embase	2. Vasculitis and genes (excluding AAV) ((exp *Vasculitis/ OR vasculit*.ti) AND (exp *Gene/ OR gene.ti OR genom*.ti OR genes.ti OR exp *genetic polymorphism/ OR polymorphism*.ti OR dna.ti OR exp *dna/ OR exp *Heredity/)) NOT ((ANCA associated vasculitis/ OR "ANCA-associated vasculitis".mp OR "ANCA associated vasculitis".mp OR "Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis".mp OR "Pauci-Immune Vasculitis".mp OR "Pauci Immune Vasculitis".mp OR "Pauci-Immune Vasculitides".mp OR "ANCA-Associated Vasculitides".mp OR "ANCA Associated Vasculitides".mp OR "ANCA-Associated Vasculitide".mp OR ("ANCA".mp AND vasculit*.mp) OR ("Anti-Neutrophil".mp AND "Cytoplasmic".mp AND "Antibody-Associated".mp AND vasculit*.mp) OR "Churg-Strauss Syndrome".mp OR "Churg Strauss Syndrome".mp OR "Allergic Granulomatous Angiitis".mp OR "Allergic Granulomatous Angiitides".mp OR "Allergic Angiitis".mp OR "Churg-Strauss Vasculitis".mp OR "eosinophilic granulomatosis with polyangiitis".mp OR ("eosinophilic".mp AND "granulomatosis".mp AND "polyangiitis".mp) OR "Microscopic Polyangiitis".mp OR "Microscopic Polyangiitides".mp OR "Wegener Granulomatosis".mp OR "Wegener's Granulomatosis".mp OR "Wegeners Granulomatosis".mp OR "granulomatosis with polyangiitis".mp OR ("granulomatosis".mp AND "polyangiitis".mp) OR "anca".mp OR (("pr3".mp OR "pr-3".mp OR "mpo".mp) AND (exp Vasculitis/ OR vasculit*.mp OR "anca".mp)) OR neutrophil cytoplasmic antibody/ OR "Antineutrophil Cytoplasmic Antibody".mp OR "Anti-Neutrophil Cytoplasmic Antibody".mp OR "Anti Neutrophil Cytoplasmic Antibody".mp OR "Anti-Neutrophil Cytoplasmic Antibodies".mp OR "Anti Neutrophil Cytoplasmic Antibodies".mp OR "Antineutrophil Cytoplasmic Antibodies".mp) AND (exp Gene/ OR gene.mp OR genom*.mp OR genes.mp OR exp genetic polymorphism/ OR polymorphism*.mp OR dna.mp OR exp dna/ OR exp *Heredity/))

Supplementary table 1. Literature search strategies (Continued)

Database	Search Strategy
Web of Science	*Two strategies:* 1. AAV and genes TS=((“ANCA-associated vasculitis” OR “ANCA associated vasculitis” OR “Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis” OR “Pauci-Immune Vasculitis” OR “Pauci Immune Vasculitis” OR “Pauci-Immune Vasculitides” OR “ANCA-Associated Vasculitides” OR “ANCA Associated Vasculitides” OR “ANCA-Associated Vasculitide” OR (“ANCA” AND vasculit*) OR (“Anti-Neutrophil” AND “Cytoplasmic” AND “Antibody-Associated” AND vasculit*) OR “Churg-Strauss Syndrome” OR “Churg Strauss Syndrome” OR “Allergic Granulomatous Angiitis” OR “Allergic Granulomatous Angiitides” OR “Allergic Angiitis” OR “Churg-Strauss Vasculitis” OR “eosinophilic granulomatosis with polyangiitis” OR (“eosinophilic” AND “granulomatosis” AND “polyangiitis”) OR “Microscopic Polyangiitis” OR “Microscopic Polyangiitides” OR “Wegener Granulomatosis” OR “Wegener’s Granulomatosis” OR “Wegeners Granulomatosis” OR “granulomatosis with polyangiitis” OR (“granulomatosis” AND “polyangiitis”) OR “anca” OR ((“pr3” OR “pr-3” OR “mpo”) AND (exp Vasculitis/ OR vasculit* OR “anca”)) OR “neutrophil cytoplasmic antibody” OR “Antineutrophil Cytoplasmic Antibody” OR “Anti-Neutrophil Cytoplasmic Antibody” OR “Anti Neutrophil Cytoplasmic Antibody” OR “Anti-Neutrophil Cytoplasmic Antibodies” OR “Anti Neutrophil Cytoplasmic Antibodies” OR “Antineutrophil Cytoplasmic Antibodies”) AND (gene OR genes OR genom* OR genetic* OR Polymorphism* OR dna)) 2. Vasculitis and genes (excluding AAV) (TI=((vasculit*) AND (gene OR genes OR genom* OR genetic* OR Polymorphism* OR dna))) NOT (TS=((“ANCA-associated vasculitis” OR “ANCA associated vasculitis” OR “Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis” OR “Pauci-Immune Vasculitis” OR “Pauci Immune Vasculitis” OR “Pauci-Immune Vasculitides” OR “ANCA-Associated Vasculitides” OR “ANCA Associated Vasculitides” OR “ANCA-Associated Vasculitide” OR (“ANCA” AND vasculit*) OR (“Anti-Neutrophil” AND “Cytoplasmic” AND “Antibody-Associated” AND vasculit*) OR “Churg-Strauss Syndrome” OR “Churg Strauss Syndrome” OR “Allergic Granulomatous Angiitis” OR “Allergic Granulomatous Angiitides” OR “Allergic Angiitis” OR “Churg-Strauss Vasculitis” OR “eosinophilic granulomatosis with polyangiitis” OR (“eosinophilic” AND “granulomatosis” AND “polyangiitis”) OR “Microscopic Polyangiitis” OR “Microscopic Polyangiitides” OR “Wegener Granulomatosis” OR “Wegener’s Granulomatosis” OR “Wegeners Granulomatosis” OR “granulomatosis with polyangiitis” OR (“granulomatosis” AND “polyangiitis”) OR “anca” OR ((“pr3” OR “pr-3” OR “mpo”) AND (exp Vasculitis/ OR vasculit* OR “anca”)) OR “neutrophil cytoplasmic antibody” OR “Antineutrophil Cytoplasmic Antibody” OR “Anti-Neutrophil Cytoplasmic Antibody” OR “Anti Neutrophil Cytoplasmic Antibody” OR “Anti-Neutrophil Cytoplasmic Antibodies” OR “Anti Neutrophil Cytoplasmic Antibodies” OR “Antineutrophil Cytoplasmic Antibodies”) AND (gene OR genes OR genom* OR genetic* OR Polymorphism* OR dna)))

Supplementary table 2. Study characteristics of the included studies

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
***CD226* rs763361 (T)**	Wieczorek et al. 2009[1**]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy subjects	761/1226	GPA (642), EGPA (119)	PR3-ANCA (438), MPO-ANCA (32), ANCA negative (43)	1.24 (1.11 – 1.39)
		Case-Control	UK	ACR and CHCC criteria	Geographically matched subjects from the general population	105/9337	GPA (all patients)	NR	1.17 (0.89 – 1.54)
	Chung et al. 2012[2**]	Case-Control	USA	ACR criteria	Geographically matched subjects with no (family) history of autoimmune disease	424/469	GPA (all patients)	PR3-ANCA (339), MPO-ANCA (44), ANCA negative (39)	1.08 (0.90 – 1.30)
		Case-Control	Canada	ACR criteria	Geographically matched subjects with no history of autoimmune disease	456/1500	GPA (all patients)	NR	1.09 (0.94 – 1.26)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5366	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.08 (0.96 – 1.21)
***CTLA-4* $(AT)_{86}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	0.18 (0.10 – 0.35)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	1.31 (0.76 – 2.25)
	Zhou et al. 2004[6]	Case-Control	USA	ACR criteria	Geographically and ethnically matched healthy, unrelated subjects	117/123	GPA (all patients)	PR3-ANCA (82), MPO-ANCA (5), PR3- and MPO-ANCA positive (2), ANCA negative (25)	0.38 (0.26 – 0.55)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.72 (0.51 – 1.02)
CTLA-4 **$(AT)_{102}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	1.71 (0.15 – 19.22)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	0.89 (0.47 – 1.68)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	1.73 (0.85 – 3.54)
CTLA-4 **$(AT)_{104}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	1.74 (0.96 – 3.13)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	1.15 (0.70 – 1.91)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	1.09 (0.70 – 1.59)
CTLA-4 **$(AT)_{106}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	2.84 (0.74 – 10.91)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	0.41 (0.14 – 1.22)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	2.63 (1.09 – 6.35)
CTLA-4 **$(AT)_{108}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	0.90 (0.42 – 1.95)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	0.89 (0.47 – 1.68)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	1.78 (1.05 – 3.03)
CTLA-4 **$(AT)_{110}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	0.48 (0.02 9.36)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	0.25 (0.01 – 4.62)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.74 (0.34 – 1.61)
CTLA-4 **$(AT)_{116}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	1.37 (0.26 – 7.26)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	0.75 (0.03 – 18.56)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.540 (0.11 – 2.62)
CTLA-4 **$(AT)_{118}$**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	3.44 (0.21 – 55.85)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	0.75 (0.08 – 7.33)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.27 (0.06 – 1.18)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
CTLA-4 $(AT)_{122}$	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	10.32 (0.42 – 256.50)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	2.32 (0.57 – 9.47)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.47 (0.05 – 4.27)
CTLA-4 $(AT)_{124}$	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	14.47 (1.59 – 131.86)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	2.28 (0.14 – 36.59)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.63 (0.13 – 3.16)
CTLA-4 $(AT)_{126}$	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	4.78 (1.04 – 21.93)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	0.45 (0.05 – 3.88)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.81 (0.21 – 3.18)
CTLA-4 $(AT)_{128}$	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	1.12 (0.05 – 27.93)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	49/111	MPA (all patients)	MPO-ANCA (all patients)	6.98 (0.72 – 67.95)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.69 (0.22 – 2.18)
***CTLA-4* rs231775 (G)**	Slot et al. 2008[8]	Case-Control	The Netherlands	CHCC criteria	Geographically and ethnically matched healthy subjects	104/185	GPA (50), MPA (24), EGPA (7), RLV (21)	PR3-ANCA (49), MPO-ANCA (34), PR3- and MPO-ANCA positive (2), ANCA negative (17)	1.53 (1.09 – 2.17)
	Kamesh et al. 2009[9]	Case-Control	UK	CHCC criteria	Ethnically matched healthy subjects with no (family) history of autoimmune disease	222/629	GPA (116), MPA (96)	NR	1.32 (1.06 – 1.65)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5365	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.09 (0.97 – 1.22)
***CTLA-4* rs3087243 (A)**	Kamesh et al. 2009[9]	Case-Control	UK	CHCC criteria	Ethnically matched healthy subjects with no (family) history of autoimmune disease	222/629	GPA (116), MPA (96)	NR	0.67 (0.54 – 0.83)
	Chung et al. 2012[2**]	Case-Control	USA	ACR criteria	Geographically matched subjects with no (family) history of autoimmune disease	424/469	GPA (all patients)	PR3-ANCA (339), MPO-ANCA (44), ANCA negative (39)	0.80 (0.66 – 0.97)
		Case-Control	Canada	ACR criteria	Geographically matched subjects with no history of autoimmune disease	456/1500	GPA (all patients)	NR	0.78 (0.67 – 0.91)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	913/5257	GPA (565), MPA (262)	PR3-ANCA (478), MPO-ANCA (264)	0.85 (0.77 - 0.94)
***FCAR* rs16986050 (G)**	Kelley et al. 2011[10**]	Case-Control	USA	ACR and CHCC criteria	Geographically matched subjects with no (family) history of autoimmune disease	445/413	GPA (all patients)	NR	0.65 (0.50 – 0.83)
		Case-Control	USA, Canada	ACR and CHCC criteria	HapMap Caucasians	190/113	GPA (all patients)	NR	0.79 (0.52 – 1.21)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5365	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.07 (0.93 – 1.24)
***FCGR2A* rs1801274 (C)**	Edberg et al. 1997[11]	Case-Control	USA, Chile, Germany, Canada	ACR and CHCC criteria	Ethnically matched healthy volunteers	147/149	GPA (all patients)	NR	0.85 (0.61 – 1.17)
	Dijstelbloem et al. 1999[12]	Case-Control	The Netherlands	ACR criteria	Ethnically matched healthy blood donors	91/154	GPA (all patients)	PR3-ANCA (all patients)	0.98 (0.68 – 1.41)
	Tse et al. 1999[13]	Case-Control	UK	CHCC criteria	Ethnically matched healthy subjects	107/100	GPA (48), MPA (54), EGPA (1), PAN (4)	PR3-ANCA (75), MPO-ANCA (32)	1.00 (0.68 – 1.48)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	50/303	MPA (all patients)	MPO-ANCA (all patients)	0.68 (0.37 – 1.24)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5365	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	0.89 (0.79 – 0.99)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	1.03 (0.73 – 1.43)
***FCGR3A* rs396991 (G)**	Dijstelbloem et al. 1999[12]	Case-Control	The Netherlands	ACR criteria	Ethnically matched healthy blood donors	91/154	GPA (all patients)	PR3-ANCA (all patients)	1.30 (0.89 – 1.91)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	50/303	MPA (all patients)	MPO-ANCA (all patients)	0.65 (0.39 – 1.07)
***FCGR3B* (NA1)**	Dijstelbloem et al. 1999[12]	Case-Control	The Netherlands	ACR criteria	Ethnically matched healthy blood donors	91/154	GPA (all patients)	PR3-ANCA (all patients)	1.05 (0.72 – 1.53)
	Tse et al. 2000[14]	Case-Control	UK	CHCC criteria	Ethnically matched healthy subjects	101/100	GPA (45), MPA (52), EGPA (1), PAN (3)	PR3-ANCA (61), MPO-ANCA (30)	1.24 (0.8 – 1.87)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	50/303	MPA (all patients)	MPO-ANCA (all patients)	1.29 (0.82 – 2.03)
	Kelley et al. 2011[10]	Case-Control	USA	ACR and CHCC criteria	Geographically matched subjects with no (family) history of autoimmune disease	673/413	GPA (all patients)	NR	0.86 (0.72 – 1.03)
***GHSR* rs509035 (A)**	Wieczorek et al. 2010[15]**	Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	454/820	GPA (all patients)	NR	1.22 (1.02 – 1.45)
		Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	179/516	GPA (all patients)	NR	0.94 (0.72 – 1.23)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	914/5259	GPA (565), MPA (262)	PR3-ANCA (478), MPO-ANCA (264)	1.00 (0.90 – 1.11)
***GHSR* rs519384 (A)**	Wieczorek et al. 2010[15**]	Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	449/813	GPA (all patients)	NR	1.17 (0.98 – 1.40)
		Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	179/515	GPA (all patients)	NR	1.01 (0.77 – 1.33)
***GHSR* rs572169 (A)**	Wieczorek et al. 2010[15**]	Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	442/812	GPA (all patients)	NR	1.21 (1.01 – 1.44)
		Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	178/492	GPA (all patients)	NR	0.96 (0.73 – 1.25)
HLA-A1	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	1.53 (0.81 – 2.87)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.84 (0.47 – 1.52)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.92 (0.42 – 2.03)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.08 (0.87 – 1.35)
HLA-A2	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	0.78 (0.42 – 1.45)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.04 (0.62 – 1.73)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.90 (0.37 – 2.23)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.87 (0.48 – 1.58)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.98 (0.81 – 1.17)
HLA-A3	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	1.73 (0.90 –3.31)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.51 (0.86 – 2.66)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.88 (0.89 – 3.98)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.04 (0.84 – 1.31)
HLA-A9	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	1.09 (0.46 – 2.59)
	Katz et al. 1979[21]	Case-Control	USA	Clinical and histological criteria	Ethnically matched subjects	31/418	GPA (all patients)	NR	69.13 (3.28 – 456.19)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.79 (0.58 – 1.06)
HLA-A10	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	1.06 (0.32 – 3.48)
	Katz et al. 1979[21]	Case-Control	USA	Clinical and histological criteria	Ethnically matched subjects	31/418	GPA (all patients)	NR	69.13 (3.28 – 1456.19)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.61 (0.33 – 1.12)
HLA-A11	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/607	GPA (all patients)	NR	1.96 (0.81 – 4.72)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.68 (0.24 – 1.90)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.71 (0.17 – 3.03)
	Papasteriades et al. 1997[22]	Case-Control	Greece	Histologically proven AAV	Geographically and ethnically matched healthy subjects	23/405	MPA (all patients)	NR	2.97 (1.19 – 7.41)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.43 (0.14 – 1.27)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.62 (0.39 – 0.99)
HLA-A24	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.91 (0.36 – 2.31)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.75 (0.34 – 1.65)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.81 (0.51 – 6.37)
HLA-A25	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.43 (0.33 – 6.17)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	2.09 (0.61 – 7.16)
HLA-A26	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.68 (0.09 – 5.07)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	2.36 (1.00. – 5.60)
	Papasteriades et al. 1997[22]	Case-Control	Greece	Histologically proven AAV	Geographically and ethnically matched healthy subjects	23/405	MPA (all patients)	NR	2.49 (1.01 – 6.18)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.39 (0.07 – 2.05)
HLA-A28	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	0.40 (0.02 – 6.68)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.32 (0.04 – 2.34)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.88 (0.57 – 1.38)
HLA-A29	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	2.11 (0.68 – 6.57)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.31 (0.04 – 2.25)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.66 (0.11 – 4.04)
HLA-A30	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.68 (0.09 – 5.07)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	2.02 (0.18 – 22.63)
HLA-A31	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.07 (0.25 – 4.54)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.55 (0.46 – 5.24)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.33 (0.03 – 3.20)
HLA-A32	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	3.73 (1.24 – 11.22)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	2.07 (0.60 – 7.04)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.24 (0.03 – 2.21)
HLA-B5	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	0.93 (0.28 – 3.02)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.56 (0.35 – 0.89)
HLA-B7	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	1.54 (0.77 – 3.08)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.74 (1.02 – 2.96)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.07 (0.52 – 2.22)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.00 (0.80 – 1.26)
HLA-B8	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	1.24 (0.60 – 2.56)
	Katz et al. 1979[21]	Case-Control	USA	Clinical and histological criteria	Ethnically matched subjects	31/418	GPA (all patients)	NR	2.30 (1.18 – 4.50)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	17/113	GPA (all patients)	NR	2.47 (1.01 – 6.05)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.03 (0.55 – 1.93)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	3.27 (1.02 – 10.50)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.11 (0.88 – 1.40)
HLA-B12	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	1.01 (0.47 – 2.15)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.96 (0.74 – 1.24)
HLA-B13	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	0.87 (0.12 – 6.50)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	2.09 (0.62 – 7.07)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.79 (0.21 – 3.03)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.63 (0.31 – 1.28)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-B14	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/701	GPA (all patients)	NR	0.32 (0.02 – 5.24)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.51 (0.16 – 1.63)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.52 (0.25 – 9.26)
	Stassen et al. 2009[19]	Case-Control	The Nether-lands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.16 (0.63 – 2.14)
HLA-B15	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.38 (0.55 – 3.43)
	Stassen et al. 2009[19]	Case-Control	The Nether-lands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.98 (0.74 – 1.29)
HLA-B18	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/549	GPA (all patients)	NR	1.16 (0.35 – 3.85)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.53 (0.13 – 2.21)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.78 (0.296 – 2.072
	Stassen et al. 2009[19]	Case-Control	The Nether-lands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.75 (0.435 – 1.283

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-B27	Strimlan et al. 1978[16]	Case-Control	USA	Histologically proven AAV	Ethnically matched healthy blood donors	31/600	GPA (all patients)	NR	0.74 (0.18 – 3.093
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.60 (0.14 – 2.503
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.06 (0.00 – 1.102
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.77 (0.48 – 1.247
HLA-B35	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	0.45 (0.11 – 1.86)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.80 (0.187 – 3.40)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.85 (0.28 – 2.62)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.81 (0.59 – 1.12)
HLA-B37	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.14 (0.01 – 2.72)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.07 (0.58 – 1.98)
HLA-B39	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.86 (0.11 – 6.51)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.33 (0.01 – 8.20)
HLA-B40	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.65 (0.52 – 5.23)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5872	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.90 (0.67 – 1.22)
HLA-B44	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.07 (0.58 – 2.00)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	2.50 (0.845 – 7.403
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	3.27 (1.02 – 10.50)
HLA-B49	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	1.27 (0.30 – 5.44)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	2.02 (0.18 – 22.63)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-B51	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.87 (0.64 – 5.49)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.61 (0.19 – 1.92)
HLA-B55	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	7.35 (2.33 – 23.14)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.07 (0.00 – 1.30)
HLA-B57	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	2.78 (1.05 – 7.32)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.00 (0.06 – 16.21)
HLA-B60	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	3.51 (0.99 – 12.36)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.19 (0.28 – 5.13)
HLA-B62	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/700	GPA (all patients)	NR	2.38 (0.70 – 8.12)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.71 (0.58 – 4.99)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-Cw1	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.45 (0.58 – 3.58)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	52/51	GPA (all patients)	NR	0.98 (0.24 – 4.03)
HLA-Cw3	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.40 (0.65 – 3.01)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	52/51	GPA (all patients)	NR	1.32 (0.62 – 2.81)
HLA-Cw7	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.50 (0.12 – 2.10)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	52/51	GPA (all patients)	NR	1.10 (0.62 – 1.96)
HLA-DPA1 **rs9277341 (C)**	Heckmann et al. 2008 [24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	282/380	GPA (all patients)	ANCA+ (250), ANCA- (27)	0.38 (0.29 – 0.51)
	Xie et al. 2013[25**]	Case-control (GWAS)	Canada, USA	ACR criteria	Geographically matched healthy subjects	459/1503	GPA (all patients)	c-ANCA (351)	0.30 (0.24 – 0.37)
		Case-control (GWAS)	USA	ACR criteria	Geographically matched healthy subjects	291/317	GPA (all patients)	c-ANCA (227)	0.43 (0.32 – 0.57)
HLA-DPB1*0101	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.20 (0.53 – 2.75)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	0.17 (0.05 – 0.63)
HLA-DPB1*0201	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	0.78 (0.49 – 1.23)
	Tsuchiya et al. 2006[28]	Case-Control	Japan	Japanese 1998 criteria	Geographically matched healthy subjects	50/77	MPA (all patients)	MPO-ANCA (all patients)	0.44 (0.21 – 0.91)
	Heckmann et al. 2008[24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	135/369	GPA (all patients)	ANCA+ (108), ANCA- (27)	1.38 (0.75 – 2.52)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.93 (0.57 – 1.51)
HLA-DPB1*0301	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.27 (0.56 – 2.89)
	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	0.02 (0.00 – 0.15)
	Heckmann et al. 2008[24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	135/369	GPA (all patients)	ANCA+ (108), ANCA- (27)	0.28 (0.14 – 0.55)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.65 (0.38 – 1.11)
	Arning et al. 2011[30**]	Case-Control	Germany	CHCC criteria	Geographically and ethnically matched healthy blood donors	482/356	GPA (389), EGPA (56), MPA (37)	NR	0.30 (0.21 – 0.44)
		Case-Control	UK	CHCC criteria	Geographically and ethnically matched healthy blood donors	193/104	GPA (102), MPA (82), EGPA (9)	NR	0.35 (0.16 – 0.77)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-DPB1*0401	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.42 (0.84 – 2.38)
	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	3.91 (2.62 – 5.84)
	Heckmann et al. 2008[24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	135/369	GPA (all patients)	ANCA+ (108), ANCA- (27)	2.15 (1.61 – 2.86)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	1.06 (0.78 – 1.45)
	Arning et al. 2011[30**]	Case-Control	Germany	CHCC criteria	Geographically and ethnically matched healthy blood donors	482/356	GPA (389), EGPA (56), MPA (37)	NR	2.34 (1.92 – 2.85)
		Case-Control	UK	CHCC criteria	Geographically and ethnically matched healthy blood donors	193/104	GPA (102), MPA (82), EGPA (9)	NR	2.02 (1.44 – 2.85)
HLA-DPB1*0402	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.82 (0.38 – 1.78)
	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	1.11 (0.65 – 1.92)
	Heckmann et al. 2008[24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	135/369	GPA (all patients)	ANCA+ (108), ANCA- (27)	1.14 (0.78 – 1.68)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	1.15 (0.75 – 1.77)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-DPB1*0501	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.34 (0.26 – 7.02)
	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	0.20 (0.01 – 4.93)
HLA-DPB1*0601	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	3.79 (0.19 – 74.15)
	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	0.60 (0.08 – 4.29)
HLA-DPB1*0901	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.52 (0.15 – 1.84)
	Jagiello et al. 2004[27]	Case-Control	Germany	ACR and CHCC criteria, biopsy	Geographically matched subjects	148/89	GPA (all patients)	PR3-ANCA (all patients)	1.81 (0.07 – 44.73)
HLA-DPB2 **rs3130215 (A)**	Heckmann et al. 2008[24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	282/380	GPA (all patients)	ANCA+ (250), ANCA- (27)	2.35 (1.88 – 2.94)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5365	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	0.88 (0.79 – 0.99)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Xie et al. 2013[25]	Case-control (GWAS)	Canada, USA	ACR criteria	Geographically matched healthy subjects	459/1503	GPA (all patients)	c-ANCA (351)	2.44 (2.10 – 2.84)
HLA-DQB1*02	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	0.98 (0.45 – 2.13)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/50	GPA (all patients)	NR	0.85 (0.41 – 1.77)
HLA-DQB1*0302	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.07 (0.53 – 2.20)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	0.94 (0.36 – 2.49)
HLA-DQB1*0303	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.84 (0.27 – 2.65)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	2.97 (0.72 – 12.23)
	Tsuchiya et al. 2006[28]	Case-Control	Japan	Japanese 1998 criteria	Geographically matched healthy subjects	50/77	MPA (all patients)	MPO-ANCA (all patients)	2.11 (1.14 – 3.90)
HLA-DQB1*04	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	1.74 (0.40 – 7.50)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/50	GPA (all patients)	NR	1.49 (0.24 – 9.08)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-DQB1*0501	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.77 (0.91 – 3.42)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	0.77 (0.32 – 1.88)
HLA-DQB1*0602	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.64 (0.29 – 1.42)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	12.86 (2.65 – 62.31)
HLA-DQ7	Zhang et al. 1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/50	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.21 (0.70 – 2.10)
	Papasteriades et al. 1997[22]	Case-Control	Greece	Histologically proven AAV	Geographically and ethnically matched healthy subjects	23/405	MPA (all patients)	NR	0.66 (0.34 – 1.30)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	0.43 (0.16 – 1.16)
HLA-DR1	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	2.17 (0.90 – 5.23)
	Papiha et al. 1992[32]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	27/105	GPA (all patients)	NR	4.37 (2.01 – 9.48)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Zhang et al.1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.89 (0.44 – 1.82)
	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	1.12 (0.48 – 2.64)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	0.68 (0.67 – 1.75)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.56 (0.23 – 1.35)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.71 (0.43 – 1.15)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5442	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.67 (0.49 – 0.92)
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	0.53 (0.34 – 0.81)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.79 (0.28 – 2.19)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-DR2	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	1.91 (0.97 – 3.75)
	Papiha et al. 1992[32]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	27/105	GPA (all patients)	NR	1.20 (0.56 – 2.54)
	Zhang et al.1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.19 (0.66 – 2.13)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.33 (0.08 – 1.38)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5442	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.97 (0.77 – 1.22)
HLA-DR3	Elkon et al. 1983[23]	Case-Control	UK	Clinical, and histological, criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	1.01 (0.52 – 1.99)
	Papiha et al. 1992[32]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	27/105	GPA (all patients)	NR	1.41 (0.59 – 3.36)
	Zhang et al.1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.75 (0.43 – 1.30)
	Papasteriades et al. 1997[22]	Case-Control	Greece	Histologically proven AAV	Geographically and ethnically matched healthy subjects	23/405	MPA (all patients)	NR	0.16 (0.02 – 1.18)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	2.54 (1.00 – 6.46)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.55 (0.53 – 4.52)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.66 (0.39 – 1.11)
	Stassen et al. 2009[19]	Case-Control	The Nether-lands	CHCC criteria	Geographically matched healthy blood donors	304/5442	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.07 (0.84 – 1.35)
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched commu-nity-based control frequen-cies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	1.30 (0.86 – 1.95)
HLA-DR4	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histo-logical criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	1.00 (0.52 – 1.92)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/300	GPA (all patients)	NR	0.96 (0.50 – 1.83)
	Papiha et al. 1992[32]	Case-Control	UK	Clinical and histo-logical criteria	Geographically matched subjects	27/105	GPA (all patients)	NR	0.81 (0.32 – 2.07)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Zhang et al.1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	1.24 (0.74 – 2.07)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	0.72 (0.27 – 1.88)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	1.25 (0.54 – 2.90)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.57 (0.61 – 4.01)
	Vaglio et al. 2007[35]	Case-Control	Italy	ACR and CHCC criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	48/350	EGPA (all patients)	MPO-ANCA (18), ANCA negative (26), undetermined (4)	1.73 (0.84 – 3.57)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/341	EGPA (all patients)	NR	1.87 (1.23 – 2.82)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5442	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	1.53 (1.25 – 1.87)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Cao et al. 2011[33]**	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	0.73 (0.51 – 1.05)
		Case-Control	USA (all African Americans)	Histologically proven AAV	USA African Americans-Bethesda database	16/112	NR	PR3-ANCA (9), MPO-ANCA (4), p-ANCA (2), ANCA negative (1)	0.62 (0.08 – 5.01)
HLA-DR5	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	0.72 (0.28 – 1.85)
	Papiha et al. 1992[32]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	27/105	GPA (all patients)	NR	0.18 (0.02 – 1.37)
	Zhang et al.1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.95 (0.45 – 2.01)
	Papasteriades et al. 1997[22]	Case-Control	Greece	Histologically proven AAV	Geographically and ethnically matched healthy subjects	23/405	MPA (all patients)	NR	1.59 (0.80 – 3.15)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5442	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.80 (0.58 – 1.09)
HLA-DR6	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	0.16 (0.04 – 0.70)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Papiha et al. 1992[32]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	27/105	GPA (all patients)	NR	0.11 (0.01 – 1.83)
	Zhang et al.1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.60 (0.27 – 1.31)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.63 (0.56 – 4.77)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5442	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.44 (0.32 – 0.59)
HLA-DR7	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	0.512 (0.22 – 1.22)
	Papiha et al. 1992[32]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	27/105	GPA (all patients)	NR	0.64 (0.23 – 1.73)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	0.99 (0.40 – 2.48)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.77 (0.34 – 1.75)
	Vaglio et al. 2007[35]	Case-Control	Italy	ACR and CHCC criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	48/350	EGPA (all patients)	MPO-ANCA (18), ANCA negative (26), undetermined (4)	2.42 (1.47 – 4.00)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/341	EGPA (all patients)	NR	1.58 (1.01 – 2.47)
	Stassen et al. 2009[19]	Case-Control	The Netherlands	CHCC criteria	Geographically matched healthy blood donors	304/5442	GPA (241), MPA (30), EGPA (12), RVL (21)	PR3-ANCA (218), MPO-ANCA (64), other (4), ANCA negative (18)	0.96 (0.72 – 1.27)
HLA-DR8	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	0.52 (0.22 – 1.22)
	Murty et al. 1991[17]	Case-Control	Ireland, UK	Histologically proven AAV	Geographically matched healthy subjects	41/300	GPA (all patients)	NR	1.05 (0.21 – 5.14)
	Zhang et al.1995[26]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	2.21 (0.67 – 7.32)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	1.63 (0.66 – 4.05)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	1.23 (0.31 – 4.91)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.00 (0.12 – 5.08)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	2.03 (1.01 – 4.05)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Stassen et al. 2009[19]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.95 (0.59 – 1.54)
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	0.76 (0.37 – 1.55)
HLA-DR9	Elkon et al. 1983[23]	Case-Control	UK	Clinical and histological criteria	Geographically matched subjects	46/113	GPA (17), EGPA (14), PAN (15)	NR	0.49 (0.02 – 10.21)
	Nakamaru et al. 1996[20]	Case-Control	Japan	Clinical, histological, serological criteria	Geographically matched healthy subjects	16/472	GPA (all patients)	c-ANCA (all patients)	3.03 (1.40 – 6.56)
	Fujii et al. 2000[36]	Case-Control	Japan	CHCC criteria	Geographically matched healthy subjects	12/472	NR	MPO-ANCA (all patients)	2.75 (1.12 – 6.76)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	0.94 (0.04 – 23.32)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.66 (0.11 – 4.04)
	Vaglio et al. 2007[35]	Case-Control	Italy	ACR and CHCC criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	48/350	EGPA (all patients)	MPO-ANCA (18), ANCA negative (26), undetermined (4)	1.83 (0.20 – 16.56)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.80 (0.17 – 3.74)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Stassen et al. 2009[19]	Case-Control	UK	CHCC criteria	Geographically matched healthy subjects	94/90	GPA (56), MPA (31), RVL (7)	PR3-ANCA (36), MPO-ANCA (19), PR3- and MPO-ANCA positive (3)	0.35 (0.11 – 1.09)
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	0.75 (0.208 – 2.72)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.68 (0.43 – 1.06)
	Tsuchiya et al. 2013[37]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	116/265	MPA (96)	MPO-ANCA (all patients)	1.74 (1.18 – 2.57)
HLA-DRB1*0401	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	2.10 (0.52 – 8.50)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.58 (0.18 – 1.90)
HLA-DRB1*0403	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.95 (0.27 – 3.40)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.16 (0.02 – 1.30)
*HLA-DRB1*0405*	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.61 (0.30 – 1.22)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	1.13 (0.52 – 2.49)
*HLA-DRB1*0406*	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.65 (0.19 – 2.22)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	1.84 (0.73 – 4.63)
*HLA-DRB1*0407*	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	2.82 (0.78 – 10.14)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.44 (0.02 – 10.77)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
HLA-DRB1*0410	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	1.67 (0.32 – 8.69)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.44 (0.02 – 10.77)
HLA-DRB1*0802	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	1.16 (0.42 – 3.18)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.26 (0.01 – 5.47)
HLA-DRB1*0803	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	1.51 (0.78 – 2.93)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	1.19 (0.61 – 2.33)
HLA-DRB1*10	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	1.43 (0.13 – 16.03)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	5.10 (0.24 – 107.55)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.40 (0.02 – 7.44)
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	0.75 (0.21 – 2.72)
HLA-DRB1*11	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	0.723 (0.26 – 2.04)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.28 (0.48 – 3.38)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.88 (0.53 – 1.48)
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	1.09 (0.66 – 1.80)
HLA-DRB1*1101	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	1.75 (1.02 – 3.00)
	Tsuchiya et al. 2013[37]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	116/265	MPA (96)	MPO-ANCA (all patients)	2.79 (0.84 – 9.23)
HLA-DRB1*12	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	2.97 (0.721 – 2.23)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	6.31 (0.75 – 53.40)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/369	EGPA (all patients)	NR	0.57 (0.17 – 1.93)
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	0.59 (0.17 – 2.06)
HLA-DRB1*1201	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.21 (0.03 – 1.60)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.46 (0.16 – 1.29)
HLA-DRB1*1202	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.37 (0.05 – 2.90)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	1.70 (0.93 – 3.13)
HLA-DRB1*13	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	0.56 (0.20 – 1.53)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.65 (0.31 – 1.38)
	Vaglio et al. 2007[35]	Case-Control	Italy	ACR and CHCC criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	48/350	EGPA (all patients)	MPO-ANCA (18), ANCA negative (26), undetermined (4)	0.23 (0.07 – 0.73)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/341	EGPA (all patients)	NR	0.50 (0.28 – 0.89)
HLA-DRB1*1302	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.54 (0.24 – 1.23)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.52 (0.16 – 1.67)
HLA-DRB1*14	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	1.07 (0.27 – 4.16)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	2.02 (0.18 – 22.63)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/341	EGPA (all patients)	NR	1.83 (0.72 – 4.65)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Cao et al. 2011[33]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	2.82 (1.05 – 7.59)
HLA-DRB1*1403	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	1.25 (0.34 – 4.60)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	1.32 (0.19 – 9.41)
HLA-DRB1*1405	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.11 (0.01 – 1.91)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.96 (0.44 – 2.13)
HLA-DRB1*15	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	0.79 (0.34 – 1.83)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.13 (0.57 – 2.26)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Cao et al. 2011[33**]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	2.72 (1.84 – 4.03)
		Case-Control	USA (all African Americans)	Histologically proven AAV	USA African Americans-Bethesda database	16/112	NR	PR3-ANCA (9), MPO-ANCA (4), p-ANCA (2), ANCA negative (1)	2.08 (0.86 – 5.03)
HLA-DRB1*1501	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	1.62 (0.79 – 3.35)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	1.69 (1.16 – 2.46)
HLA-DRB1*1502	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.67 (0.32 – 1.39)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.35 (0.10 – 1.27)
HLA-DRB1*16	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3 (all patients)	0.40 (0.02 – 7.80)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.14 (0.01 – 2.72)
	Cao et al. 2011[33**]	Case-Control	USA (96 Caucarians and 41 African Americans)	Histologically proven AAV	Ethnically matched community-based control frequencies (Healthy Organ Donor-North Carolina Services)	137/379	NR	PR3-ANCA (90), MPO-ANCA (47)	1.39 (0.34 – 5.59)
		Case-Control	USA (all African Americans)	Histologically proven AAV	USA African Americans-Bethesda database	16/112	NR	PR3-ANCA (9), MPO-ANCA (4), p-ANCA (2), ANCA negative (1)	2.42 (0.47 – 12.55)
HLA-DRB1*1602	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	64/265	GPA (3), MPA (50), EGPA (8), PAN (3)	MPO-ANCA (all patients)	0.24 (0.01 – 4.171)
	Luo et al. 2011[34]	Case-Control	China	EMA algorithm	Ethnically matched healthy blood donors	152/200	GPA (45), MPA (107)	PR3-ANCA (19), MPO-ANCA (130), PR3- and MPO-ANCA positive (3)	0.12 (0.01 – 2.14)
HLA-DRB3	Spencer et al. 1992[38]	Case-Control	UK	Clinical criteria	Geographically and ethnically matched subjects	59/1103	GPA (34), MPA (25)	c-ANCA (47), p-ANCA (10), ANCA negative (2)	0.29 (0.11 – 0.79)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.41 (0.76 – 2.60)
	Vaglio et al. 2007[35]	Case-Control	Italy	ACR and CHCC criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	48/350	EGPA (all patients)	MPO-ANCA (18), ANCA negative (26), undetermined (4)	0.54 (0.35 – 0.84)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/341	EGPA (all patients)	NR	0.61 (0.44 – 0.86)
HLA-DRB4	Spencer et al. 1992[38]	Case-Control	UK	Clinical criteria	Geographically and ethnically matched subjects	59/1103	GPA (34), MPA (25)	c-ANCA (47), p-ANCA (10), ANCA negative (2)	1.38 (0.89 – 2.13)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	0.89 (0.46 – 1.72)
	Vaglio et al. 2007[35]	Case-Control	Italy	ACR and CHCC criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	48/350	EGPA (all patients)	MPO-ANCA (18), ANCA negative (26), undetermined (4)	2.49 (1.58 – 3.90)
	Wieczorek et al. 2008[29]	Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	102/341	EGPA (all patients)	NR	1.87 (1.34 – 2.61)
HLA-DRB5	Spencer et al. 1992[38]	Case-Control	UK	Clinical criteria	Geographically and ethnically matched subjects	59/1103	GPA (34), MPA (25)	c-ANCA (47), p-ANCA (10), ANCA negative (2)	1.35 (0.69 – 2.63)
	Von Vietinghoff et al. 2006[18]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	51/51	GPA (all patients)	NR	1.07 (0.82 – 2.18)
HSD17B8 **rs421446 (C)**	Heckmann et al. 2008[24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	282/369	GPA (all patients)	ANCA+ (250), ANCA- (27)	0.43 (0.32 – 0.57)
	Xie et al. 2013[25]	Case-control (GWAS)	Canada, USA	ACR criteria	Geographically matched healthy subjects	459/1503	GPA (all patients)	c-ANCA (351)	0.39 (0.32 – 0.48)
IL-1β **(A2)**	Huang et al. 2000[4]	Case-Control	Sweden	ACR criteria	Geographically and ethnically matched healthy subjects	32/109	GPA (all patients)	PR3-ANCA (29)	1.21 (0.55 – 2.63)
	Borgmann et al. 2003[39]	Case-Control	Germany	CHCC criteria	Geographically matched healthy, unrelated subjects	109/196	NR	PR3-ANCA (79), MPO-ANCA (30)	0.89 (0.61 – 1.29)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Zhou et al. 2004[6]	Case-Control	USA	ACR criteria	Geographically and ethnically matched healthy, unrelated subjects	117/123	GPA (all patients)	PR3-ANCA (82), MPO-ANCA (5), PR3- and MPO-ANCA positive (2), ANCA negative (25)	1.16 (0.72 – 1.85)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	914/5259	GPA (565), MPA (262)	PR3-ANCA (478), MPO-ANCA (264)	1.00 (0.89 – 1.12)
***IL1RN**1**	Borgmann et al. 2003[39]	Case-Control	Germany	CHCC criteria	Geographically matched healthy, unrelated subjects	109/234	NR	PR3-ANCA (79), MPO-ANCA (30)	1.12 (0.79 –1.59)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	1.00 (0.69 – 1.44)
***IL1RN**2**	Borgmann et al. 2003[39]	Case-Control	Germany	CHCC criteria	Geographically matched healthy, unrelated subjects	109/234	NR	PR3-ANCA (79), MPO-ANCA (30)	1.00 (0.69 – 1.43)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	1.01 (0.69 – 1.48)
***IL1RN**3**	Borgmann et al. 2003[39]	Case-Control	Germany	CHCC criteria	Geographically matched healthy, unrelated subjects	109/234	NR	PR3-ANCA (79), MPO-ANCA (30)	0.30 (0.07 – 1.33)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	1.06 (0.35 – 3.20)
***IL1RN**4**	Borgmann et al. 2003[39]	Case-Control	Germany	CHCC criteria	Geographically matched healthy, unrelated subjects	109/234	NR	PR3-ANCA (79), MPO-ANCA (30)	0.71 (0.03 – 17.58)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.95 (0.09 – 10.56)
***IL1RN*5**	Borgmann et al. 2003[39]	Case-Control	Germany	CHCC criteria	Geographically matched healthy, unrelated subjects	109/234	NR	PR3-ANCA (79), MPO-ANCA (30)	1.07 (0.10 – 11.91)
	Persson et al. 2013[7]	Case-Control	Sweden	ACR and CHCC criteria, EMA algorithm	Blood donors	105/200	GPA (61), MPA (44)	PR3-ANCA (62), MPO-ANCA (43)	0.63 (0.03 – 15.60)
***IL-6* rs1800795 (C)**	Zhou et al. 2004[6]	Case-Control	USA	ACR criteria	Geographically and ethnically matched healthy, unrelated subjects	117/123	GPA (all patients)	PR3-ANCA (82), MPO-ANCA (5), PR3- and MPO-ANCA positive (2), ANCA negative (25)	1.00 (0.70 – 1.44)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	1.37 (0.76 – 2.48)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	914/5259	GPA (565), MPA (262)	PR3-ANCA (478), MPO-ANCA (264)	1.04 (0.94 – 1.15)
	Husmann et al. 2014[40]	Case-Control	Germany	EMA algorithm	Geographically matched healthy blood donors	863/1344	GPA (646), MPA (53), EGPA (164)	NR	0.95 (0.85 – 1.08)
***IL-10* rs1800872 (A)**	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	1.10 (0.55 – 2.12)
	Wieczorek et al. 2008[41]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	506/507	GPA (403), EGPA (103)	ANCA+ (389), ANCA- (110)	1.00 (0.81 – 1.23)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
***IL-10* rs1800896 (G)**	Murakozy et al. 2001[42]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	39/72	GPA (all patients)	NR	0.59 (0.34 – 1.03)
	Bartfai et al. 2003[43]	Case-Control	Caucasian patients	ACR and CHCC criteria	Ethnically matched blood donors	161/153	GPA (125), MPA (36)	NR	0.74 (0.54 – 1.02)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	1.18 (0.67 – 2.10)
	Wieczorek et al. 2008[41]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	506/507	GPA (403), EGPA (103)	ANCA+ (389), ANCA- (110)	0.88 (0.74 – 1.05)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5360	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.04 (0.93 – 1.17)
***IRF5* rs10954213 (G)**	Wieczorek et al. 2010[44]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	627/902	GPA (all patients)	NR	0.31 (0.26 – 0.37)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5365	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.04 (0.93 – 1.17)
	Kawasaki et al. 2013[45]	Case-Control	Japan	EMA algorithm	Geographically matched healthy, unrelated subjects	232/710	GPA (28), MPA (177), EGPA (15), unclassified (12)	MPO (all patients)	1.28 (1.04 – 1.58)
***LEPR* rs8179183 (C)**	Wieczorek et al. 2010[15***]	Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	456/818	GPA (all patients)	NR	0.72 (0.58 – 0.90)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
		Case-Control	Germany	CHCC criteria	Geographically matched healthy subjects	226/508	GPA (all patients)	NR	0.71 (0.52 – 0.97)
		Case-Control	Germany	ACR criteria	Geographically matched healthy subjects	196/1327	EGPA (all patients)	NR	1.41 (1.10 – 1.81)
MPO **rs2333227 (A)**	Reynolds et al. 2002[46]	Case-Control	The Netherlands	CHCC criteria	Ethnically matched healthy subjects	142/192	GPA (96), MPA (26), EGPA (8), iRPGN (12)	PR3-ANCA (92), MPO-ANCA (50)	1.07 (0.70 – 1.63)
	Fiebeler et al. 2004[47]	Case-Control	Germany	Clinical criteria	Geographically matched healthy subjects	119/270	GPA (63), MPA (56)	PR3-ANCA (71), MPO-ANCA (48)	0.90 (0.62 – 1.32)
	Rajp et al. 2007[48]	Case-Control	UK	CHCC criteria and histological criteria	Ethnically matched healthy subjects	134/150	GPA (69), MPA (65)	PR3-ANCA (91), MPO-ANCA (43)	0.92 (0.61 – 1.39)
PCD1 **rs1156882 (A)**	Slot et al. 2008[8]	Case-Control	The Netherlands	CHCC criteria	Geographically and ethnically matched healthy subjects	102/204	GPA (50), MPA (24), EGPA (7), RLV (21)	PR3-ANCA (49), MPO-ANCA (34), PR3- and MPO-ANCA positive (2), ANCA negative (17)	0.94 (0.52 – 1.70)
	Sakthivel et al. 2009[49]	Case-Control	Sweden	Clinical criteria	Geographically and ethnically matched blood donors	66/275	GPA (all patients)	NR	0.78 (0.37 – 1.64)
PTPN22 **rs2476601 (A)**	Jagiello et al. 2005[50]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy subjects	199/399	GPA (all patients)	c-ANCA+ (168), p-ANCA+ (1), ANCA negative (30)	1.75 (1.23 – 2.49)
	Chung et al. 2012[2**]	Case-Control	USA	ACR criteria	Geographically matched subjects with no (family) history of autoimmune disease	424/469	GPA (all patients)	PR3-ANCA (339), MPO-ANCA (44), ANCA negative (39)	1.25 (0.91 – 1.71)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
		Case-Control	Canada	ACR criteria	Geographically matched subjects with no history of autoimmune disease	456/1500	GPA (all patients)	NR	1.40 (1.11 – 1.78)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5365	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.35 (1.14 – 1.60)
	Martorana et al. 2012[51]	Case-Control	Italy	ACR and CHCC criteria	Geographically matched healthy subjects with no history of autoimmune disease	344/945	GPA (143), EGPA (99), MPA (102)	c-ANCA (126), p-ANCA (163), ANCA negative (39)	1.40 (0.97 – 2.04)
***RING1/ RXRB* rs213213 (A)**	Heckmann et al. 2008[24]	Case-Control	Germany	ACR criteria	Geographically matched healthy blood donors	279/369	GPA (all patients)	ANCA+ (250), ANCA- (27)	2.13 (1.70 – 2.67)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5366	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.55 (1.38 – 1.74)
	Xie et al. 2013[25]	Case-control (GWAS)	Canada, USA	ACR criteria	Geographically matched healthy subjects	459/1503	GPA (all patients)	c-ANCA (351)	1.83 (1.57 – 2.13)
***RXRB* rs6531 (C)**	Szyld et al. 2006[52]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy subjects	187/201	GPA (all patients)	ANCA+ (151), ANCA- (36)	0.60 (0.45 – 0.80)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	911/5251	GPA (565), MPA (262)	PR3-ANCA (478), MPO-ANCA (264)	1.79 (1.61 – 1.98)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Xie et al. 2013[25]	Case-control (GWAS)	Canada, USA	ACR criteria	Geographically matched healthy subjects	459/1503	GPA (all patients)	c-ANCA (351)	1.81 (1.55 – 2.11)
***RXRB* rs9277935 (T)**	Wieczorek et al. 2009[53]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	282/380	GPA (all patients)	NR	0.39 (0.29 – 0.54)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5350	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	0.51 (0.43 – 0.61)
	Xie et al. 2013[25]	Case-control (GWAS)	Canada, USA	ACR criteria	Geographically matched healthy subjects	459/1503	GPA (all patients)	c-ANCA (351)	0.34 (0.27 – 0.44)
***SERPINA1* S allele**	Lhotta et al. 1994[54]	Case-Control	Austria	ACR	Geographically matched healthy subjects	32/868	GPA (29), MPA (2), iRPGN (1)	c-ANCA (all patients)	0.69 (0.09 – 5.11)
	Griffith et al. 1996[55]	Case-Control	UK	CHCC criteria	Geographically and ethnically matched unrelated subjects	102/2310	GPA (51), MPA (29), EGPA (9), PAN (2), iRPGN (11)	c-ANCA (70), p-ANCA (32)	1.42 (0.79 – 2.52)
	Mahr et al. 2010[56]	Case-Control	USA	ACR criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	433/421	GPA (all patients)	PR3-ANCA (339), MPO-ANCA (44), ANCA negative (39)	1.68 (1.08 – 2.63)
	Morris et al. 2011[57]	Case-Control	Germany (531), France (81), UK (244)	CHCC criteria	Geographically and ethnically matched healthy subjects with no (family) history of autoimmune disease	856/1505	GPA (723), MPA (133)	c-ANCA (605), p-ANCA (150), ANCA negative (54)	1.11 (0.81 – 1.51)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Chorostowska-Wynimko et al. 2013[58]	Case-Control	Poland	Clinical, histological, and serological criteria	Geographically matched neonates born alive	51/658	GPA (all patients)	c-ANCA (43), p-ANCA (2), ANCA negative (4)	2.61 (0.56 – 12.08)
***SERPINA1* Z allele**	Lhotta et al. 1994[54]	Case-Control	Austria	ACR criteria	Geographically matched healthy subjects	32/868	GPA (29), MPA (2), iRPGN (1)	c-ANCA (all patients)	8.76 (3.63 – 21.17)
	Griffith et al. 1996[55]	Case-Control	UK	CHCC criteria	Geographically and ethnically matched unrelated subjects	102/2310	GPA (51), MPA (29), EGPA (9), PAN (2), iRPGN (11)	c-ANCA (70), p-ANCA (32)	2.20 (1.05 – 4.62)
	Callea et al. 1997[59]	Case- Control	Italy	CHCC criteria	Healthy blood donors	84/200	GPA (33), MPA (28), iRPGN (23)	c-ANCA (38), p-ANCA (46)	4.90 (1.21 – 19.83)
	Borgmann et al. 2001[60]	Case-Control	Germany	ACR and CHCC criteria	Geographically and ethnically matched healthy, unrelated subjects	97/752	GPA (all patients)	c-ANCA (all patients)	3.36 (1.45 – 7.79)
	Mahr et al. 2010[56]	Case-Control	USA	ACR criteria	Geographically matched healthy subjects with no (family) history of autoimmune disease	433/421	GPA (all patients)	PR3-ANCA (339), MPO-ANCA (44), ANCA negative (39)	1.99 (1.12 – 3.52)
	Morris et al. 2011[57]	Case-Control	Germany (531), France (81), UK (244)	CHCC criteria	Geographically and ethnically matched healthy subjects with no (family) history of autoimmune disease	856/1505	GPA (723), MPA (133)	c-ANCA (605), p-ANCA (150), ANCA negative (54)	2.25 (1.60 – 3.18)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Previously unpublished Lyons et al. 2012 GWAS data[3**]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	562/805	GPA (391), MPA (143)	PR3-ANCA (322), MPO-ANCA (166)	2.59 (1.66 – 4.02)
		Case-control (GWAS)	Europe	EMA algorithm, supported by either serology or histology	Geographically matched subjects	1445/1062	NR	NR	4.19 (2.62 – 6.69)
	Chorostowska-Wynimko et al. 2013[58]	Case-Control	Poland	Clinical, histological, serological criteria	Geographically matched neonates born alive	51/658	GPA (all patients)	c-ANCA (43), p-ANCA (2), ANCA negative (4)	2.19 (0.63 – 7.55)
***STAT4* rs7574865 (T)**	Wieczorek et al. 2010[44]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	612/880	GPA (all patients)	NR	1.01 (0.85 – 1.20)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5366	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.18 (1.04 – 1.35)
	Kawasaki et al. 2013[45]	Case-Control	Japan	EMA algorithm	Geographically matched healthy, unrelated subjects	232/710	GPA (28), MPA (177), EGPA (15), unclassified (12)	MPO (all patients)	1.10 (0.89 – 1.37)
***TGF*-β_1 rs1800471 (C)**	Murakozy et al. 2001[42]	Case-Control	Germany	ACR and CHCC criteria	Geographically matched healthy blood donors	39/72	GPA (all patients)	NR	2.20 (0.89 – 5.44)
	Bartfai et al. 2003[43]	Case-Control	Caucasian patients	ACR and CHCC criteria	Ethnically matched blood donors	161/96	GPA (125), MPA (36)	NR	1.06 (0.52 – 2.14)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	1.86 (0.73 – 4.71)
***TNFα* rs1800629 (A)**	Mascher et al. 1997[61]	Case-Control	Germany	ACR and CHCC criteria	Healthy blood donors	35/111	GPA (all patients)	c-ANCA (32), p-ANCA (1), ANCA negative (1)	1.54 (0.77 – 3.10)
	Zhou et al. 2004[6]	Case-Control	USA	ACR criteria	Geographically and ethnically matched healthy, unrelated subjects	117/123	GPA (all patients)	PR3-ANCA (82), MPO-ANCA (5), PR3- and MPO-ANCA positive (2), ANCA negative (25)	0.88 (0.52 – 1.50)
	Spriewald et al. 2005[31]	Case-Control	Germany	ACR and CHCC criteria	Ethnically matched healthy subjects	32/91	GPA (all patients)	PR3-ANCA (all patients)	2.22 (0.93 – 5.29)
	Previously unpublished Lyons et al. 2012 GWAS data[3]	Case-control (GWAS)	UK	EMA algorithm, supported by either serology or histology	Wellcome Trust Case Control Consortium (UK)	676/5366	GPA (394), MPA (156)	PR3-ANCA (326), MPO-ANCA (167)	1.06 (0.92 – 1.23)
***TNFRII* 196R**	Tsuchiya et al. 2003[5]	Case-Control	Japan	CHCC and Japanese 1998 criteria	Geographically matched healthy subjects	50/262	MPA (all patients)	MPO-ANCA (all patients)	0.67 (0.31 – 1.45)

Supplementary table 2. Study characteristics of the included studies (Continued)

Variants	Article	Study details		Definitions		Participants (n)			OR (95% CI)
		Design	Setting	Cases	Controls	Cases / Controls	Diagnoses	ANCA serotype	Allele level
	Zhou et al. 2004[6]	Case-Control	USA	ACR criteria	Geographically and ethnically matched healthy, unrelated subjects	117/123	GPA (all patients)	PR3-ANCA (82), MPO-ANCA (5), PR3- and MPO-ANCA positive (2), ANCA negative (25)	1.05 (0.70 – 1.57)
***TLR9* rs352162 (T)**	Husmann et al. 2014[40**]	Case-Control	Germany	EMA algorithm	Geographically matched healthy blood donors	863/1344	GPA (646), MPA (53), EGPA (164)	NR	1.89 (1.67 – 2.14)
			The Netherlands, UK	EMA algorithm	Geographically and ethnically matched healthy subjects	426/554	GPA (273), MPA (100), EGPA (53)	NR	1.07 (0.90 – 1.28)
***TLR9* rs352140 (T)**	Husmann et al. 2014[40**]	Case-Control	Germany	EMA algorithm	Geographically matched healthy blood donors	863/1344	GPA (646), MPA (53), EGPA (164)	NR	1.16 (1.03 – 1.31)
			The Netherlands, UK	EMA algorithm	Geographically and ethnically matched healthy subjects	426/554	GPA (273), MPA (100), EGPA (53)	NR	1.06 (0.89 – 1.27)
***TLR9* rs352139 (T)**	Husmann et al. 2014[40**]	Case-Control	Germany	EMA algorithm	Geographically matched healthy blood donors	863/1344	GPA (646), MPA (53), EGPA (164)	NR	1.12 (0.99 – 1.27)
			The Netherlands, UK	EMA algorithm	Geographically and ethnically matched healthy subjects	426/554	GPA (273), MPA (100), EGPA (53)	NR	1.08 (0.91 – 1.30)
***TLR9* rs5743836 (G)**	Husmann et al. 2014[40**]	Case-Control	Germany	EMA algorithm	Geographically matched healthy blood donors	863/1344	GPA (646), MPA (53), EGPA (164)	NR	0.82 (0.69 – 0.98)
			The Netherlands, UK	EMA algorithm	Geographically and ethnically matched healthy subjects	426/554	GPA (273), MPA (100), EGPA (53)	NR	1.22 (0.94 – 1.58)

ACR, American College of Rheumatology; ANCA, anti-neutrophil cytoplasm antibody; AAV, ANCA-associated vasculitis; c-ANCA, cytoplasmic ANCA; CHCC, Chapel Hill Consensus Conference; EGPA, eosinophilic granulomatosis with polyangiitis; EMA, European Medicines Agency; GPA, granulomatosis with polyangiitis; GWAS, genome-wide association study; iRPGN, idiopathic rapidly progressive glomerulonephritis; MPA, microscopic polyangiitis; MPO-ANCA, myeloperoxidase ANCA; NR, not reported; PAN, polyarteritis nodosa; p-ANCA, perinuclear ANCA; NR, not reported; RLV, renal limited vasculitis; PR3-ANCA, proteinase 3 ANCA; UK, United Kingdom; USA, United States of America. **Two cohorts described in the same publication; ***Three cohorts described in the same publication.

Supplementary table 3. Genetic variants not associated with AAV after meta-analysis

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry
CTLA-4 $(AT)_{102}$	3	186/420	1.20 (0.75 – 1.90)	0.449	0	0.375	0.986
CTLA-4 $(AT)_{104}$	3	186/420	1.21 (0.92 – 1.60)	0.180	0	0.390	0.227
CTLA-4 $(AT)_{106}$	3	186/420	1.30 (0.74 – 2.29)	0.359	75	0.018	0.674
CTLA-4 $(AT)_{108}$	3	186/420	0.88 (0.50 – 1.56)	0.963	0	0.963	0.473
CTLA-4 $(AT)_{110}$	3	186/420	0.74 (0.34 – 1.61)	0.441	0	0.667	0.171
CTLA-4 $(AT)_{116}$	3	186/420	0.80 (0.27 – 2.33)	0.677	0	0.724	0.786
CTLA-4 $(AT)_{118}$	3	186/420	0.46 (0.16 – 1.35)	0.158	26	0.258	0.155
CTLA-4 $(AT)_{122}$	3	186/420	1.70 (0.62 – 4.71)	0.304	26	0.260	0.557
CTLA-4 $(AT)_{124}$	3	186/420	1.97 (0.73 – 5.31)	0.178	61	0.080	0.637
CTLA-4 $(AT)_{126}$	3	186/420	1.25 (0.53 – 2.94)	0.609	53	0.121	0.701
CTLA-4 $(AT)_{128}$	3	186/420	1.15 (0.47 – 2.82)	0.760	37	0.204	0.826
FCAR rs16986050 (G)	2	1311/5891	0.93 (0.82 – 1.05)	0.211	84	0.002	0.523
FCGR3A rs396991 (G)	2	141/457	1.00 (0.74 – 1.34)	0.978	79	0.030	N/A
FCGR3B (NA1)	4	982/970	0.96 (0.84 – 1.11)	0.590	37	0.191	0.210
GHSR rs509035 (A)	2	1547/6595	1.04 (0.96 – 1.14)	0.342	52	0.123	0.874
GHSR rs519384 (A)	1	628/1328	1.12 (0.96 – 1.30)	0.140	0	0.389	N/A
GHSR rs572169 (A)	1	620/1304	1.12 (0.97 – 1.30)	0.118	50	0.158	N/A
HLA-A1	4	427/7324	1.07 (0.89 – 1.30)	0.455	0	0.567	0.996
HLA-A2	5	443/7796	0.96 (0.82 – 1.12)	0.582	0	0.956	0.335
HLA-A3	4	427/7324	1.18 (0.97 – 1.42)	0.092	35	0.201	0.273
HLA-A9	3	366/6991	0.85 (0.65 – 1.13)	0.266	77	0.014	0.311
HLA-A10	3	366/6991	0.79 (0.48 – 1.30)	0.356	78	0.010	0.273

Supplementary table 3. Genetic variants not associated with AAV after meta-analysis (Continued)

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry
HLA-A11	6	466/8107	0.97 (0.52 – 1.81)	0.924	65	0.013	0.273
HLA-A24	3	108/1223	0.94 (0.56 – 1.60)	0.830	0	0.510	0.090
HLA-A25	2	92/751	1.82 (0.72 – 4.58)	0.206	0	0.699	N/A
HLA-A26	4	131/1628	1.56 (0.89 – 2.74)	0.124	43	0.154	0.143
HLA-A28	3	376/6962	0.80 (0.52 – 1.23)	0.302	0	0.538	0.066
HLA-A29	3	123/988	0.88 (0.38 – 2.03)	0.760	42	0.178	0.801
HLA-A30	2	92/751	1.03 (0.25 – 4.28)	0.964	0	0.496	N/A
HLA-A31	3	108/1223	1.02 (0.43 – 2.44)	0.969	0	0.482	0.169
HLA-A32	3	108/1223	1.62 (0.74 – 3.54)	0.231	62	0.074	0.996
HLA-B7	4	427/7324	1.11 (0.91 – 1.35)	0.294	30	0.232	0.234
HLA-B12	2	335/6573	0.97 (0.76 – 1.23)	0.775	0	0.911	N/A
HLA-B13	4	427/7324	0.78 (0.46 – 1.34)	0.373	0	0.413	0.319
HLA-B14	4	427/7324	0.90 (0.54 – 1.49)	0.676	0	0.487	0.615
HLA-B15	2	355/5923	1.01 (0.77 – 1.31)	0.955	0	0.482	N/A
HLA-B18	4	427/7172	0.76 (0.50 – 1.16)	0.204	0	0.865	0.756
HLA-B27	4	412/7223	0.67 (0.44 – 1.02)	0.061	1	0.388	0.336
HLA-B35	4	412/7095	0.78 (0.58 – 1.06)	0.108	0	0.885	0.527
HLA-B37	2	355/5923	0.93 (0.51 – 1.70)	0.814	44	0.182	N/A
HLA-B39	2	67/523	0.65 (0.11 – 3.68)	0.622	0	0.619	N/A
HLA-B40	2	355/5923	0.94 (0.70 – 1.25)	0.652	0	0.319	N/A
HLA-B44	3	108/1223	1.53 (0.96 – 2.44)	0.072	46	0.157	0.225
HLA-B49	2	92/751	1.46 (0.43 – 4.92)	0.542	0	0.747	N/A

Supplementary table 3. Genetic variants not associated with AAV after meta-analysis (Continued)

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry
HLA-B51	2	67/523	1.03 (0.46 – 2.32)	0.943	50	0.158	N/A
HLA-B55	2	67/523	1.07 (0.40 – 2.88)	0.887	93	<0.001	N/A
HLA-B57	2	92/751	2.37 (0.92 – 6.11)	0.075	0	0.493	N/A
HLA-B60	2	57/1172	1.98 (0.77 – 5.09)	0.154	20	0.263	N/A
HLA-B62	2	57/1172	1.95 (0.87 – 4.37)	0.107	0	0.688	N/A
HLA-Cw1	2	68/523	1.28 (0.59 – 2.77)	0.534	0	0.650	N/A
HLA-Cw3	2	68/523	1.36 (0.79 – 2.33)	0.267	0	0.907	N/A
HLA-Cw7	2	68/523	0.96 (0.57 – 1.62)	0.877	3	0.309	N/A
*HLA-DPB1*0101*	2	242/139	0.65 (0.34 – 1.22)	0.178	84	0.013	N/A
*HLA-DPB1*0201*	4	435/904	0.85 (0.65 – 1.12)	0.241	49	0.118	0.708
*HLA-DPB1*0402*	4	479/877	1.10 (0.87 – 1.40)	0.433	0	0.893	0.074
*HLA-DPB1*0501*	2	242/139	0.86 (0.22 – 3.33)	0.823	7	0.300	N/A
*HLA-DPB1*0601*	2	242/139	1.25 (0.28 – 5.67)	0.769	7	0.300	N/A
*HLA-DPB1*0901*	2	242/139	0.63 (0.20 – 2.01)	0.438	0	0.476	N/A
*HLA-DQB1*02*	2	83/141	0.91 (0.53 – 1.55)	0.720	0	0.796	N/A
*HLA-DQB1*0302*	2	126/141	1.03 (0.58 – 1.82)	0.931	0	0.832	N/A
*HLA-DQB1*04*	2	83/141	1.63 (0.52 – 5.11)	0.404	0	0.893	N/A
*HLA-DQB1*0501*	2	209/282	1.32 (0.79 – 2.20)	0.287	53	0.143	N/A
*HLA-DQB1*0602*	2	126/141	1.36 (0.70 – 2.64)	0.360	91	0.001	N/A
HLA-DQ7	3	149/546	0.82 (0.56 – 1.20)	0.302	49	0.142	0.252
HLA-DR1	10	1009/7105	0.93 (0.64 – 1.34)	0.684	70	<0.001	0.105
HLA-DR2	5	487/6222	1.10 (0.80 – 1.54)	0.554	37	0.176	0.757

Supplementary table 3. Genetic variants not associated with AAV after meta-analysis (Continued)

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry
HLA-DR3	9	816/7045	1.05 (0.81 – 1.36)	0.740	41	0.096	0.946
HLA-DR4	11	914/7696	1.19 (0.95 – 1.50)	0.132	47	0.037	0.342
HLA-DR5	5	494/6155	0.90 (0.61 – 1.31)	0.570	32	0.206	0.965
HLA-DR7	7	610/6493	1.08 (0.73 – 1.60)	0.164	66	0.007	0.932
HLA-DR8	9	823/7157	1.10 (0.80 – 1.51)	0.552	15	0.307	0.375
HLA-DR9	11	1016/8204	1.16 (0.71 – 1.90)	0.560	60	0.005	0.925
*HLA-DRB1*0401*	2	216/465	0.93 (0.38 – 2.28)	0.869	48	0.167	N/A
*HLA-DRB1*0403*	2	216/465	0.49 (0.17 – 1.42)	0.190	53	0.144	N/A
*HLA-DRB1*0405*	2	216/465	0.78 (0.47 – 1.30)	0.344	27	0.242	N/A
*HLA-DRB1*0406*	2	216/465	1.22 (0.61 – 2.5)	0.580	44	0.182	N/A
*HLA-DRB1*0407*	2	216/465	1.95 (0.61 – 6.29)	0.263	13	0.283	N/A
*HLA-DRB1*0410*	2	216/465	1.17 (0.27 – 5.05)	0.832	0	0.463	N/A
*HLA-DRB1*0802*	2	216/465	0.94 (0.36 – 2.43)	0.897	0	0.359	N/A
*HLA-DRB1*0803*	2	216/465	1.33 (0.83 – 2.14)	0.236	0	0.622	N/A
*HLA-DRB1*10*	4	322/890	0.98 (0.39 – 2.46)	0.969	0	0.627	0.596
*HLA-DRB1*11*	4	322/890	0.98 (0.71 – 1.34)	0.818	0	0.818	1.000
*HLA-DRB1*12*	4	322/890	1.12 (0.60 – 2.09)	0.712	54	0.088	0.121
*HLA-DRB1*1302*	2	216/465	0.54 (0.27 – 1.05)	0.068	0	0.952	N/A
*HLA-DRB1*1202*	2	216/465	1.42 (0.81 – 2.49)	0.219	49	0.160	N/A
*HLA-DRB1*1403*	2	216/465	1.27 (0.43 – 3.76)	0.666	0	0.964	N/A
*HLA-DRB1*1405*	2	216/465	0.66 (0.32 – 1.37)	0.265	57	0.125	N/A
*HLA-DRB1*1502*	2	216/465	0.57 (0.30 – 1.07)	0.079	0	0.398	N/A

Supplementary table 3. Genetic variants not associated with AAV after meta-analysis (Continued)

Variant by gene (minor allele)	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis	I^2 (%)	P value for heterogeneity	P value for funnel plot asymmetry
*HLA-DRB1*16*	3	236/633	0.91 (0.37 – 2.27)	0.842	15	0.315	0.436
*HLA-DRB1*1602*	2	216/465	0.17 (0.02 – 1.29)	0.086	0	0.734	N/A
HLA-DRB5	2	110/1154	1.20 (0.73 – 1.97)	0.470	0	0.643	N/A
*IL-1*β (A2)	4	1172/5687	1.00 (0.90 – 1.12)	0.995	0	0.805	0.645
*IL1RN*1*	2	214/434	1.06 (0.82 – 1.37)	0.662	0	0.659	N/A
*IL1RN*2*	2	214/434	1.00 (0.77 – 1.31)	0.980	0	0.956	N/A
*IL1RN*3*	2	214/434	0.61 (0.26 – 1.44)	0.257	45	0.176	N/A
*IL1RN*4*	2	214/434	0.85 (0.13 – 5.82)	0.872	0	0.888	N/A
*IL1RN*5*	2	214/434	0.88 (0.13 – 5.94)	0.892	0	0.796	N/A
IL-6 rs1800795 (C)	4	1926/6817	1.01 (0.94 – 1.09)	0.793	0	0.537	0.584
IL-10 rs1800872 (A)	2	538/598	1.01 (0.83 – 1.22)	0.963	0	0.795	N/A
IL-10 rs1800896 (G)	5	1414/6183	0.90 (0.76 – 1.07)	0.232	55	0.063	0.285
LEPR rs8179183 (C)	1	878/2653	0.89 (0.77 – 1.03)	0.118	89	<0.001	0.780
MPO rs2333227 (A)	3	395/299	0.96 (0.76 – 1.21)	0.696	0	0.822	0.478
PCD1 rs1156882 (A)	2	168/479	0.88 (0.55 – 1.38)	0.568	0	0.701	N/A
TGF-β_1 rs1800471 (C)	3	232/259	1.49 (0.92 – 2.41)	0.107	0	0.401	0.220
*TNF*α rs1800629 (A)	4	860/5691	1.14 (0.88 – 1.45)	0.327	29	0.236	0.389
TNFRII 196R	2	167/385	0.94 (0.66 – 1.34)	0.749	0	0.317	N/A
TLR9 rs5743836 (G)	1	1289/1898	0.93 (0.81 – 1.08)	0.343	83	0.014	N/A

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
CD226 rs763361 (T)				
GPA	3	2021/17898	1.19 (1.11 – 1.28)	<0.001
MPA	1	156/5366	1.04 (0.83 – 1.31)	0.739
EGPA	1	119/1226	1.19 (0.91 – 1.56)	0.208
PR3-ANCA	2	764/6592	1.22 (1.09 – 1.36)	<0.001
MPO-ANCA	2	199/6592	1.20 (0.99 – 1.46)	0.066
CTLA-4 $(AT)_{86}$				
GPA	3	210/432	0.44 (0.34 – 0.57)	<0.001
MPA	2	93/311	0.87 (0.60 – 1.25)	0.450
PR3-ANCA	1	62/200	0.71 (0.47 – 1.09)	0.122
MPO-ANCA	2	92/311	0.94 (0.65 – 1.35)	0.733
CTLA-4 $(AT)_{102}$				
GPA	2	93/309	0.94 (0.45 – 1.94)	0.856
MPA	2	93/311	0.86 (0.51 – 1.45)	0.574
PR3-ANCA	1	62/200	0.87 (0.41 – 1.87)	0.722
MPO-ANCA	2	92/311	0.69 (0.26 – 1.81)	0.456
CTLA-4 $(AT)_{104}$				
GPA	2	93/309	0.84 (0.55 – 1.27)	0.398
MPA	2	93/311	0.87 (0.58 – 1.29)	0.474
PR3-ANCA	1	62/200	0.42 (0.22 – 0.79)	0.007
MPO-ANCA	2	92/311	0.85 (0.57 – 1.26)	0.412
CTLA-4 $(AT)_{106}$				
GPA	2	93/309	1.69 (0.67 – 4.25)	0.264
MPA	2	93/311	0.64 (0.28 – 1.46)	0.287
PR3-ANCA	1	62/200	1.83 (0.60 – 5.55)	0.289
MPO-ANCA	2	92/311	0.53 (0.22 – 1.30)	0.168
CTLA-4 $(AT)_{108}$				
GPA	2	93/309	0.40 (0.12 – 1.35)	0.141
MPA	2	93/311	0.75 (0.36 – 1.54)	0.431
PR3-ANCA	1	62/200	0.30 (0.07 – 1.28)	0.103
MPO-ANCA	2	92/311	0.66 (0.31 – 1.41)	0.291
CTLA-4 $(AT)_{110}$				
GPA	2	93/309	0.55 (0.18 – 1.74)	0.311
MPA	2	93/311	0.26 (0.05 – 1.36)	0.109
PR3-ANCA	1	62/200	0.56 (0.16 – 1.94)	0.359
MPO-ANCA	2	92/311	0.26 (0.05 – 1.38)	0.113

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
CTLA-4 $(AT)_{116}$				
GPA	2	93/309	0.66 (0.17 – 2.60)	0.553
MPA	2	93/311	0.41 (0.05 – 3.38)	0.408
PR3-ANCA	1	62/200	0.21 (0.01 – 3.72)	0.288
MPO-ANCA	2	92/311	0.69 (0.12 – 3.98)	0.673
CTLA-4 $(AT)_{118}$				
GPA	2	93/309	0.32 (0.06 – 1.68)	0.176
MPA	2	93/311	0.31 (0.06 – 1.69)	0.175
PR3-ANCA	1	62/200	0.11 (0.01 – 1.81)	0.121
MPO-ANCA	2	92/311	0.44 (0.10 – 1.97)	0.283
CTLA-4 $(AT)_{122}$				
GPA	2	93/309	1.32 (0.26 – 6.80)	0.742
MPA	2	93/311	1.57 (0.48 – 5.19)	0.456
PR3-ANCA	1	62/200	0.35 (0.02 – 6.62)	0.487
MPO-ANCA	2	92/311	3.69 (1.40 – 9.71)	0.008
CTLA-4 $(AT)_{124}$				
GPA	2	93/309	1.69 (0.67 – 4.25)	0.264
MPA	2	93/311	0.64 (0.28 – 1.46)	0.287
PR3-ANCA	1	62/200	1.83 (0.60 – 5.55)	0.289
MPO-ANCA	2	92/311	0.53 (0.22 – 1.30)	0.168
CTLA-4 $(AT)_{126}$				
GPA	2	93/309	1.43 (0.47 – 4.35)	0.526
MPA	2	93/311	0.38 (0.08 – 2.11)	0.266
PR3-ANCA	1	62/200	0.21 (0.01 – 3.72)	0.288
MPO-ANCA	2	92/311	0.54 (0.12 – 2.46)	0.428
CTLA-4 $(AT)_{128}$				
GPA	2	93/309	0.39 (0.07 – 2.14)	0.278
MPA	2	93/311	1.27 (0.39 – 4.20)	0.694
PR3-ANCA	1	62/200	0.29 (0.04 – 2.25)	0.235
MPO-ANCA	2	92/311	1.29 (0.39 – 4.28)	0.674
CTLA-4 rs231775 (G)				
GPA	2	510/5994	1.05 (0.92 – 1.20)	0.430
MPA	2	252/5994	1.17 (0.97 – 1.40)	0.100
PR3-ANCA	1	326/5365	1.00 (0.85 – 1.18)	0.983
MPO-ANCA	1	167/5365	0.96 (0.77 – 1.21)	0.747

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
CTLA-4 rs3087243 (A)				
GPA	3	1561/7855	0.80 (0.73 – 0.87)	<0.001
MPA	2	358/5886	0.79 (0.68 – 0.92)	0.003
PR3-ANCA	1	478/5257	0.81 (0.71 – 0.93)	0.003
MPO-ANCA	1	264/5257	0.96 (0.80 – 1.15)	0.658
FCAR rs16986050 (G)				
GPA	2	1029/5891	0.92 (0.80 – 1.05)	0.214
MPA	1	394/5365	0.93 (0.69 – 1.26)	0.647
PR3-ANCA	1	326/5365	1.13 (0.93 – 1.38)	0.211
MPO-ANCA	1	167/5365	1.00 (0.75 – 1.33)	0.988
FCGR2A rs1801274 (C)				
GPA	5	1082/5969	0.99 (0.94 – 1.05)	0.827
MPA	4	800/5766	0.97 (0.81 – 1.15)	0.698
PR3-ANCA	4	883/5820	0.91 (0.79 – 1.03)	0.138
MPO-ANCA	4	844/5969	0.80 (0.67 – 0.95)	0.011
FCGR3A rs396991 (G)				
GPA	1	91/154	1.30 (0.89 – 1.91)	0.172
MPA	1	50/303	0.65 (0.39 – 1.07)	0.090
PR3-ANCA	1	91/154	1.30 (0.89 – 1.91)	0.172
MPO-ANCA	1	50/303	0.65 (0.39 – 1.07)	0.090
FCGR3B (NA1)				
GPA	3	865/667	0.93 (0.80 – 1.08)	0.343
MPA	2	75/200	1.52 (1.03 – 2.25)	0.035
PR3-ANCA	2	143/254	1.07 (0.79 – 1.45)	0.664
MPO-ANCA	2	121/403	1.19 (0.86 – 1.64)	0.287
GHSR rs509035 (A)				
GPA	2	1198/6595	1.05 (0.96 – 1.16)	0.292
MPA	1	262/5259	1.00 (0.82 – 1.20)	0.966
PR3-ANCA	1	478/5259	1.05 (0.91 – 1.21)	0.521
MPO-ANCA	1	264/5259	0.83 (0.68 – 1.01)	0.057
HLA-A2				
GPA	5	380/7796	0.96 (0.81 – 1.14)	0.633
c-ANCA	1	16/96	0.90 (0.37 – 2.23)	0.826
HLA-A11				
GPA	5	380/7702	0.61 (0.41 – 0.90)	0.014

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
MPA	1	23/405	2.97 (1.19 – 7.41)	0.020
c-ANCA	1	16/472	0.71 (0.17 – 3.03)	0.644
HLA-A24				
GPA	3	108/1223	0.94 (0.56 – 1.60)	0.830
c-ANCA	1	16/472	0.75 (0.34 – 1.65)	0.476
HLA-A26				
GPA	3	108/1223	1.24 (0.61 – 2.54)	0.550
MPA	1	23/405	2.49 (1.00 – 6.18)	0.049
c-ANCA	1	16/472	2.36 (1.00 – 5.60)	0.051
HLA-A31				
GPA	3	108/1223	1.02 (0.43 – 2.44)	0.969
c-ANCA	1	16/472	1.55 (0.46 – 5.24)	0.479
HLA-A32				
GPA	3	108/1223	1.62 (0.74 – 3.54)	0.231
c-ANCA	1	16/472	2.07 (0.61 – 7.04)	0.246
HLA-B35				
GPA	4	349/7095	0.62 (0.44 – 0.89)	0.010
c-ANCA	1	16/472	0.80 (0.19 – 3.40)	0.757
HLA-B39				
GPA	2	67/523	0.65 (0.11 – 3.68)	0.622
c-ANCA	1	16/472	0.86 (0.11 – 6.51)	0.887
HLA-B44				
GPA	3	108/1223	1.53 (0.96 – 2.44)	0.072
c-ANCA	1	16/472	2.50 (0.85 – 7.40)	0.098
HLA-B51				
GPA	2	67/523	1.03 (0.46 – 2.32)	0.943
c-ANCA	1	16/472	1.87 (0.64 – 5.49)	0.255
HLA-B55				
GPA	2	67/523	1.07 (0.40 – 2.88)	0.887
c-ANCA	1	16/472	7.35 (2.33 – 23.14)	0.001
HLA-B60				
GPA	2	57/1172	1.98 (0.77 – 5.09)	0.154
c-ANCA	1	16/472	1.19 (0.28 – 5.13)	0.814
HLA-B62				
GPA	2	57/1172	2.27 (1.05 – 4.90)	0.036

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
c-ANCA	1	16/472	2.21 (0.83 – 5.91)	0.114
HLA-Cw1				
GPA	2	68/523	1.28 (0.59 – 2.77)	0.534
c-ANCA	1	16/472	1.45 (0.58 – 3.58)	0.426
HLA-Cw3				
GPA	2	68/523	1.36 (0.79 – 2.33)	0.267
c-ANCA	1	16/472	1.40 (0.65 – 3.01)	0.384
HLA-Cw7				
GPA	2	68/523	0.96 (0.57 – 1.62)	0.877
c-ANCA	1	16/472	0.50 (0.12 – 2.10)	0.340
HLA-DPA1 rs9277341 (C)				
GPA	2	1032/2200	0.35 (0.30 – 0.41)	<0.001
c-ANCA	1	578/1820	0.27 (0.22 – 0.33)	<0.001
*HLA-DPB1*0101*				
GPA	1	148/89	0.17 (0.05 – 0.63)	0.008
PR3-ANCA	2	183/139	0.45 (0.20 – 0.99)	0.048
MPO-ANCA	1	22/50	1.01 (0.29 – 3.48)	0.986
*HLA-DPB1*0201*				
GPA	2	283/458	0.67 (0.45 – 0.99)	0.042
MPA	1	50/77	1.38 (0.75 – 2.52)	0.298
EGPA	1	102/369	0.93 (0.57 – 1.51)	0.759
PR3-ANCA	1	148/89	0.44 (0.21 – 0.91)	0.027
MPO-ANCA	1	50/77	1.38 (0.75 – 2.52)	0.298
*HLA-DPB1*0301*				
GPA	3	774/918	0.23 (0.16 – 0.32)	<0.001
MPA	1	119/460	0.67 (0.37 – 1.21)	0.186
EGPA	2	167/829	0.47 (0.29 – 0.75)	0.001
PR3-ANCA	2	183/139	0.19 (0.09 – 0.39)	<0.001
MPO-ANCA	1	22/50	1.71 (0.74 – 3.94)	0.210
ANCA negative	1	27/369	0.58 (0.21 – 1.65)	0.311
*HLA-DPB1*0401*				
GPA	3	774/918	2.89 (2.50 – 3.35)	<0.001
MPA	1	119/460	1.09 (0.80 – 1.49)	0.597
EGPA	2	167/829	1.08 (0.85 – 1.36)	0.548

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
PR3-ANCA	2	183/139	3.93 (2.75 – 5.62)	<0.001
MPO-ANCA	1	22/50	1.43 (0.67 – 3.02)	0.351
ANCA negative	1	27/369	1.26 (0.73 – 2.20)	0.407
*HLA-DPB1*0402*				
GPA	2	283/458	1.13 (0.82 – 1.55)	0.439
EGPA	1	102/369	1.15 (0.75 – 1.75)	0.533
PR3-ANCA	2	183/139	1.11 (0.69 – 1.77)	0.674
MPO-ANCA	1	22/50	0.94 (0.31 – 2.85)	0.913
*HLA-DPB1*0501*				
GPA	1	148/89	0.20 (0.01 – 4.93)	0.324
PR3-ANCA	2	183/139	1.11 (0.26 – 4.76)	0.886
MPO-ANCA	1	22/50	1.14 (0.10 – 12.91)	0.916
*HLA-DPB1*0601*				
GPA	1	148/89	0.60 (0.08 – 4.29)	0.610
PR3-ANCA	2	183/139	1.12 (0.23 – 5.51)	0.887
MPO-ANCA	1	22/50	6.93 (0.28 – 173.53)	0.239
*HLA-DPB1*0901*				
GPA	1	148/89	1.81 (0.07 – 44.73)	0.716
PR3-ANCA	2	183/139	0.98 (0.27 – 3.62)	0.978
MPO-ANCA	1	22/50	0.91 (0.17 – 4.85)	0.907
HLA-DPB2 rs3130215 (A)				
GPA	3	1135/7249	1.37 (0.88 – 2.13)	0.160
MPA	1	156/5366	1.33(1.06 – 1.66)	0.013
PR3-ANCA	1	326/5366	0.65 (0.55 – 0.77)	<0.001
MPO-ANCA	1	167/5366	1.27 (1.02 – 1.58)	0.032
*HLA-DQB1*02*				
GPA	2	83/141	0.91 (0.53 – 1.55)	0.720
PR3-ANCA	1	32/91	0.98 (0.45 – 2.14)	0.954
*HLA-DQB1*0302*				
GPA	1	32/91	0.94 (0.36 – 2.49)	0.905
PR3-ANCA	2	67/141	1.03 (0.54 – 1.98)	0.924
MPO-ANCA	1	22/50	1.06 (0.37 – 2.99)	0.917
*HLA-DQB1*0303*				
GPA	1	32/91	2.97 (0.72 – 12.23)	0.132

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
MPA	1	50/77	2.11 (1.14 – 3.90)	0.018
PR3-ANCA	2	67/141	0.79 (0.31 – 2.05)	0.634
MPO-ANCA	2	72/127	1.91 (1.12 – 3.26)	0.017
*HLA-DQB1*04*				
GPA	2	83/141	1.63 (0.52 – 5.11)	0.404
PR3-ANCA	1	32/91	1.74 (0.40 – 7.50)	0.457
*HLA-DQB1*0501*				
GPA	1	32/91	0.77 (0.32 – 1.88)	0.568
PR3-ANCA	2	67/141	1.05 (0.58 – 1.91)	0.865
MPO-ANCA	1	22/50	2.05 (0.84 – 4.97)	0.113
*HLA-DQB1*0602*				
GPA	1	32/91	0.89 (0.38 – 2.05)	0.779
PR3-ANCA	2	67/141	0.80 (0.42 – 1.54)	0.504
MPO-ANCA	1	22/50	0.35 (0.08 – 1.63)	0.181
HLA-DQ7				
GPA	1	32/91	0.43 (0.16 – 1.16)	0.095
MPA	1	23/405	0.66 (0.34 – 1.30)	0.234
PR3-ANCA	2	67/141	0.98 (0.58 – 1.66)	0.940
MPO-ANCA	1	22/50	1.26 (0.57 – 2.77)	0.569
HLA-DR1				
GPA	6	413/6002	0.76 (0.58 – 1.00)	0.054
MPA	4	223/6020	1.12 (0.72 – 1.74)	0.612
EGPA	3	128/5924	0.82 (0.53 – 1.26)	0.360
PR3-ANCA	3	157/560	0.57 (0.38 – 0.86)	0.008
MPO-ANCA	3	133/734	0.78 (0.49 – 1.25)	0.297
HLA-DR2				
GPA	4	301/6132	1.36 (1.08 – 1.72)	0.010
EGPA	1	14/113	1.40 (0.45 – 4.39)	0.561
PR3-ANCA	1	35/90	1.34 (0.63 – 2.86)	0.442
MPO-ANCA	1	22/90	1.03 (0.39 – 2.69)	0.958
c-ANCA	1	16/472	0.33 (0.08 – 1.38)	0.128
HLA-DR3				
GPA	5	368/5802	1.24 (1.00 – 1.56)	0.056
MPA	2	38/518	0.45 (0.17 – 1.14)	0.092
EGPA	2	116/482	0.70 (0.43 – 1.13)	0.139

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
PR3-ANCA	3	157/560	1.29 (0.90 – 1.86)	0.170
MPO-ANCA	2	69/469	0.85 (0.49 – 1.48)	0.574
HLA-DR4				
GPA	7	425/6424	1.32 (1.09 – 1.60)	0.004
MPA	2	66/5555	1.50 (0.98 – 2.30)	0.060
EGPA	4	176/6246	1.70 (1.23 – 2.34)	0.001
PR3-ANCA	3	157/560	0.98 (0.71 – 1.36)	0.917
MPO-ANCA	2	69/202	2.51 (1.46 – 4.32)	0.001
c-ANCA	1	16/472	0.72 (0.27 – 1.88)	0.498
HLA-DR5				
GPA	3	285/5660	0.76 (0.54 – 1.06)	0.102
MPA	1	23/405	1.59 (0.80 – 3.15)	0.182
EGPA	1	14/113	1.24 (0.34 – 4.46)	0.746
PR3-ANCA	1	35/90	0.85 (0.30 – 2.42)	0.756
MPO-ANCA	1	22/90	0.52 (0.12 – 2.38)	0.402
HLA-DR6				
GPA	4	301/6132	0.45 (0.33 – 0.62)	<0.001
MPA	1	30/5442	0.29 (0.09 – 0.92)	0.035
EGPA	2	26/5555	0.19 (0.04 – 0.95)	0.043
PR3-ANCA	1	35/90	0.28 (0.06 – 1.25)	0.096
MPO-ANCA	1	22/90	0.70 (0.20 – 2.51)	0.586
c-ANCA	1	16/472	1.63 (0.56 – 4.77)	0.371
HLA-DR7				
GPA	5	368/5802	0.92 (0.71 – 1.19)	0.508
MPA	1	15/113	0.70 (0.20 – 2.44)	0.575
EGPA	3	164/804	1.71 (1.24 – 2.37)	0.001
PR3-ANCA	1	32/91	1.00 (0.40 – 2.48)	0.991
HLA-DR8				
GPA	5	381/6206	0.89 (0.57 – 1.39)	0.610
MPA	1	51/5442	0.31 (0.04 – 2.21)	0.242
EGPA	2	114/5811	3.08 (1.75 – 5.41)	<0.001
PR3-ANCA	3	157/560	0.68 (0.33 – 1.42)	0.308
MPO-ANCA	2	69/469	1.70 (0.80 – 3.60)	0.170
c-ANCA	1	16/472	1.63 (0.66 – 4.05)	0.291
HLA-DR9				

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
GPA	6	402/6369	0.90 (0.58 – 1.39)	0.623
MPA	3	218/578	1.14 (0.83 – 1.56)	0.423
EGPA	3	164/832	1.07 (0.33 – 3.42)	0.915
PR3-ANCA	2	122/470	0.47 (0.09 – 2.58)	0.383
MPO-ANCA	3	175/1116	1.83 (1.29 – 2.60)	0.001
c-ANCA	1	16/472	3.03 (1.40 – 6.56)	0.005
*HLA-DRB1*0401*				
GPA	1	45/200	0.49 (0.06 – 3.90)	0.499
MPA	2	157/465	1.42 (0.60 – 3.41)	0.426
MPO-ANCA	1	64/265	2.10 (0.52 – 8.50)	0.300
*HLA-DRB1*0403*				
GPA	1	45/200	0.26 (0.02 – 4.46)	0.349
MPA	2	157/465	0.48 (0.14 – 1.59)	0.227
MPO-ANCA	1	64/265	0.95 (0.27 – 3.40)	0.943
*HLA-DRB1*0405*				
GPA	1	45/200	0.15 (0.01 – 2.49)	0.184
MPA	2	157/465	0.95 (0.56 – 1.62)	0.862
MPO-ANCA	1	64/265	0.61 (0.30 – 1.22)	0.159
*HLA-DRB1*0406*				
GPA	1	45/200	2.28 (0.67 – 7.74)	0.187
MPA	2	157/465	1.31 (0.62 – 2.78)	0.483
MPO-ANCA	1	64/265	0.65 (0.19 – 2.22)	0.487
*HLA-DRB1*0407*				
GPA	1	45/200	1.47 (0.06 – 36.42)	0.813
MPA	2	157/465	1.95 (0.54 – 6.99)	0.306
MPO-ANCA	1	64/265	2.82 (0.78 – 10.14)	0.113
*HLA-DRB1*0410*				
GPA	1	45/200	1.47 (0.06 – 36.42)	0.813
MPA	2	157/465	1.53 (0.35 – 6.64)	0.569
MPO-ANCA	1	64/265	1.67 (0.32 – 8.69)	0.544
*HLA-DRB1*0802*				
GPA	1	45/200	0.88 (0.04 – 18.50)	0.935
MPA	2	157/465	0.99 (0.35 – 2.79)	0.984
MPO-ANCA	1	64/265	1.16 (0.42 – 3.18)	0.778
*HLA-DRB1*0803*				

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
GPA	1	45/200	1.18 (0.43 – 3.25)	0.749
MPA	2	157/465	1.25 (0.73 – 2.13)	0.413
MPO-ANCA	1	64/265	1.51 (0.78 – 2.93)	0.226
*HLA-DRB1*10*				
GPA	2	83/142	2.61 (0.45 – 15.24)	0.286
EGPA	1	102/369	0.40 (0.02 – 7.44)	0.538
PR3-ANCA	2	122/470	0.59 (0.13 – 2.65)	0.487
MPO-ANCA	1	47/379	1.48 (0.32 – 6.76)	0.616
*HLA-DRB1*11*				
GPA	2	83/142	0.97 (0.49 – 1.94)	0.932
EGPA	1	102/369	0.88 (0.53 – 1.48)	0.632
PR3-ANCA	2	122/470	0.97 (0.58 – 1.62)	0.908
MPO-ANCA	1	47/379	1.10 (0.51 – 2.38)	0.805
*HLA-DRB1*1101*				
GPA	1	45/200	0.16 (0.02 – 1.21)	0.076
MPA	2	223/465	2.57 (1.56 – 4.23)	<0.001
MPO-ANCA	1	116/265	2.79 (0.84 – 9.23)	0.093
*HLA-DRB1*12*				
GPA	2	83/142	4.06 (1.27 – 12.99)	0.018
EGPA	1	102/369	0.57 (0.17 – 1.93)	0.362
PR3-ANCA	2	122/470	1.01 (0.36 – 2.81)	0.983
MPO-ANCA	1	47/379	1.16 (0.26 – 5.16)	0.850
*HLA-DRB1*1201*				
GPA	1	45/200	0.15 (0.01 – 2.49)	0.184
MPA	2	157/465	0.51 (0.21 – 1.26)	0.146
MPO-ANCA	1	64/265	0.21 (0.03 – 1.60)	0.132
*HLA-DRB1*1202*				
GPA	1	45/200	2.92 (1.37 – 6.23)	0.005
MPA	2	157/465	1.07 (0.55 – 2.09)	0.838
PR3-ANCA	1	19/200	1.63 (0.46 – 5.75)	0.449
MPO-ANCA	2	89/465	1.00 (0.38 – 2.61)	1.000
*HLA-DRB1*13*				
GPA	2	83/142	0.61 (0.34 – 1.12)	0.111
EGPA	2	150/691	0.40 (0.24 – 0.68)	0.001
PR3-ANCA	1	32/91	0.56 (0.20 – 1.53)	0.257

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
*HLA-DRB1*1302*				
GPA	1	45/200	0.89 (0.19 – 4.12)	0.878
MPA	2	157/465	0.46 (0.20 – 1.02)	0.055
MPO-ANCA	1	64/265	0.54 (0.24 – 1.23)	0.142
*HLA-DRB1*14*				
GPA	2	83/142	1.26 (0.40 – 4.01)	0.698
EGPA	1	102/341	1.83 (0.72 – 4.65)	0.205
PR3-ANCA	2	122/470	2.00 (0.87 – 4.56)	0.101
MPO-ANCA	1	47/379	2.04 (0.43 – 9.74)	0.372
*HLA-DRB1*1403*				
GPA	1	45/200	2.24 (0.20 – 24.93)	0.513
MPA	2	157/465	0.81 (0.21 – 3.19)	0.767
MPO-ANCA	1	64/265	1.25 (0.34 – 4.60)	0.739
*HLA-DRB1*1405*				
GPA	1	45/200	1.51 (0.53 – 4.27)	0.437
MPA	2	157/465	0.45 (0.18 – 1.15)	0.096
MPO-ANCA	1	64/265	0.11 (0.01 – 1.91)	0.131
*HLA-DRB1*15*				
GPA	2	83/142	0.97 (0.57 – 1.65)	0.921
PR3-ANCA	2	131/582	2.82 (2.00 – 3.96)	<0.001
MPO-ANCA	1	51/491	0.80 (0.37 – 1.73)	0.566
*HLA-DRB1*1501*				
GPA	1	45/200	1.70 (0.98 – 2.94)	0.059
MPA	2	157/465	1.65 (1.14 – 2.38)	0.007
PR3-ANCA	1	19/200	0.98 (0.40 – 2.45)	0.973
MPO-ANCA	2	89/465	2.03 (1.26 – 3.29)	0.004
*HLA-DRB1*1502*				
GPA	1	45/200	0.80 (0.18 – 3.69)	0.779
MPA	2	157/465	0.60 (0.30 – 1.21)	0.154
MPO-ANCA	1	64/265	0.67 (0.32 – 1.39)	0.279
*HLA-DRB1*16*				
GPA	2	83/142	0.23 (0.03 – 1.85)	0.166
PR3-ANCA	2	131/582	1.00 (0.29 – 3.41)	0.996
MPO-ANCA	1	51/491	3.55 (1.02 – 12.38)	0.047
*HLA-DRB1*1602*				

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
GPA	1	45/200	0.40 (0.02 – 7.25)	0.533
MPA	2	157/465	0.23 (0.03 – 1.72)	0.151
MPO-ANCA	1	64/265	0.24 (0.01 – 4.17)	0.327
HLA-DRB3				
GPA	1	51/51	1.41 (0.76 – 2.60)	0.276
EGPA	2	150/691	0.58 (0.45 – 0.76)	<0.001
HLA-DRB4				
GPA	1	51/51	0.89 (0.46 – 1.72)	0.738
EGPA	2	150/691	2.06 (1.57 – 2.69)	<0.001
*IL-1*β (A2)				
GPA	3	714/5491	1.07 (0.93 – 1.22)	0.336
MPA	1	262/5259	0.89 (0.72 – 1.10)	0.286
PR3-ANCA	2	557/5455	1.01 (0.87 – 1.17)	0.930
MPO-ANCA	2	294/5455	1.00 (0.82 – 1.21)	0.971
*IL1RN**1				
GPA	1	61/200	0.88 (0.57 – 1.37)	0.573
MPA	1	105/200	1.21 (0.83 – 1.77)	0.325
PR3-ANCA	2	122/434	1.12 (0.82 – 1.54)	0.476
MPO-ANCA	2	92/434	0.98 (0.69 – 1.40)	0.916
*IL1RN**2				
GPA	1	61/200	1.22 (0.78 – 1.92)	0.380
MPA	1	105/200	0.77 (0.52 – 1.16)	0.212
PR3-ANCA	2	122/434	0.95 (0.68 – 1.31)	0.748
MPO-ANCA	2	92/434	1.02 (0.71 – 1.47)	0.921
*IL1RN**3				
GPA	1	61/200	0.72 (0.15 – 3.40)	0.682
MPA	1	105/200	1.50 (0.55 – 4.08)	0.429
PR3-ANCA	2	122/434	0.61 (0.21 – 1.76)	0.358
MPO-ANCA	2	92/434	0.71 (0.22 – 2.29)	0.571
*IL1RN**4				
GPA	1	61/200	0.65 (0.03 – 13.64)	0.782
MPA	1	105/200	1.91 (0.27 – 13.68)	0.518
PR3-ANCA	2	122/434	0.95 (0.11 – 8.63)	0.964
MPO-ANCA	2	92/434	3.08 (0.58 – 16.42)	0.188
*IL1RN**5				

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
GPA	1	61/200	1.09 (0.04 – 26.86)	0.959
MPA	1	105/200	0.63 (0.03 – 15.60)	0.779
PR3-ANCA	2	122/434	0.87 (0.10 – 7.74)	0.901
MPO-ANCA	2	92/434	2.18 (0.32 – 14.79)	0.424
IL-6 rs1800795 (C)				
GPA	4	1360/1558	0.99 (0.94 – 1.04)	0.750
MPA	2	315/6593	0.98 (0.84 – 1.16)	0.829
EGPA	1	426/6593	0.96 (0.76 – 1.21)	0.730
PR3-ANCA	3	NR/6694	0.98 (0.89 – 1.09)	0.751
MPO-ANCA	2	NR/6603	1.04 (0.90 – 1.20)	0.616
IL-10 rs1800872 (A)				
GPA	2	435/598	1.03 (0.84 – 1.27)	0.762
EGPA	1	103/507	0.89 (0.62 – 1.27)	0.512
PR3-ANCA	1	32/91	1.10 (0.55 – 2.20)	0.793
IL-10 rs1800896 (G)				
GPA	5	993/6183	0.95 (0.85 – 1.05)	0.293
MPA	2	192/5513	0.95 (0.78 – 1.17)	0.655
EGPA	1	103/507	0.68 (0.50 – 0.92)	0.012
PR3-ANCA	2	358/5451	1.05 (0.90 – 1.22)	0.527
MPO-ANCA	1	167/5360	0.96 (0.77 – 1.19)	0.693
IRF5 rs10954213 (G)				
GPA	2	1021/6267	0.66 (0.59 – 0.74)	<0.001
MPA	2	333/6075	1.22 (1.03 – 1.44)	0.018
PR3-ANCA	1	326/5365	1.09 (0.93 – 1.28)	0.293
MPO-ANCA	2	399/6075	1.12 (0.96 – 1.31)	0.142
LEPR rs8179183 (C)				
GPA	1	682/1326	0.72 (0.60 – 0.86)	<0.001
EGPA	1	196/1327	1.41 (1.10 – 1.81)	0.007
MPO rs2333227 (A)				
GPA	1	69/150	0.70 (0.41 – 1.21)	0.204
MPA	1	65/150	1.18 (0.72 – 1.93)	0.523
PR3-ANCA	3	258/549	1.00 (0.77 – 1.31)	0.977
MPO-ANCA	3	141/549	0.94 (0.68 – 1.31)	0.719
PTPN22 rs2476601 (A)				
GPA	4	1616/8678	1.43 (1.26 – 1.62)	<0.001

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
MPA	2	258/6310	1.42 (1.06 – 1.89)	0.018
EGPA	1	99/945	0.52 (0.21 – 1.29)	0.158
PR3-ANCA	2	419/6310	1.42 (1.14 – 1.77)	0.002
MPO-ANCA	2	183/6310	1.47 (1.08 – 2.00)	0.014
RING1/RXRB rs213213 (A)				
GPA	3	1132/7238	1.91 (1.73 – 2.10)	<0.001
MPA	1	156/5366	1.15 (0.90 – 1.45)	0.264
PR3-ANCA	1	326/5366	2.06 (1.75 – 2.41)	<0.001
MPO-ANCA	1	167/5366	1.05 (0.83 – 1.32)	0.687
RXRB rs6531 (C)				
GPA	3	1211/6955	1.70 (1.55 – 1.86)	<0.001
MPA	1	262/5251	1.38 (1.15 – 1.66)	0.001
PR3-ANCA	1	478/5251	2.19 (1.92 – 2.51)	<0.001
MPO-ANCA	1	264/5251	1.21 (1.00 – 1.46)	0.046
ANCA negative	1	36/201	1.02 (0.59 – 1.74)	0.948
RXRB rs9277935 (T)				
GPA	3	1135/7233	0.37 (0.31 – 0.43)	<0.001
MPA	1	156/5350	0.93 (0.70 – 1.24)	0.629
PR3-ANCA	1	326/5350	0.24 (0.17 – 0.33)	<0.001
MPO-ANCA	1	167/5350	1.18 (0.92 – 1.53)	0.193
SERPINA1 S allele				
GPA	2	484/1079	1.72 (1.12 – 2.66)	0.014
c-ANCA	3	145/3836	1.92 (1.12 – 3.29)	0.017
SERPINA1 Z allele				
GPA	4	972/2636	2.40 (1.73 - 3.33)	<0.001
MPA	1	143/805	1.60 (0.76 - 3.39)	0.218
PR3-ANCA	1	322/805	2.58 (1.57 - 4.25)	<0.001
MPO-ANCA	1	166/805	2.01 (1.04 - 3.87)	0.037
c-ANCA	5	280/4788	3.53 (2.28 – 5.49)	<0.001
p-ANCA	2	78/2510	3.13 (1.21 – 8.13)	0.019
STAT4 rs7574865 (T)				
GPA	2	1288/6246	1.06 (0.94 – 1.20)	0.331
MPA	1	676/5366	1.12 (0.86 – 1.46)	0.392
PR3-ANCA	1	676/5366	1.06 (0.88 – 1.28)	0.547

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
MPO-ANCA	2	908/6076	1.24 (1.05 – 1.46)	0.009
TGF-β_1 rs1800471 (C)				
GPA	3	196/259	1.52 (0.93 – 2.48)	0.096
MPA	1	36/96	1.03 (0.35 – 2.99)	0.960
PR3-ANCA	1	32/91	1.86 (0.73 – 4.71)	0.193
*TNF*α rs1800629 (A)				
GPA	4	578/5691	1.15 (0.98 – 1.35)	0.098
MPA	1	156/5366	1.13 (0.95 – 1.35)	0.182
PR3-ANCA	2	357/5457	1.21 (0.93 – 1.57)	0.149
MPO-ANCA	1	167/5366	0.88 (0.66 – 1.17)	0.375
TNFRII 196R				
GPA	1	177/123	1.05 (0.70 – 1.57)	0.819
MPA	1	50/262	0.67 (0.31 – 1.45)	0.313
MPO-ANCA	1	50/262	0.67 (0.31 – 1.45)	0.313
TLR9 rs352162 (T)				
GPA	1	919/1898	1.20 (1.07 – 1.34)	0.001
MPA	1	153/1898	0.74 (0.58 – 0.95)	0.020
EGPA	1	217/1898	1.28 (1.05 – 1.57)	0.015
PR3-ANCA	1	NR/NR	1.30 (1.14 – 1.47)	<0.001
MPO-ANCA	1	NR/NR	0.79 (0.65 – 0.97)	0.028
ANCA negative	1	NR/NR	1.22 (0.99 – 1.50)	0.060
TLR9 rs352140 (T)				
GPA	1	919/1898	1.20 (1.07 – 1.35)	0.001
MPA	1	153/1898	0.71 (0.55 – 0.91)	0.006
EGPA	1	217/1898	1.17 (0.96 – 1.43)	0.129
PR3-ANCA	1	NR/NR	1.28 (1.12 – 1.45)	<0.001
MPO-ANCA	1	NR/NR	0.75 (0.62 – 0.91)	0.004
ANCA negative	1	NR/NR	1.19 (0.97 – 1.47)	0.097
TLR9 rs352139 (T)				
GPA	1	919/1898	1.18 (1.06 – 1.32)	0.003
MPA	1	153/1898	0.68 (0.52 – 0.87)	0.003
EGPA	1	217/1898	1.21 (0.99 – 1.47)	0.057
PR3-ANCA	1	NR/NR	1.23 (1.09 – 1.40)	0.001
MPO-ANCA	1	NR/NR	0.78 (0.63 – 0.96)	0.017
ANCA negative	1	NR/NR	1.20 (0.98 – 1.47)	0.086

Supplementary table 4. Results of the meta-analyses stratified by diagnostic and serologic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
TLR9 rs5743836 (G)				
GPA	1	919/1898	0.83 (0.70 – 0.99)	0.037
MPA	1	153/1898	1.78 (1.32 – 2.39)	<0.001
EGPA	1	217/1898	0.90 (0.68 – 1.21)	0.499
PR3-ANCA	1	NR/NR	0.83 (0.70 – 1.00)	0.045
MPO-ANCA	1	NR/NR	1.20 (0.91 – 1.58)	0.207
ANCA negative	1	NR/NR	0.99 (0.74 – 1.34)	0.970

Supplementary table 5. Results of the meta-analyses stratified by ethnic subgroups

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
CTLA-4 $(AT)_{86}$				
Asian	1	49/111	1.31 (0.76 – 2.25)	0.333
Caucasian	3	254/432	0.46 (0.36 – 0.58)	<0.001
CTLA-4 $(AT)_{102}$				
Asian	1	49/111	0.89 (0.47 – 1.68)	0.714
Caucasian	2	137/309	1.73 (0.87 – 3.44)	0.117
CTLA-4 $(AT)_{104}$				
Asian	1	49/111	1.15 (0.70 – 1.91)	0.578
Caucasian	2	137/309	1.24 (0.88 – 1.73)	0.215
CTLA-4 $(AT)_{106}$				
Asian	1	49/111	0.41 (0.14 – 1.22)	0.109
Caucasian	2	137/309	2.69 (1.28 – 5.63)	0.009
CTLA-4 $(AT)_{108}$				
Asian	1	49/111	0.82 (0.33 – 2.02)	0.670
Caucasian	2	137/309	0.92 (0.44 – 1.92)	0.831
CTLA-4 $(AT)_{110}$				
Asian	1	49/111	0.25 (0.01 – 4.62)	0.349
Caucasian	2	137/309	0.84 (0.37 – 1.91)	0.679
CTLA-4 $(AT)_{116}$				
Asian	1	49/111	0.75 (0.03 – 18.56)	0.860
Caucasian	2	137/309	0.80 (0.26 – 2.50)	0.705
CTLA-4 $(AT)_{118}$				
Asian	1	49/111	0.75 (0.08 – 7.33)	0.807
Caucasian	2	137/309	0.41 (0.12 – 1.39)	0.152

Supplementary table 5. Results of the meta-analyses stratified by ethnic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
CTLA-4 $(AT)_{122}$				
Asian	1	49/111	2.32 (0.57 – 9.45)	0.241
Caucasian	2	137/309	1.22 (0.27 – 5.49)	0.799
CTLA-4 $(AT)_{124}$				
Asian	1	49/111	2.28 (0.14 – 36.80)	0.562
Caucasian	2	137/309	1.94 (0.67 – 5.58)	0.222
CTLA-4 $(AT)_{126}$				
Asian	1	49/111	0.45 (0.05 – 3.88)	0.466
Caucasian	2	137/309	1.65 (0.63 – 4.32)	0.305
CTLA-4 $(AT)_{128}$				
Asian	1	49/111	6.98 (0.72 – 67.95)	0.094
Caucasian	2	137/309	0.72 (0.24 – 2.15)	0.560
FCGR2A rs1801274 (C)				
Asian	1	50/303	0.68 (0.37 – 1.24)	0.208
Caucasian	5	1126/5969	0.91 (0.82 – 1.00)	0.043
FCGR3A rs396991 (G)				
Asian	1	50/303	0.65 (0.39 – 1.07)	0.090
Caucasian	1	91/154	1.30 (0.89 – 1.91)	0.172
FCGR3B (NA1)				
Asian	1	50/303	1.29 (0.82 – 2.03)	0.267
Caucasian	3	865/667	0.93 (0.80 – 1.08)	0.343
HLA-A2				
Asian	1	16/472	0.90 (0.37 – 2.23)	0.826
Caucasian	4	427/7324	0.96 (0.81 – 1.13)	0.595
HLA-A11				
Asian	1	16/472	0.71 (0.17 – 3.03)	0.644
Caucasian	5	450/7635	1.01 (0.50 – 2.05)	0.978
HLA-A24				
Asian	1	16/472	0.75 (0.34 – 1.65)	0.476
Caucasian	2	92/751	1.16 (0.57 – 2.38)	0.683
HLA-A26				
Asian	1	16/472	2.36 (1.00 – 5.60)	0.051
Caucasian	3	115/1156	1.21 (0.58 – 2.55)	0.612
HLA-A31				
Asian	1	16/472	1.55 (0.46 – 5.24)	0.479
Caucasian	2	92/751	0.73 (0.21 – 2.52)	0.613

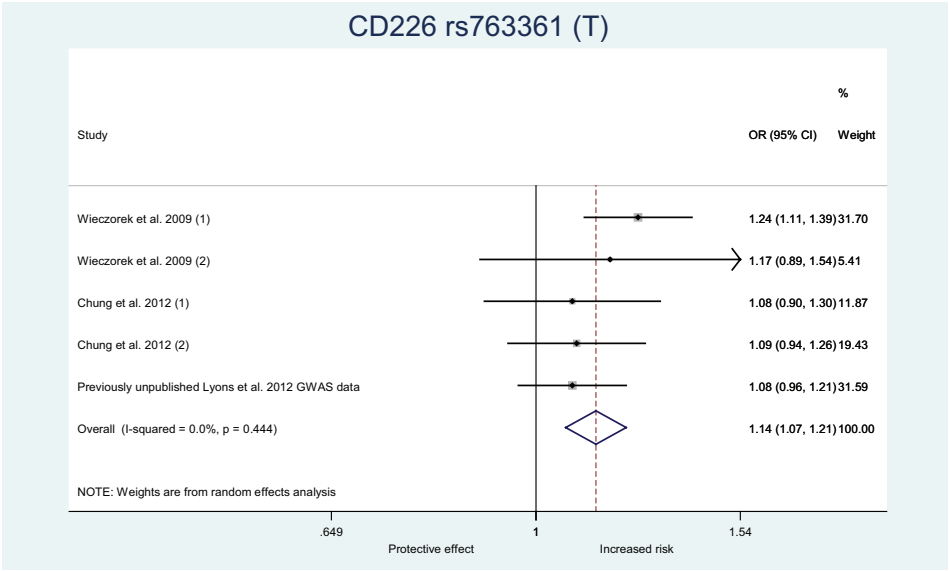
Supplementary table 5. Results of the meta-analyses stratified by ethnic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
HLA-A32				
Asian	1	16/472	2.07 (0.61 – 7.04)	0.246
Caucasian	2	92/751	1.41 (0.52 – 3.84)	0.500
HLA-B35				
Asian	1	16/472	0.80 (0.17 – 3.40)	0.757
Caucasian	3	396/6623	0.78 (0.58 – 1.06)	0.115
HLA-B39				
Asian	1	16/472	0.86 (0.11 – 6.51)	0.887
Caucasian	1	51/51	0.33 (0.01 – 8.20)	0.499
HLA-B44				
Asian	1	16/472	2.50 (0.85 – 7.40)	0.098
Caucasian	2	92/751	1.41(0.85 – 2.35)	0.188
HLA-B51				
Asian	1	16/472	1.87 (0.64 – 5.49)	0.255
Caucasian	1	51/51	0.61 (0.19 – 1.92)	0.394
HLA-B55				
Asian	1	16/472	7.35 (2.34 – 23.14)	0.001
Caucasian	1	51/51	0.07 (0.00 – 1.30)	0.075
HLA-B60				
Asian	1	16/472	1.19 (0.28 – 5.13)	0.814
Caucasian	1	41/700	3.51 (0.99 – 12.36)	0.051
HLA-B62				
Asian	1	16/472	1.71 (0.58 – 4.99)	0.331
Caucasian	1	41/700	2.38 (0.70 – 8.12)	0.166
HLA-Cw1				
Asian	1	16/472	1.45 (0.58 – 3.58)	0.426
Caucasian	1	52/51	0.98 (0.24 – 4.03)	0.978
HLA-Cw3				
Asian	1	16/472	1.40 (0.66 – 3.01)	0.384
Caucasian	1	52/51	1.32 (0.62 – 2.81)	0.479
HLA-Cw7				
Asian	1	16/472	0.50 (0.12 – 2.10)	0.340
Caucasian	1	52/51	1.10 (0.62 – 1.96)	0.735
*HLA-DPB1*0201*				
Asian	1	50/77	1.38 (0.75 – 2.52)	0.298
Caucasian	3	385/827	0.76 (0.56 – 1.03)	0.073

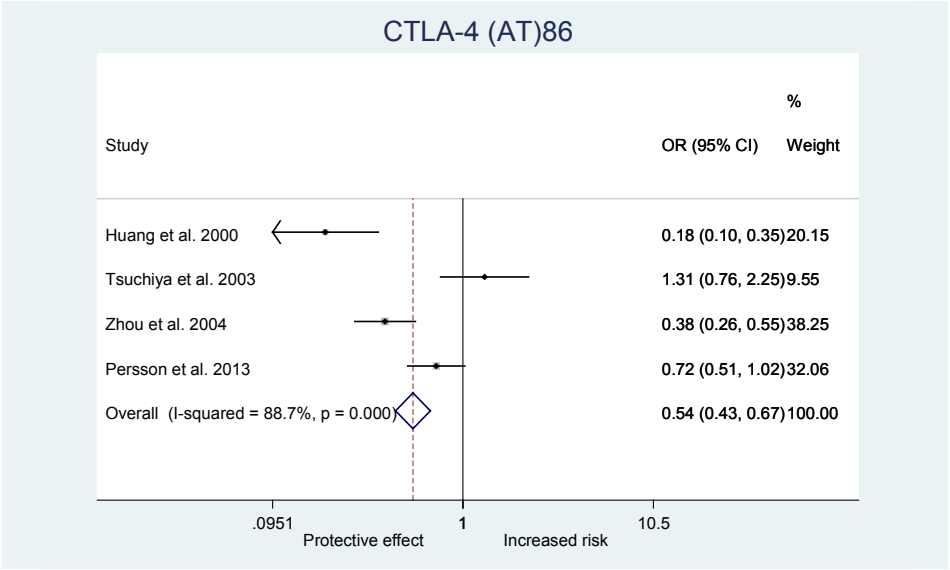
Supplementary table 5. Results of the meta-analyses stratified by ethnic subgroups (Continued)

Variant by gene	Publications (n)	Cases (n) / Controls (n)	OR (95% CI)	P value for meta-analysis
*HLA-DQB1**0303				
Asian	1	50/77	2.11 (1.14 – 3.90)	0.018
Caucasian	2	126/141	1.35 (0.55 – 3.33)	0.515
HLA-DR1				
Asian	1	64/265	1.12 (0.48 – 2.64)	0.793
Caucasian	9	945/6840	0.91 (0.61 – 1.36)	0.651
HLA-DR2				
Asian	1	16/472	0.33 (0.08 – 1.38)	0.128
Caucasian	4	471/5750	1.06 (0.87 – 1.29)	0.565
HLA-DR4				
Asian	1	16/472	0.72 (0.27 – 1.88)	0.498
Caucasian	10	882/7262	1.23 (0.97 – 1.57)	0.088
HLA-DR6				
Asian	1	16/472	1.63 (0.56 – 4.77)	0.371
Caucasian	4	471/5750	0.43 (0.30 – 0.61)	<0.001
HLA-DR8				
Asian	1	16/472	1.63 (0.66 – 4.05)	0.291
Caucasian	8	597/1483	1.05 (0.75 – 1.49)	0.768
HLA-DR9				
Asian	4	296/1409	1.67 (0.83 – 3.34)	0.148
Caucasian	7	510/1443	0.63 (0.34 – 1.19)	0.152
IRF5 rs10954213 (G)				
Asian	1	232/710	1.28 (1.04 – 1.58)	0.022
Caucasian	2	1303/6267	0.69 (0.63 – 0.76)	<0.001
STAT4 rs7574865 (T)				
Asian	1	232/710	1.10 (0.89 – 1.37)	0.388
Caucasian	2	1288/6246	1.11 (1.00 – 1.24)	0.044
TNFRII 196R				
Asian	1	50/262	0.67 (0.31 – 1.45)	0.313
Caucasian	1	117/123	1.05 (0.70 – 1.57)	0.819

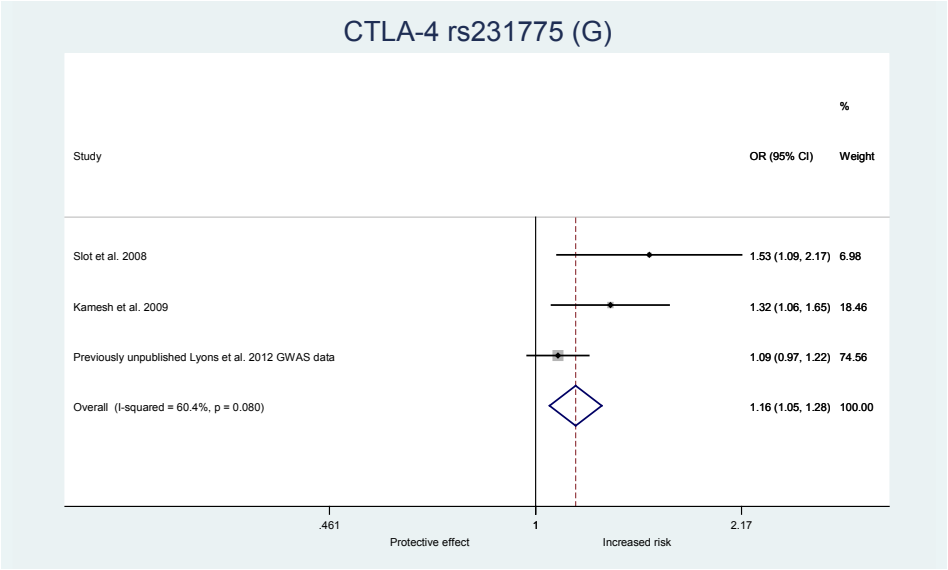
Supplementary figure 1. Forest plots



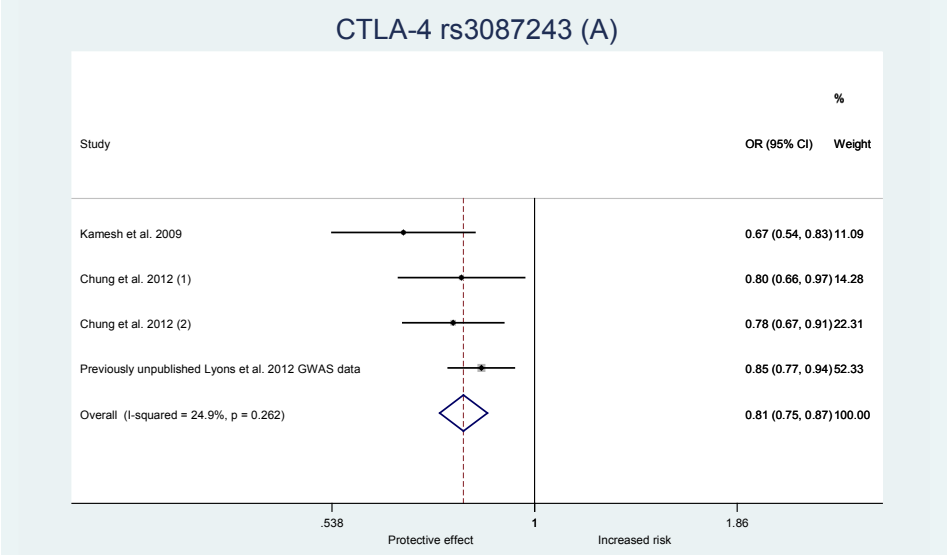
CD226 rs763361 (T) forest plot. Harbold test: N/A, Egger test: p=0.792.
References: [1]**, [2]**, [3]



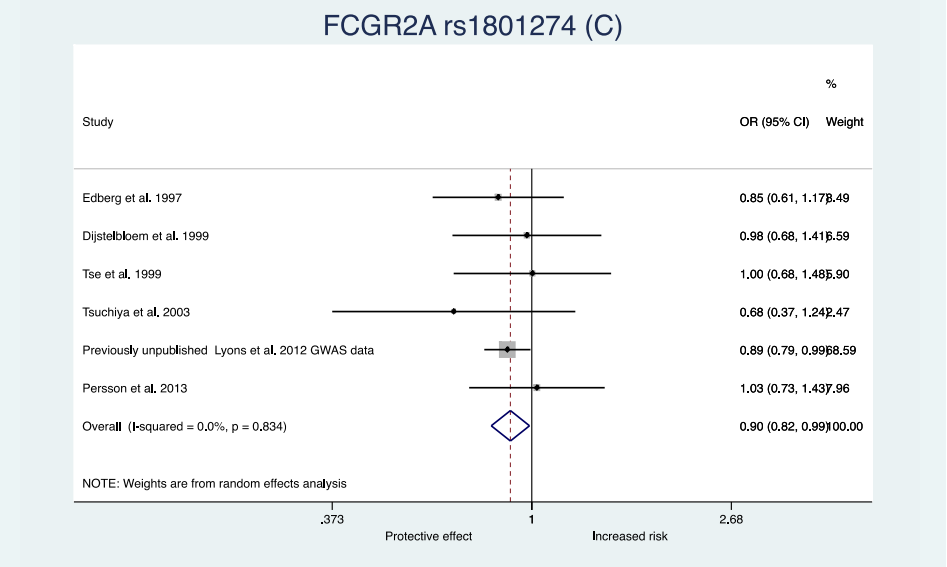
CTLA-4 $(AT)_{86}$ forest plot. Harbold test: p=0.946, Egger test: p=0.788.
References: [4], [5], [6], [7]



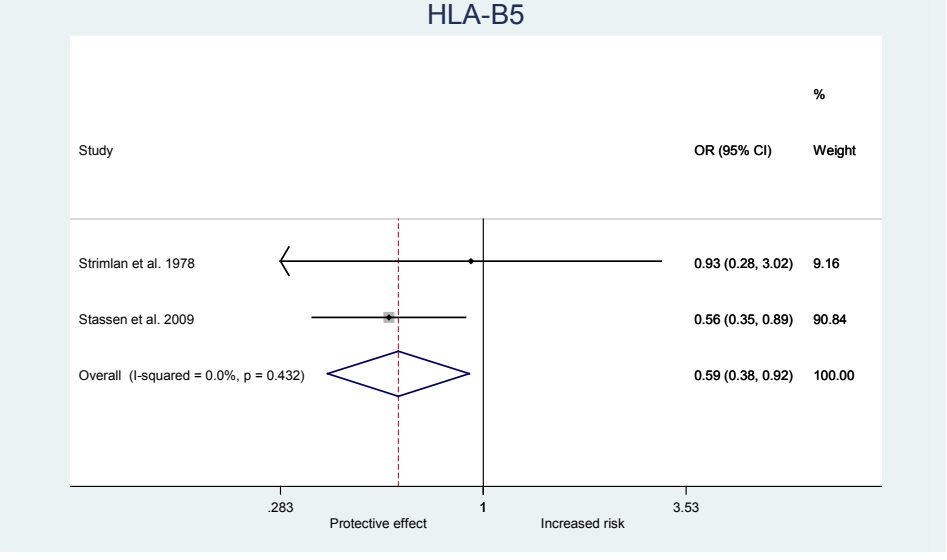
CTLA-4 rs231775 (G) forest plot. Harbold test: p=0.080, Egger test: p=0.081.
References: [8], [9], [3]



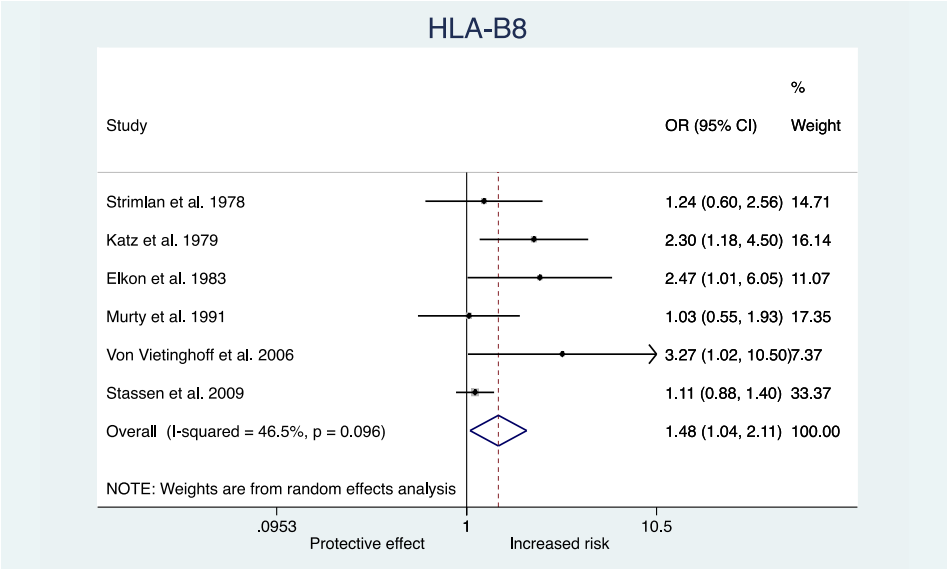
CTLA-4 rs3087243 (A) forest plot. Harbold test: N/A, Egger test: p=0.122.
References: [9], [2]**, [3]



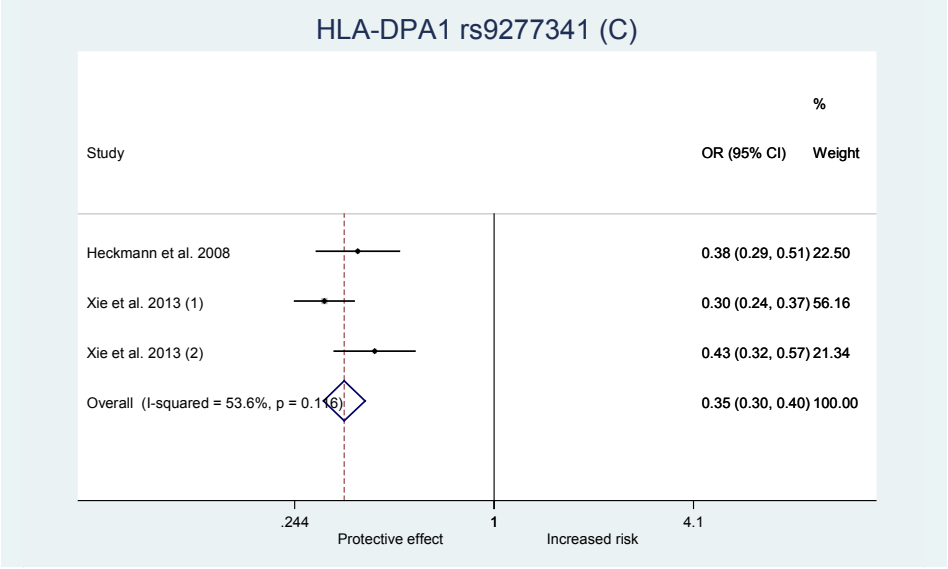
FCGR2A rs1801274 (C) forest plot. Harbold test: p=0.788, Egger test: p=0.829.
References: [11], [12], [13], [5], [7], [3]



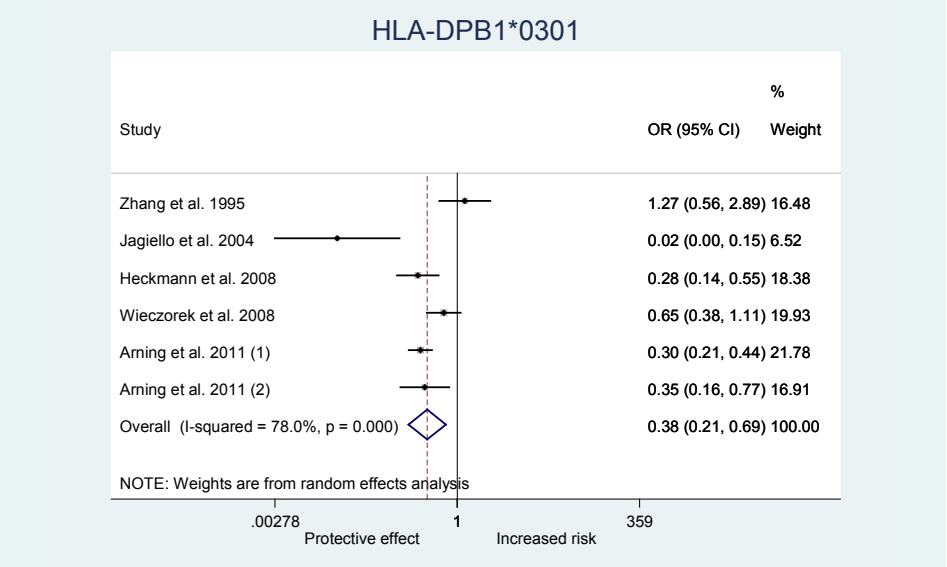
HLA-B5 forest plot. Harbold test: N/A, Egger test: N/A.
References: [16], [19]



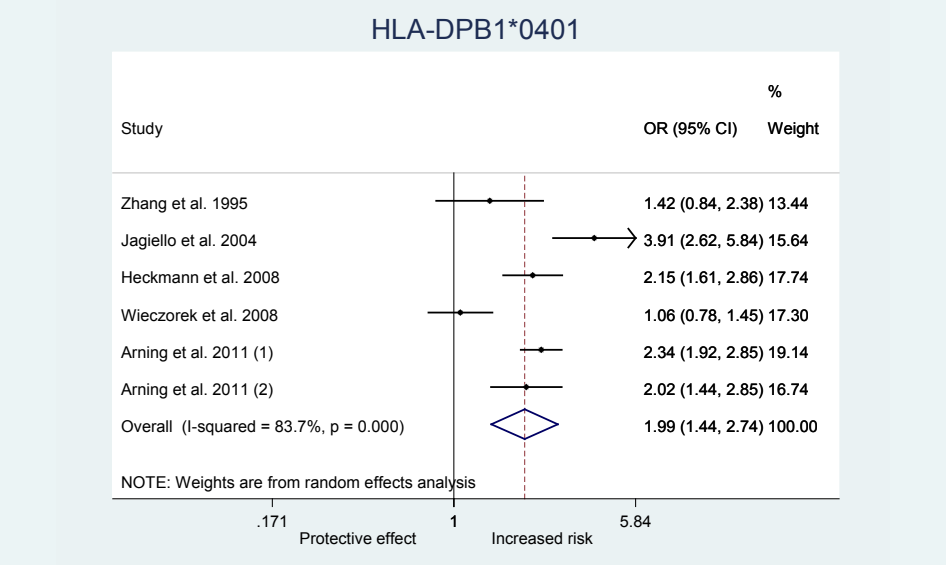
HLA-B8 forest plot. Harbold test: p=0.063, Egger test: p=0.077.
References: [16], [21], [23], [17], [18], [19]



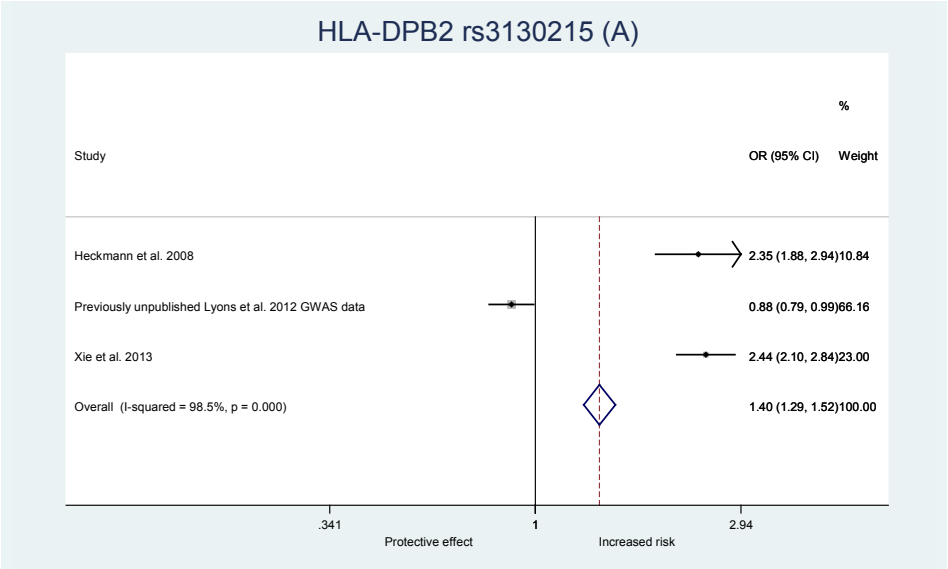
HLA-DPA1 rs9277341 (C) forest plot. Harbold test: p=0.215, Egger test: p=0.219.
References: [24], [25]**



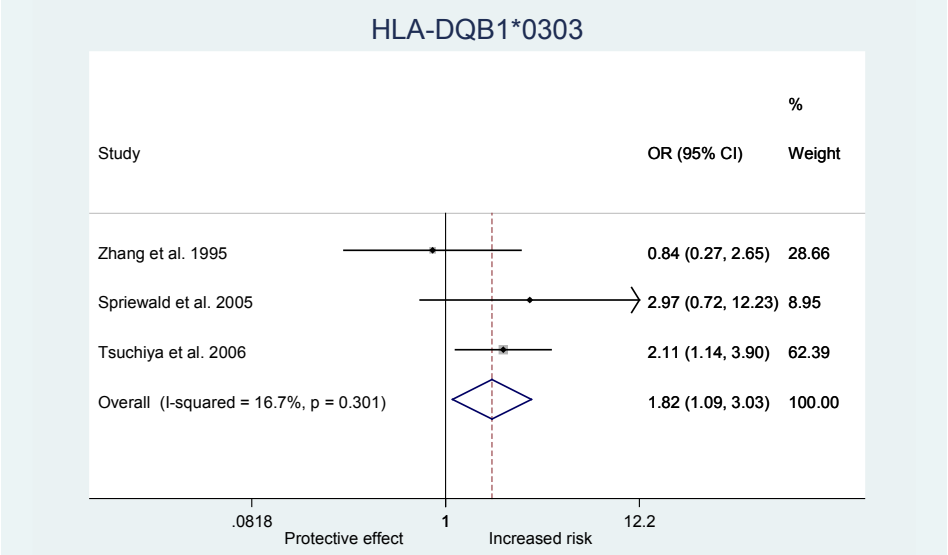
*HLA-DPB1*0301* forest plot. Harbold test: p=0.938, Egger test: p=0.744.
References: [26], [27], [24], [29], [30]**



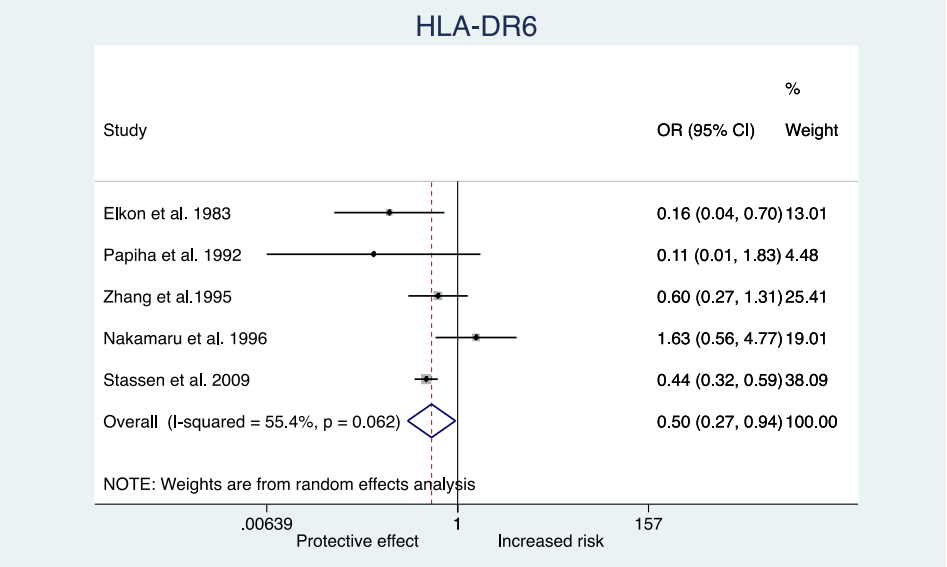
*HLA-DPB1*0401* forest plot. Harbold test: p=0.738, Egger test: p=0.759.
References: [26], [27], [24], [29], [30]**



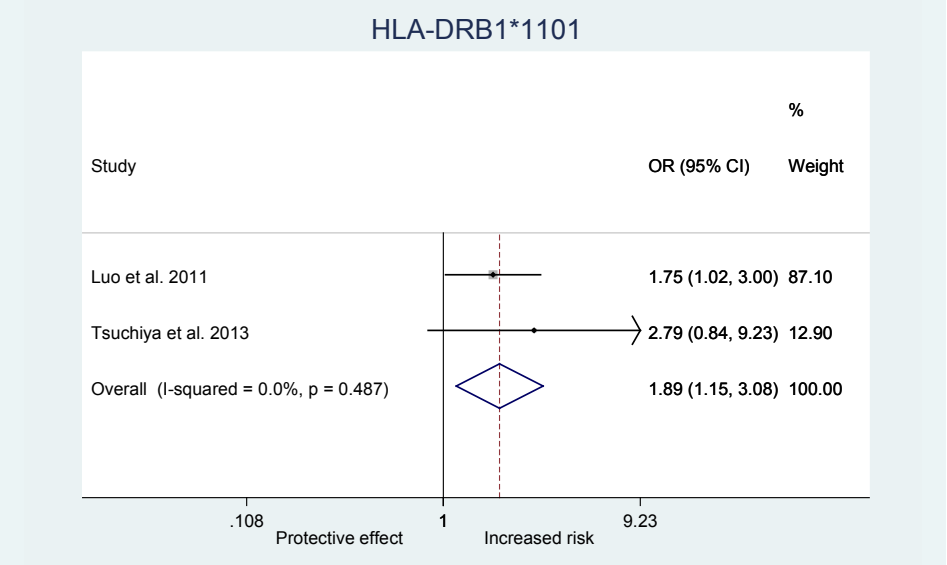
HLA-DPB2 rs3130215 (A) forest plot. Harbold test: p=0.446, Egger test: p=0.431.
References: [24], [3], [25]



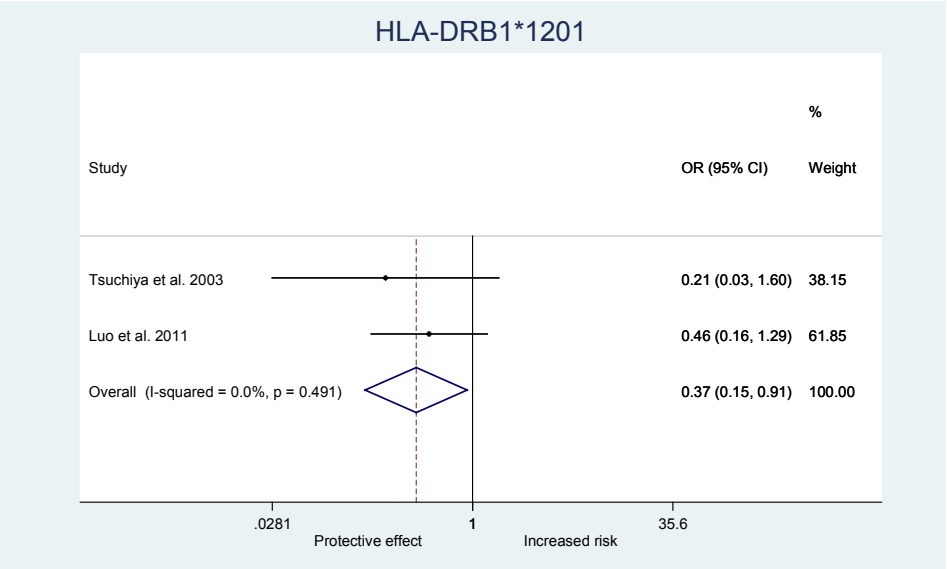
*HLA-DQB1*0303* forest plot. Harbold test: p=0.916, Egger test: p=0.834.
References: [26], [31], [28]



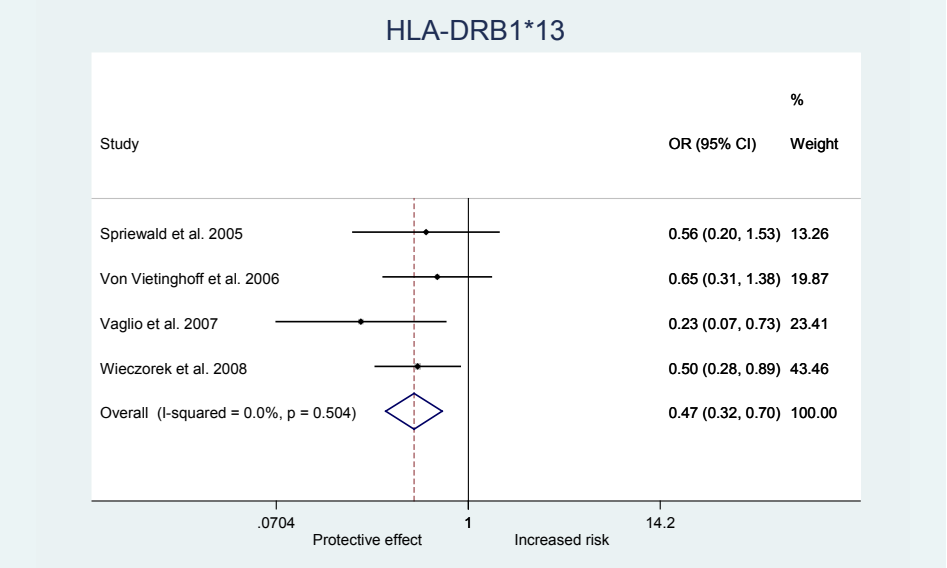
HLA-DR6 forest plot. Harbold test: p=0.997, Egger test: p=0.989.
References: [23], [32], [26], [20], [19]



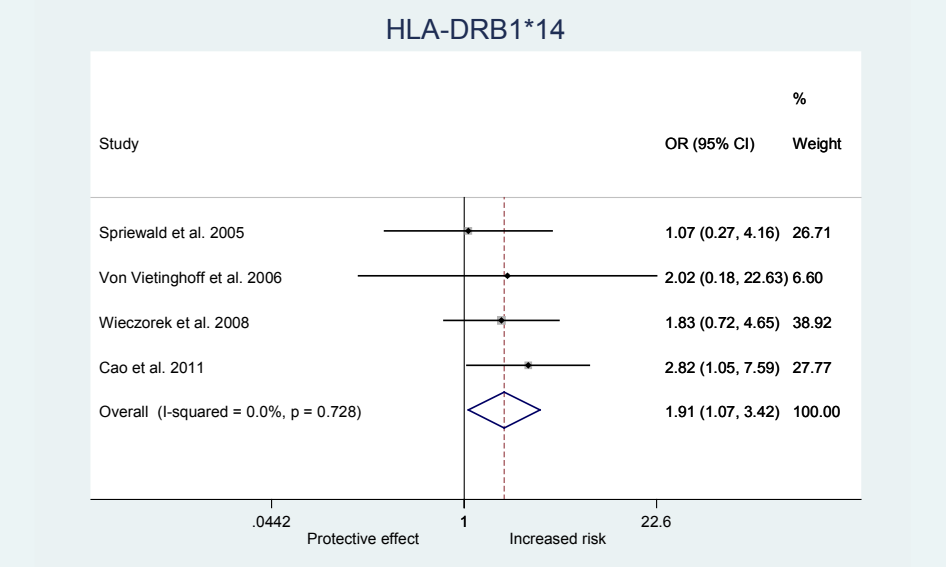
*HLA-DRB1*1101* forest plot. Harbold test: N/A, Egger test: N/A.
References: [34], [37]



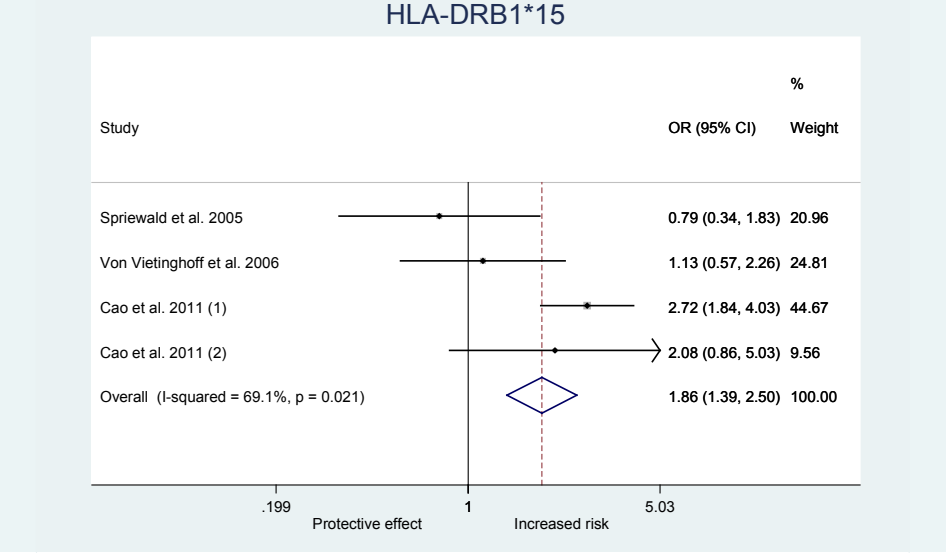
*HLA-DRB1*1201* forest plot. Harbold test: N/A, Egger test: N/A.
References: [5], [34]



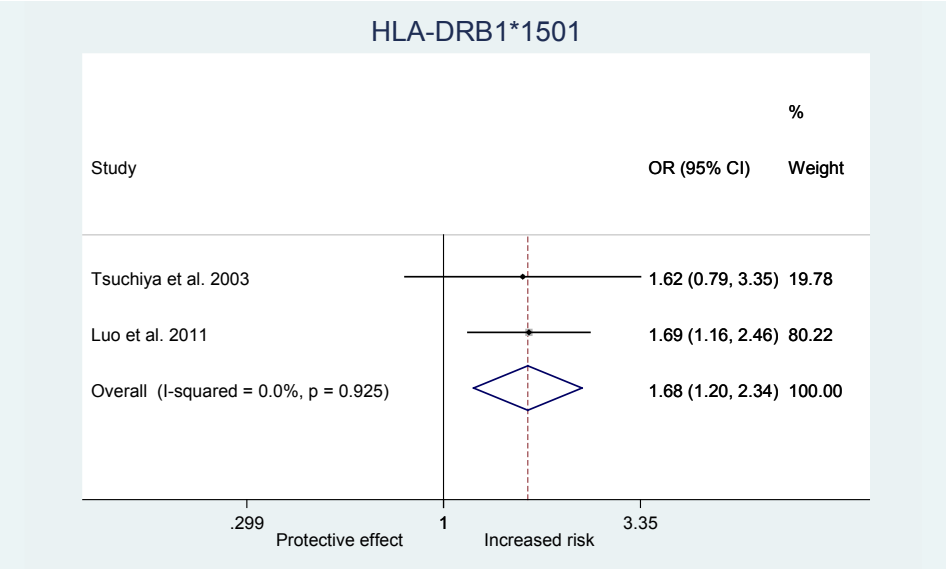
*HLA-DRB1*13* forest plot. Harbold test: p=0.884, Egger test: p=0.467.
References: [31], [18], [35], [29]



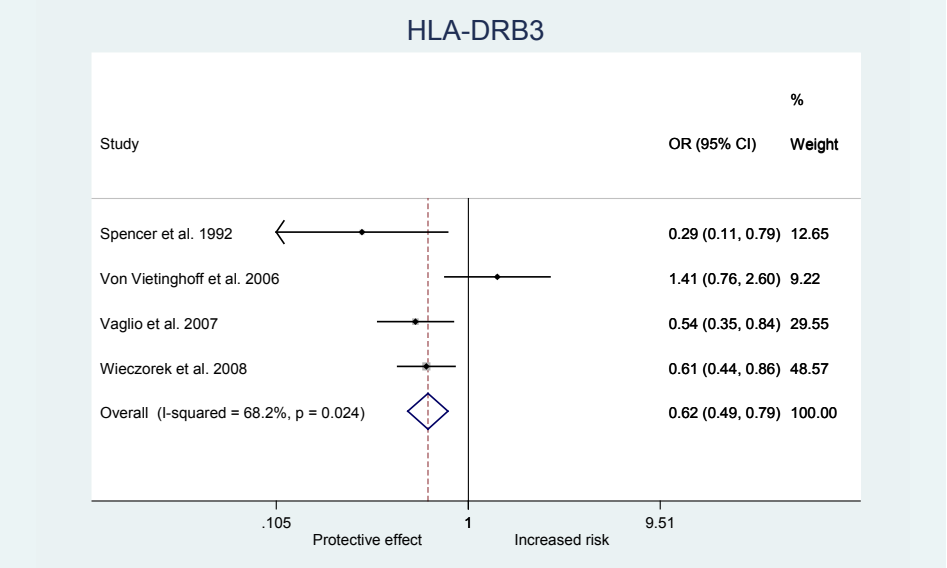
*HLA-DRB1*14* forest plot. Harbold test: p=0.700, Egger test: p=0.706.
References: [31], [18], [29], [33]



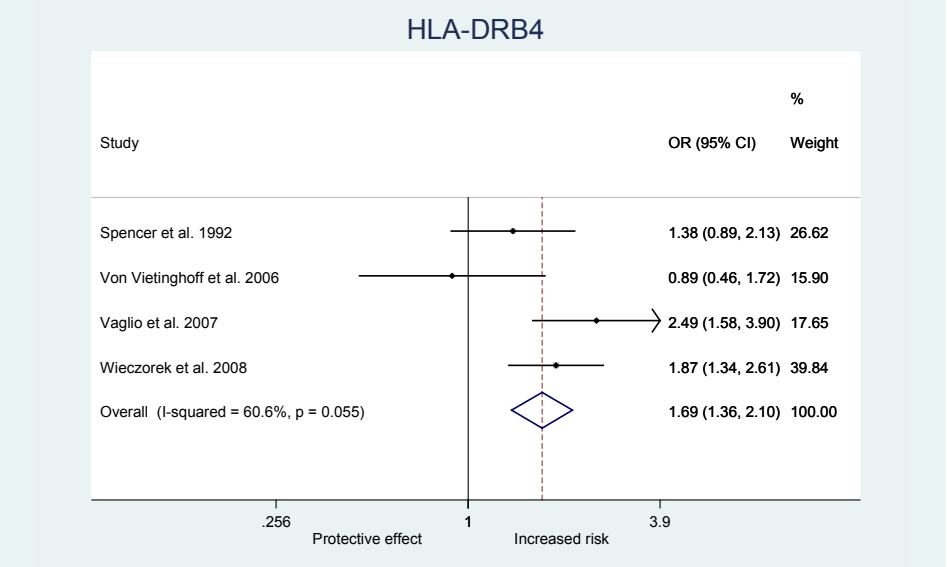
*HLA-DRB1*15* forest plot. Harbold test: p=0.347, Egger test: p=0.214.
References: [31], [18], [33]**



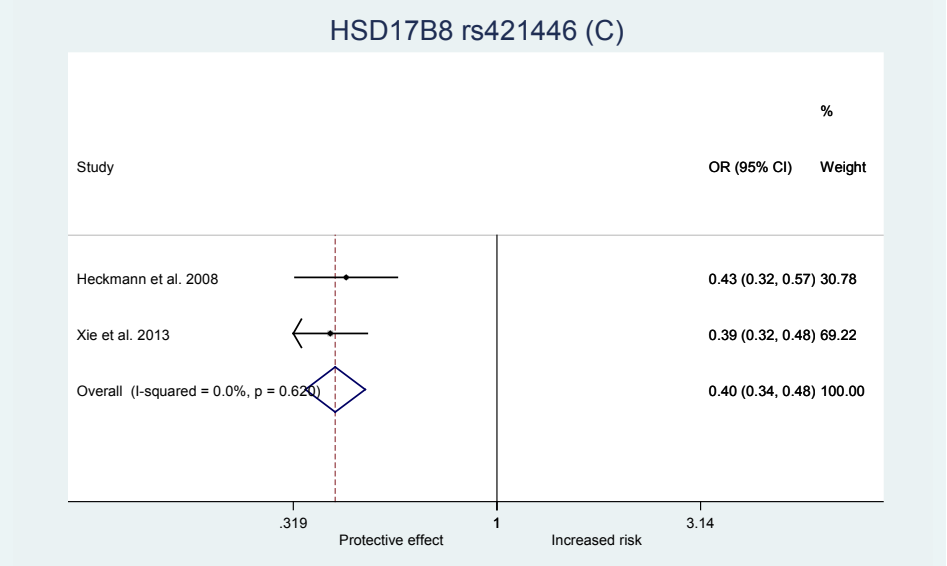
*HLA-DRB1*1501* forest plot. Harbold test: N/A, Egger test: N/A.
References: [5], [34]



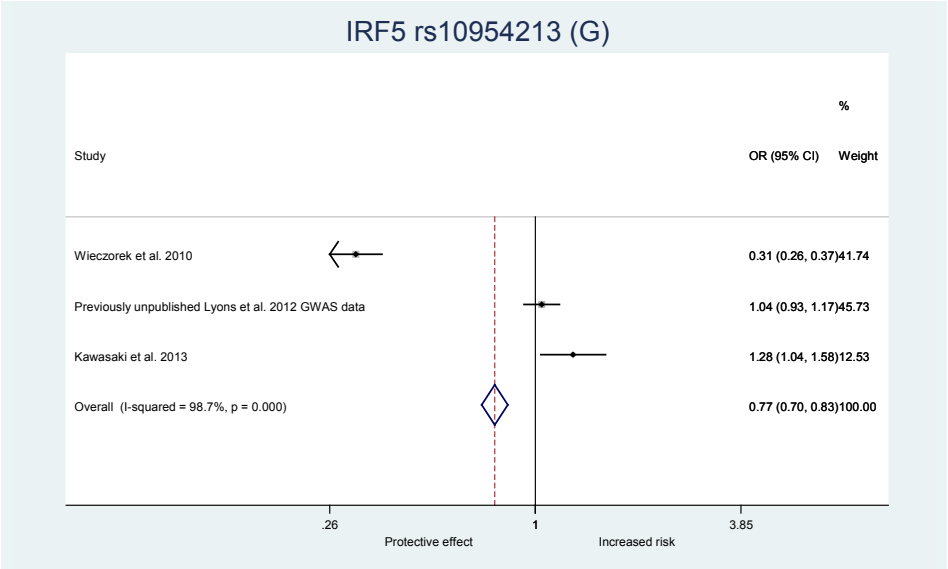
HLA-DRB3 forest plot. Harbold test: p=0.689, Egger test: p=0.958.
References: [38], [18], [35], [29]



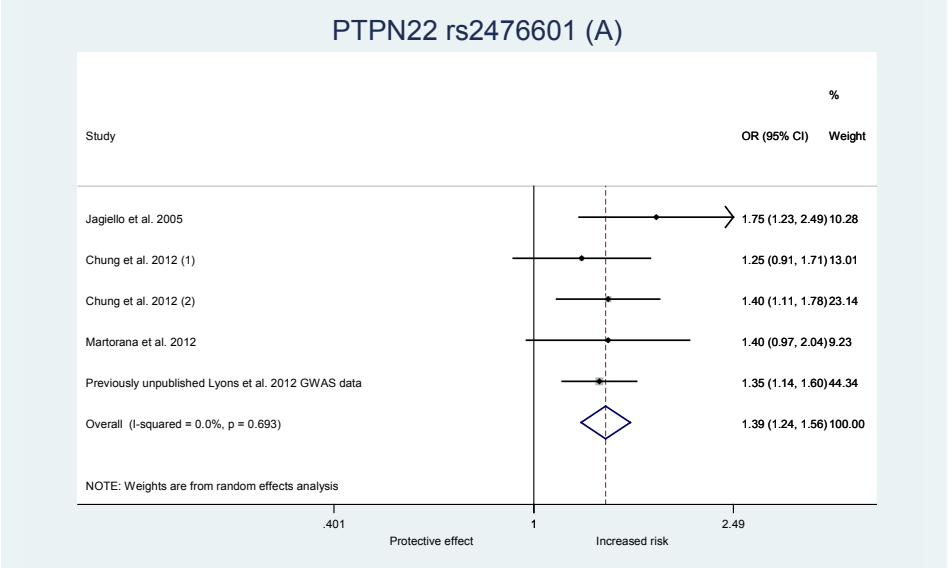
HLA-DRB4 forest plot. Harbold test: p=0.533, Egger test: p=0.388.
References: [38], [18], [35], [29]



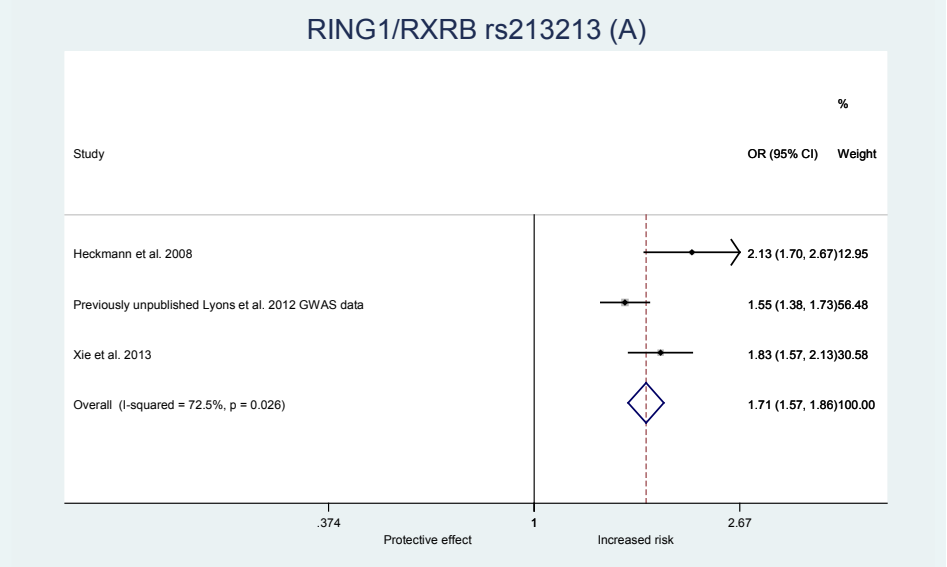
HSD17B8 rs421446 (C) forest plot. Harbold test: N/A, Egger test: N/A.
References: [24], [25]



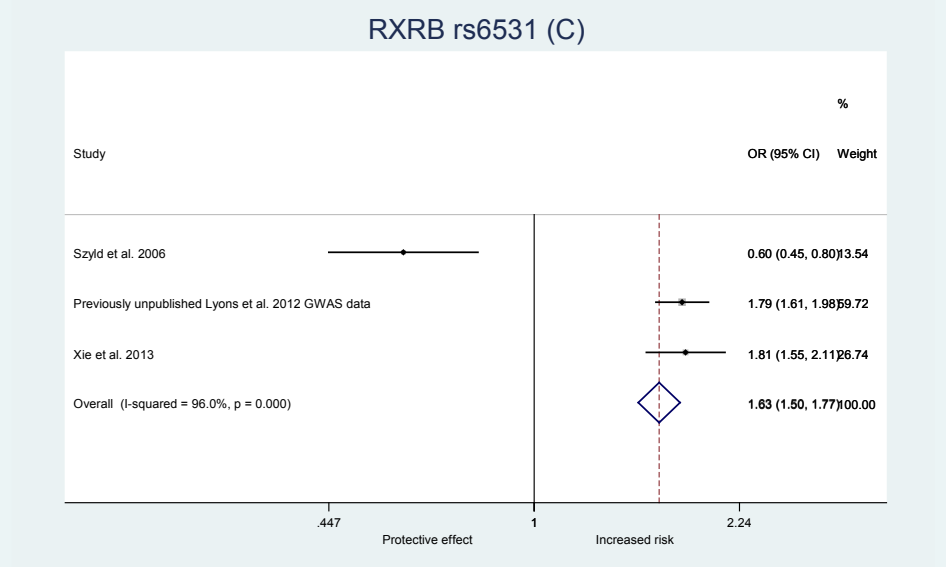
IRF5 rs10954213 (G) forest plot. Harbold test: p=0.948, Egger test: p=0.833.
References: [44], [3], [45]



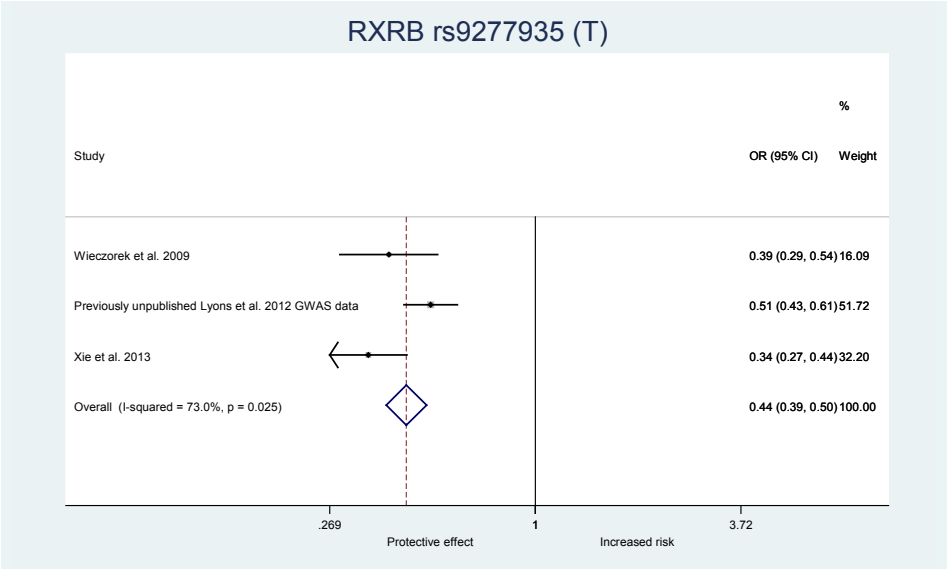
PTPN22 rs2476601 (A) forest plot. Harbold test: N/A, Egger test: p=0.500.
References: [50], [2**], [51], [3]



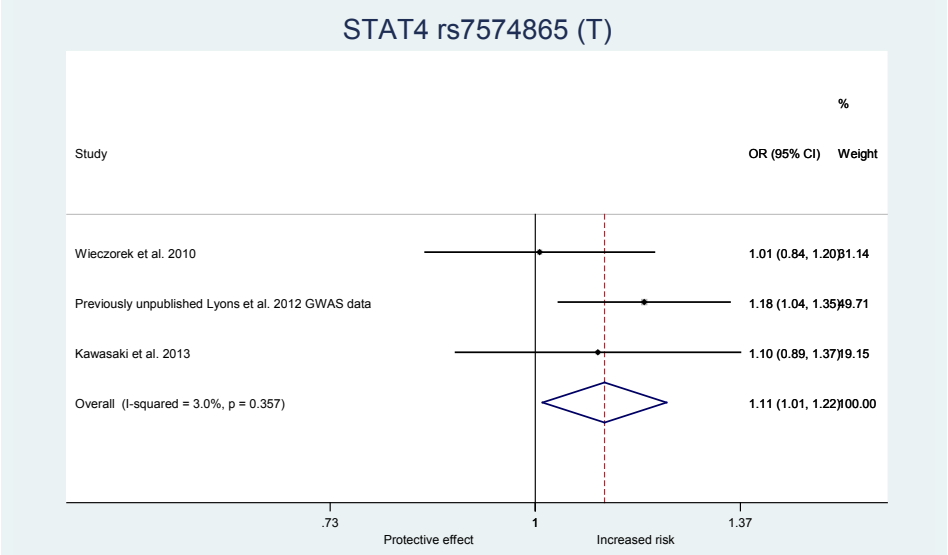
RING1/RXRB rs213213 (A) forest plot. Harbold test: p=0.187, Egger test: p=0.169.
References: [24], [3], [25]



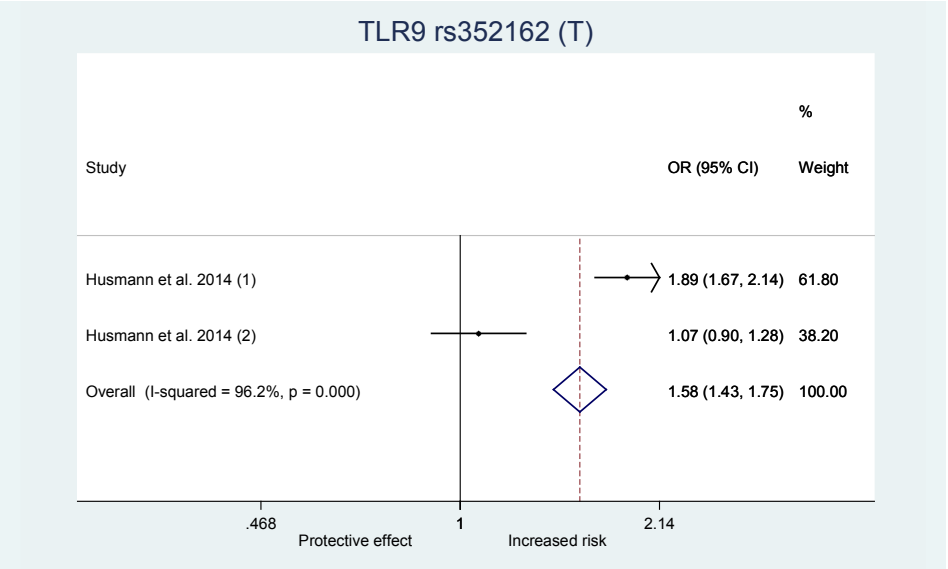
RXRB rs6531 (C) forest plot. Harbold test: p=0.292, Egger test: p=0.301.
References: [52], [3], [25]



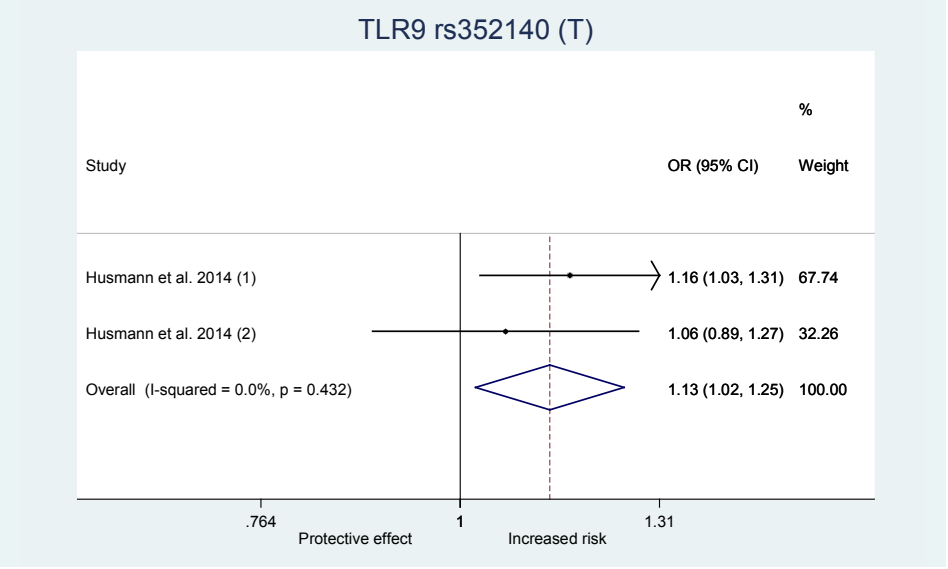
RXRB rs9277935 (T) forest plot. Harbold test: p=0.393, Egger test: p=0.406.
References: [53], [3], [25]



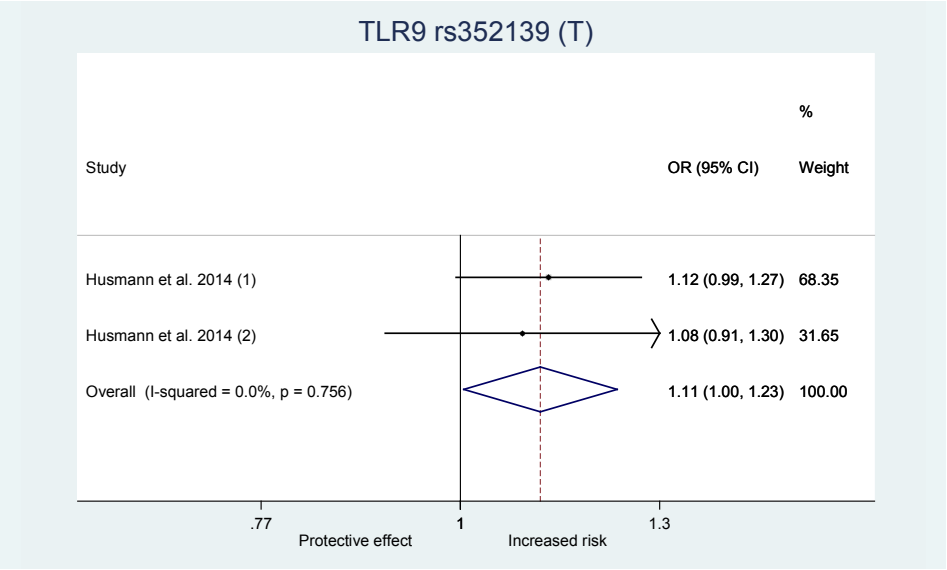
STAT4 rs7574865 (T) forest plot. Harbold test: p=0.590, Egger test: p=0.567.
References: [44], [45], [3]



TLR9 rs352162 (T) forest plot. Harbold test: N/A, Egger test: N/A.
References: [40]**



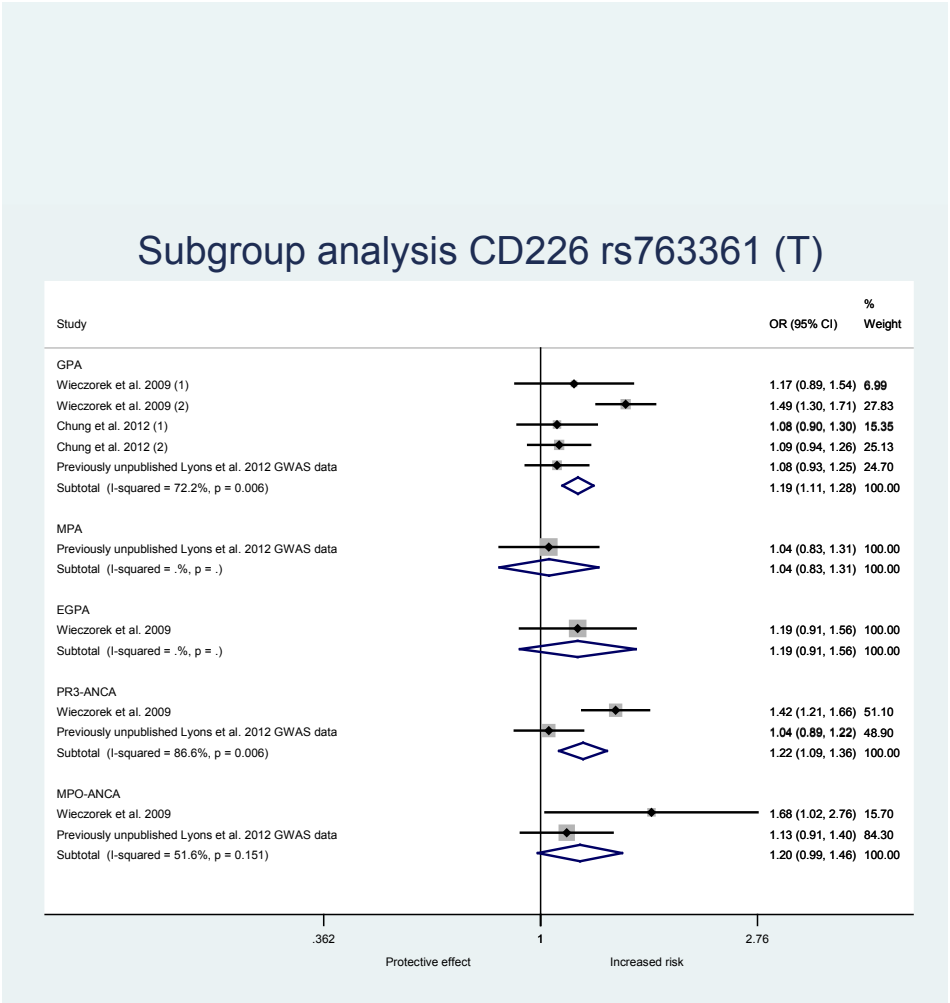
TLR9 rs352140 (T) forest plot. Harbold test: N/A, Egger test: N/A.
References: [40]**



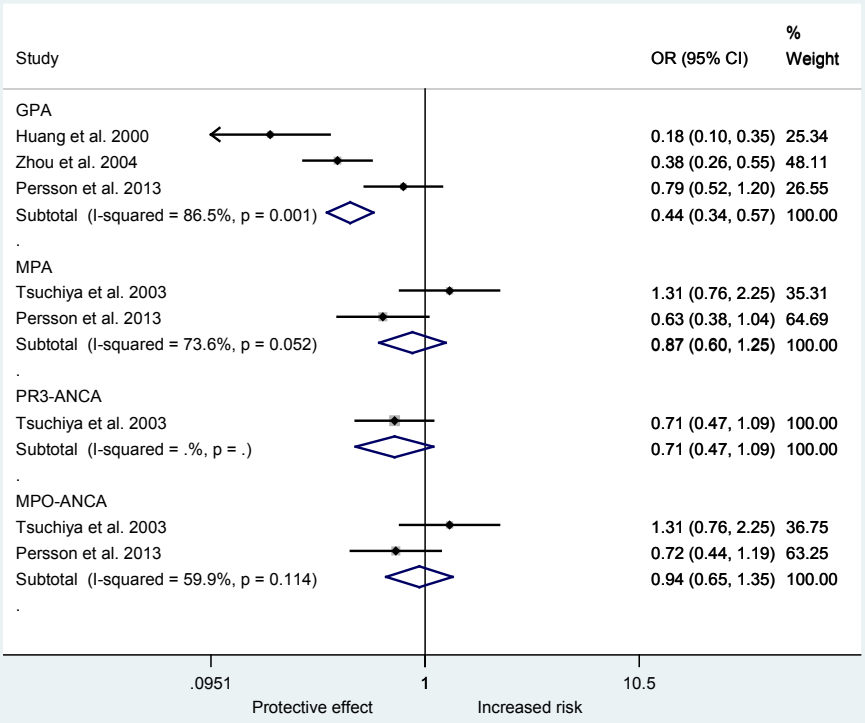
TLR9 rs352139 (T) forest plot. Harbold test: N/A, Egger test: N/A.
References: [40]**

**Two cohorts described in the same publication.
***Three cohorts described in the same publication.
N/A, not applicable

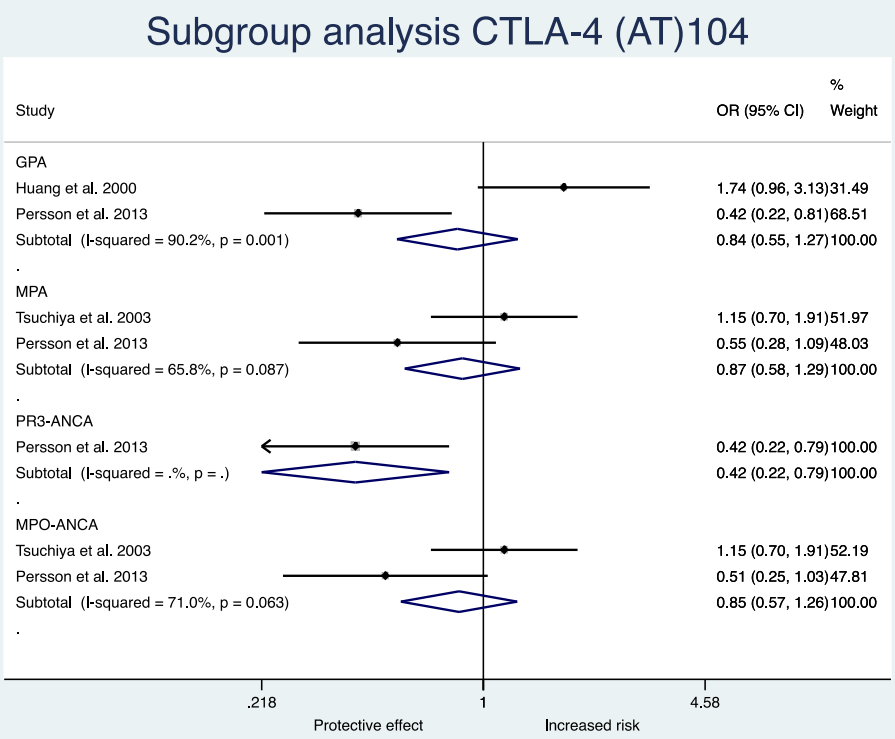
Supplementary figure 2. Forest plots by diagnostic and serologic subgroups



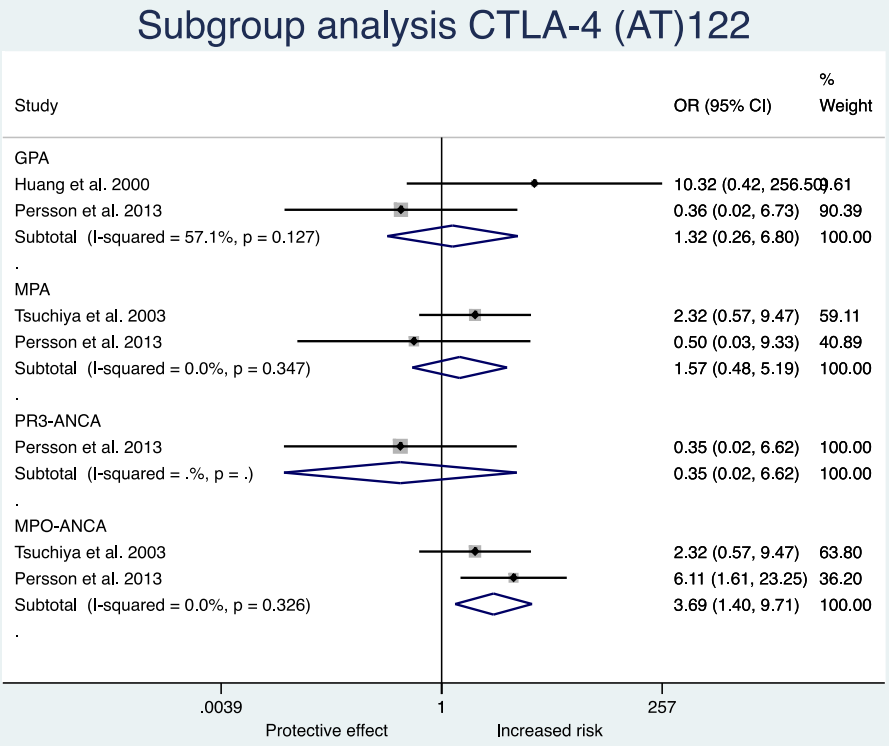
References: [1]**, [2]**, [3]



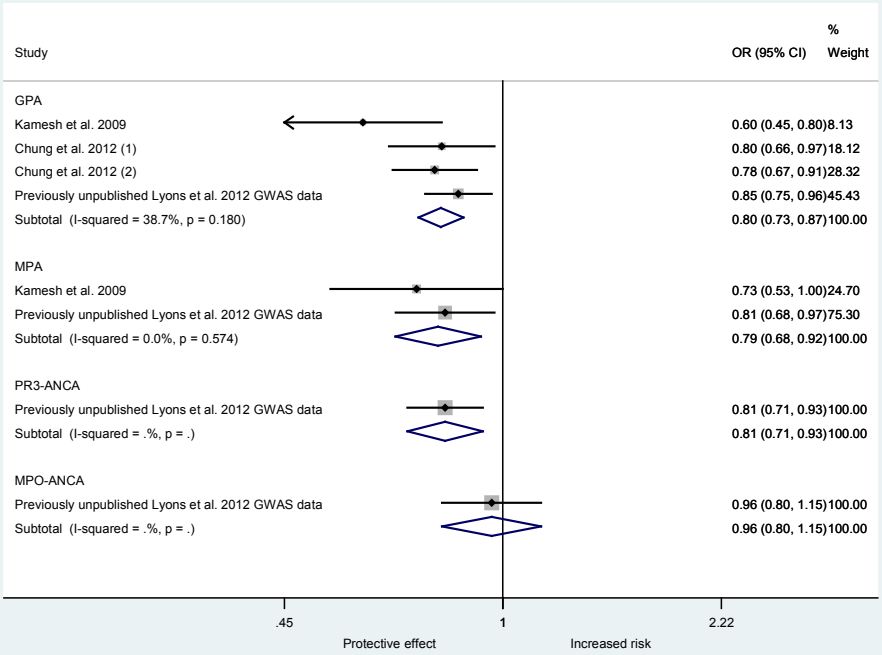
References: [4], [5], [6], [7]



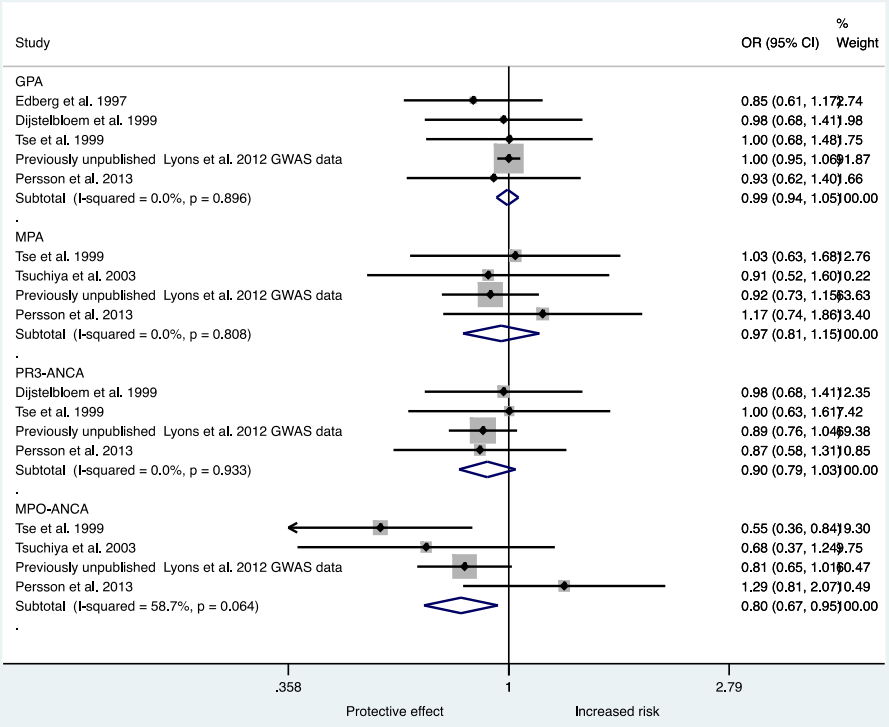
References: [4], [7], [5]



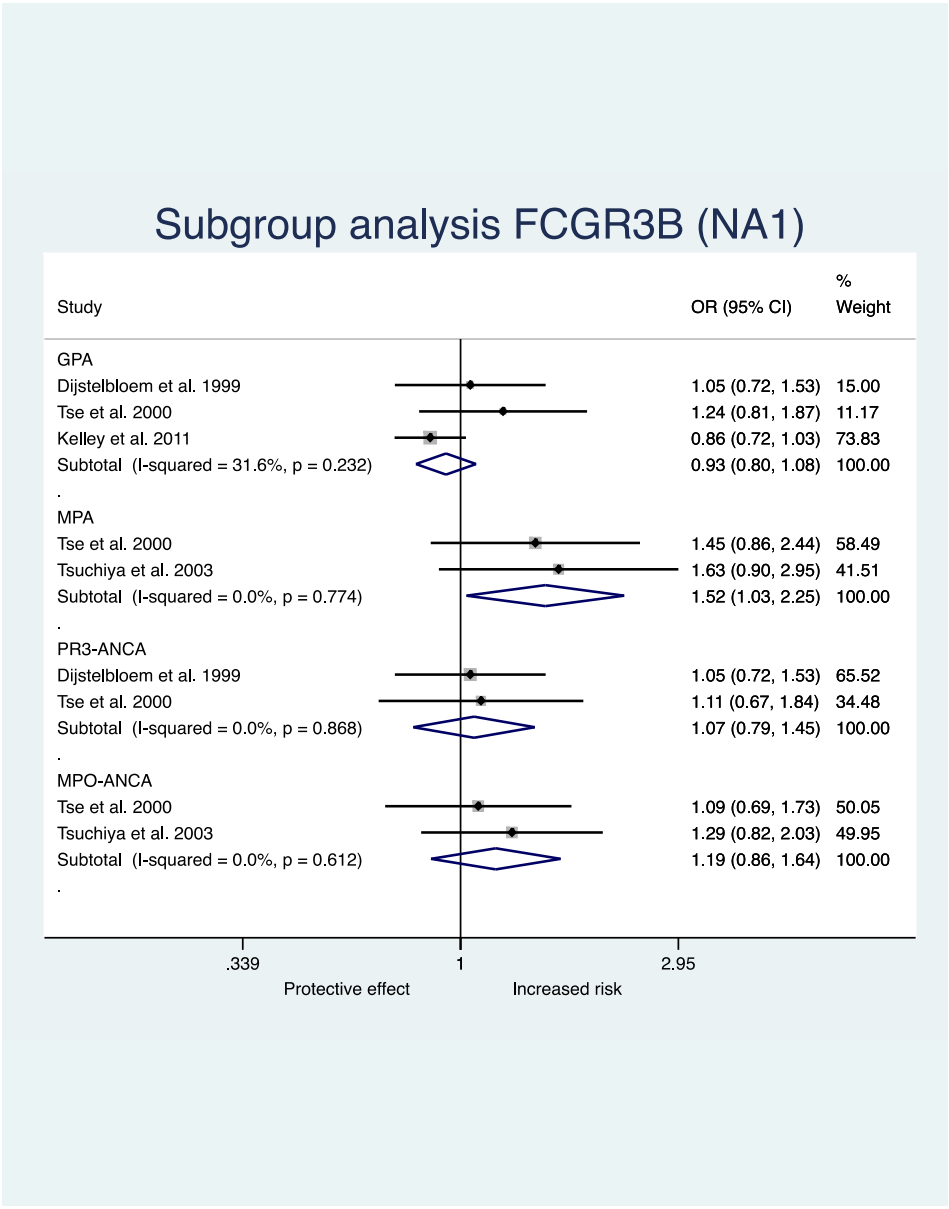
References: [4], [7], [5]



References: [9], [2]**, [3]

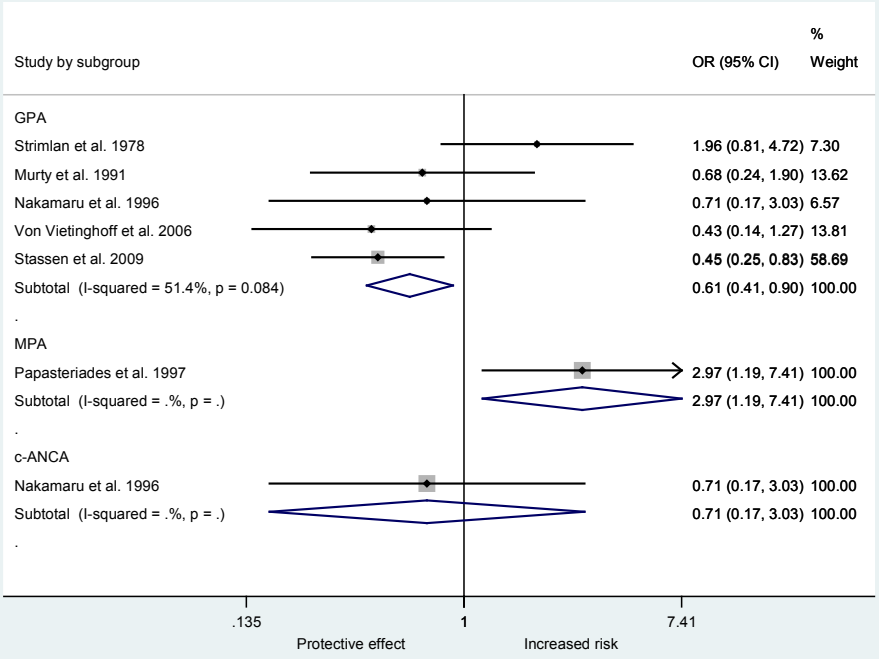


References: [11], [12], [13], [5], [7], [3]

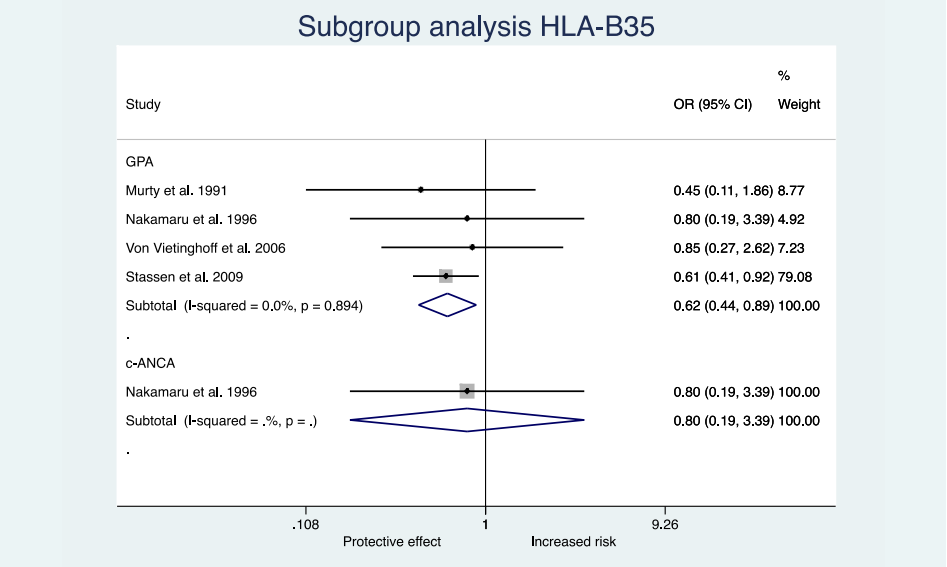


References: [12], [14], [10], [5]

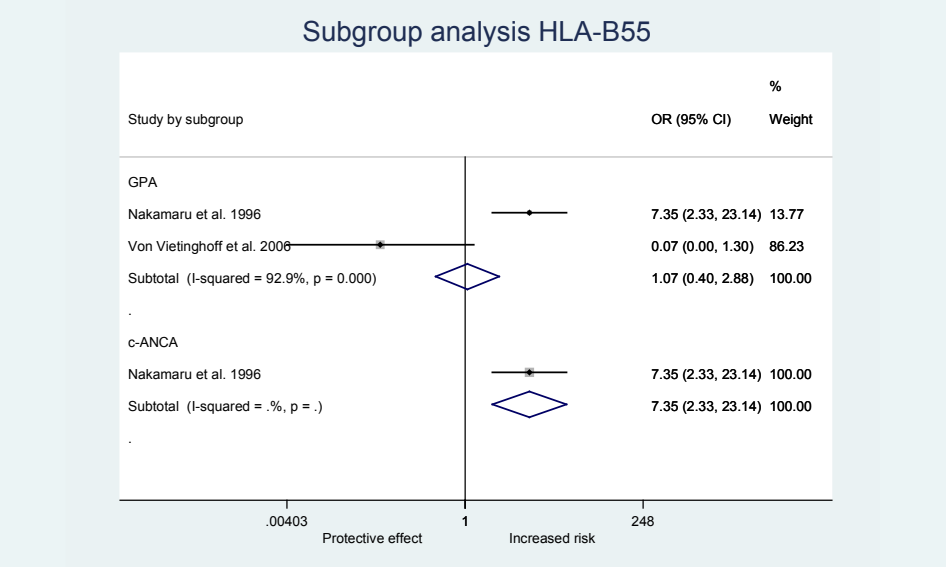
Subgroup analysis HLA-A11



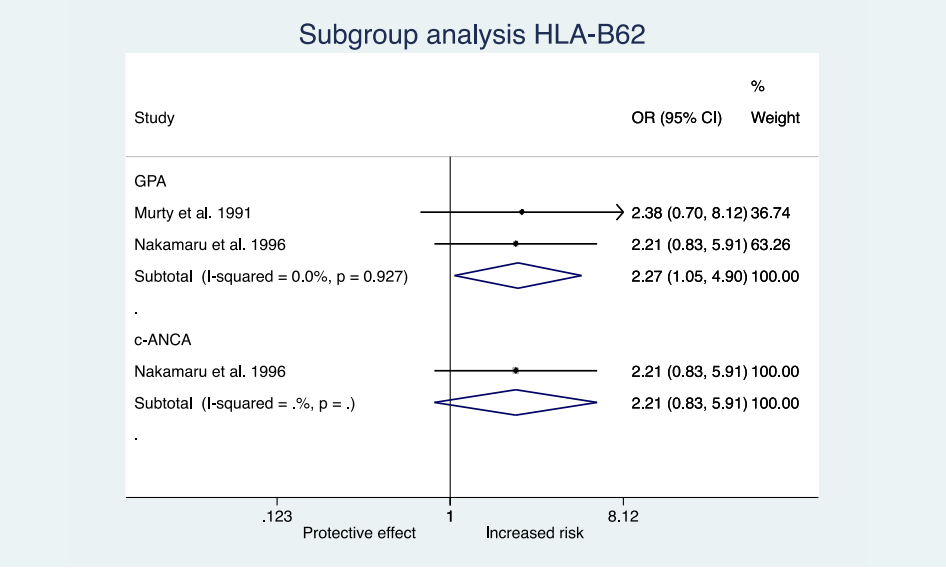
References: [16], [17], [20], [22], [18], [19]



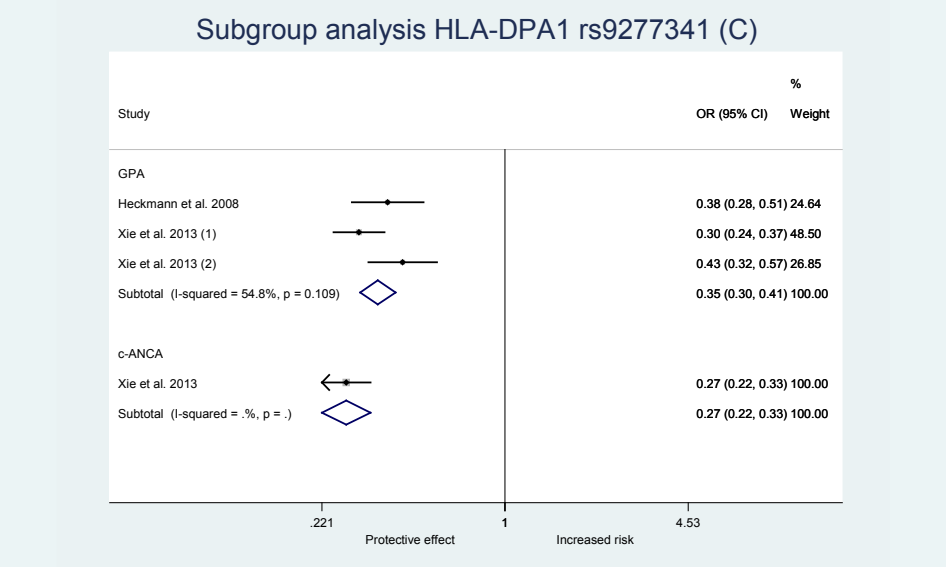
References: [17], [20], [18], [19]



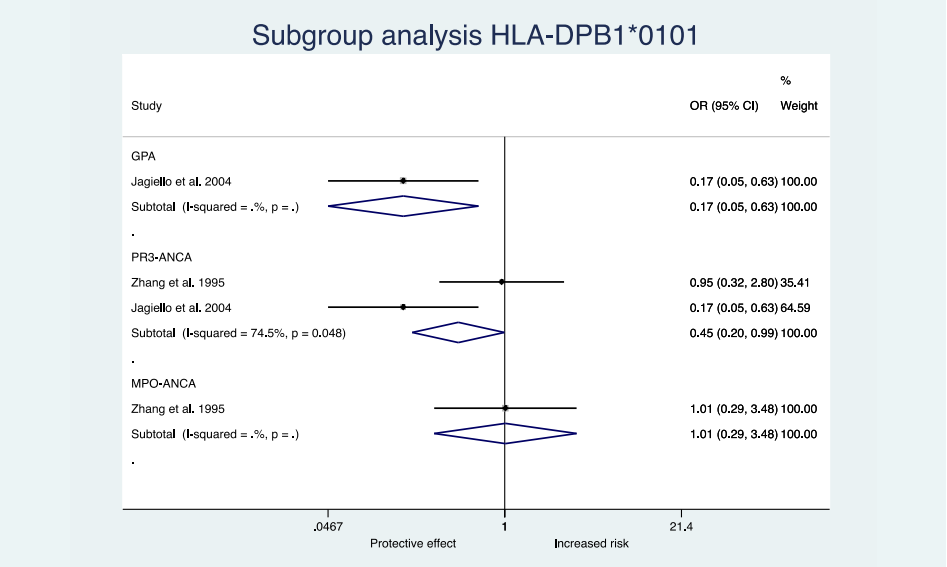
References: [20], [18]



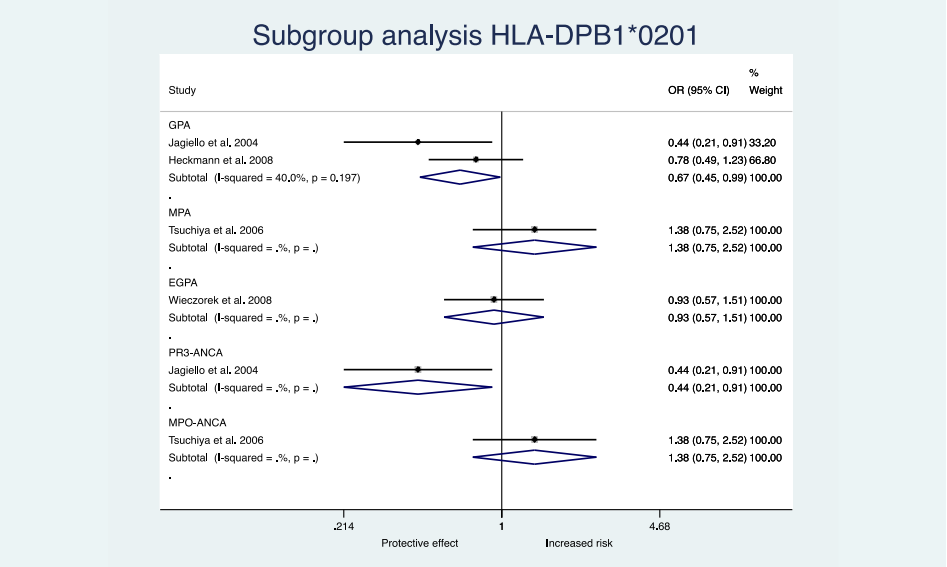
References: [17], [20]



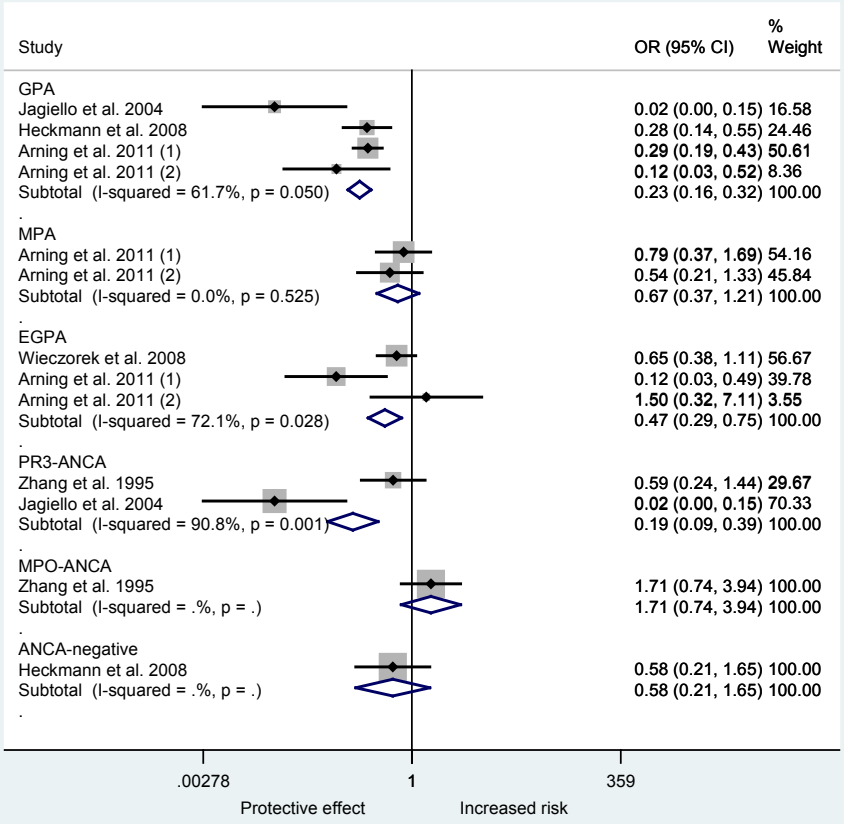
References: [24], [25]**



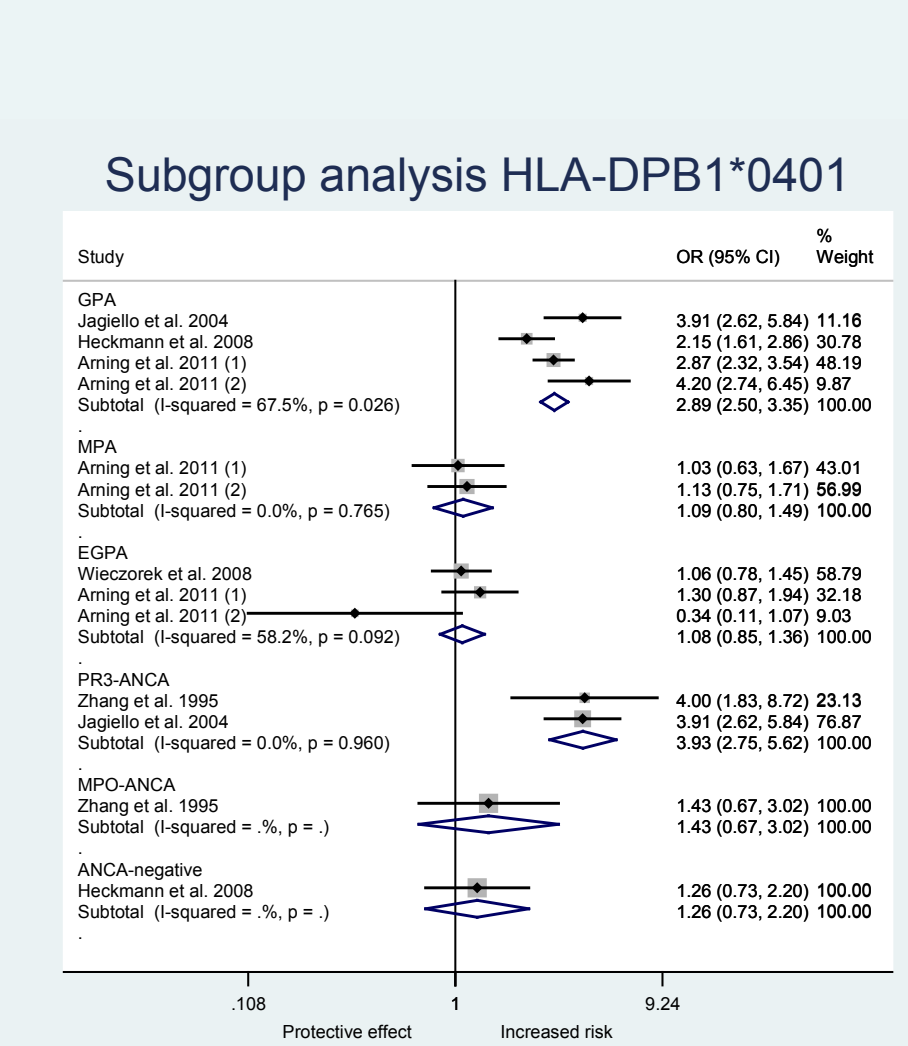
References: [27], [26]



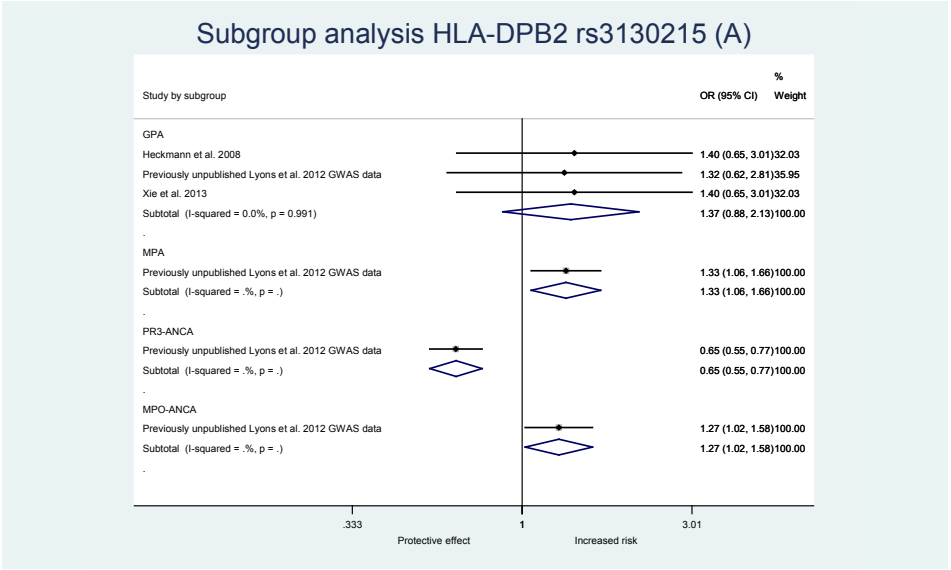
References: [27], [24], [28], [29]



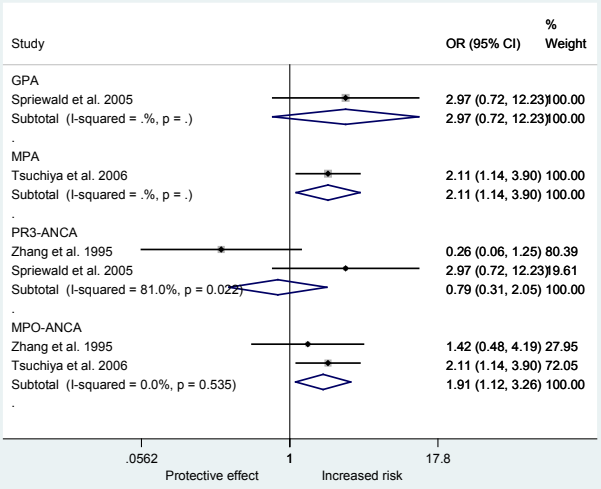
References: [26], [27], [24], [29], [30]**



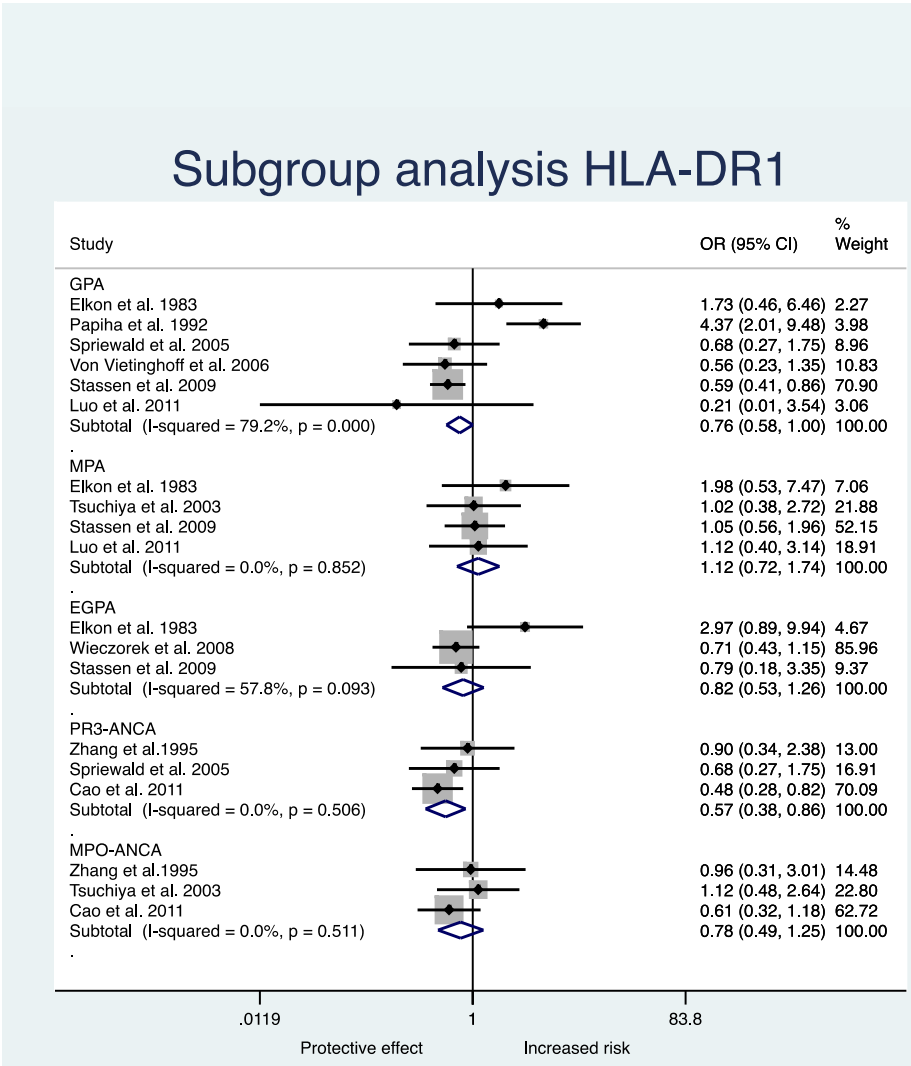
References: [26], [27], [24], [29], [30]**



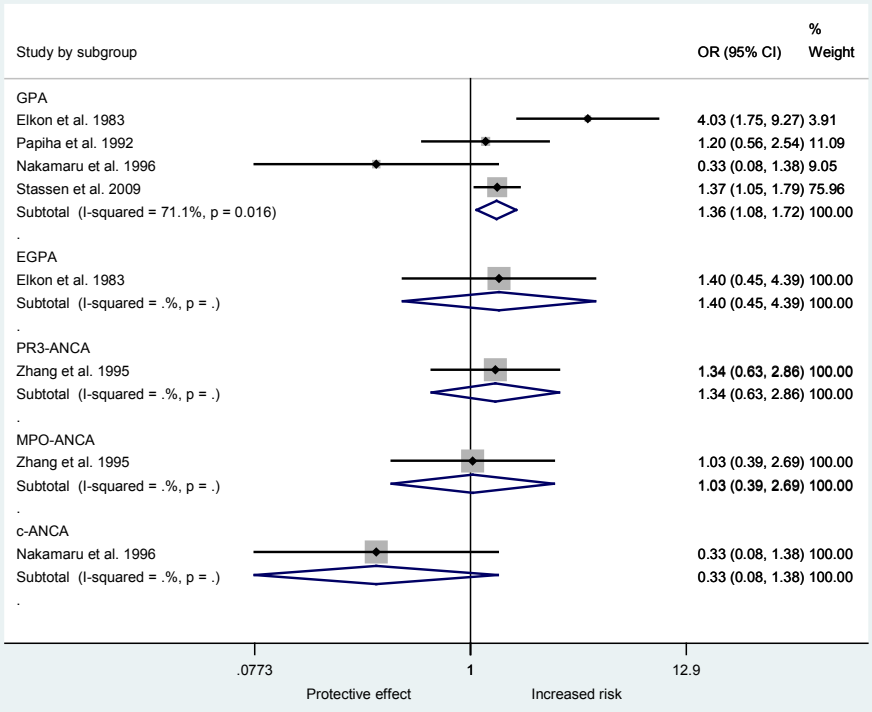
References: [24], [3], [25]



References: [26], [31], [28]

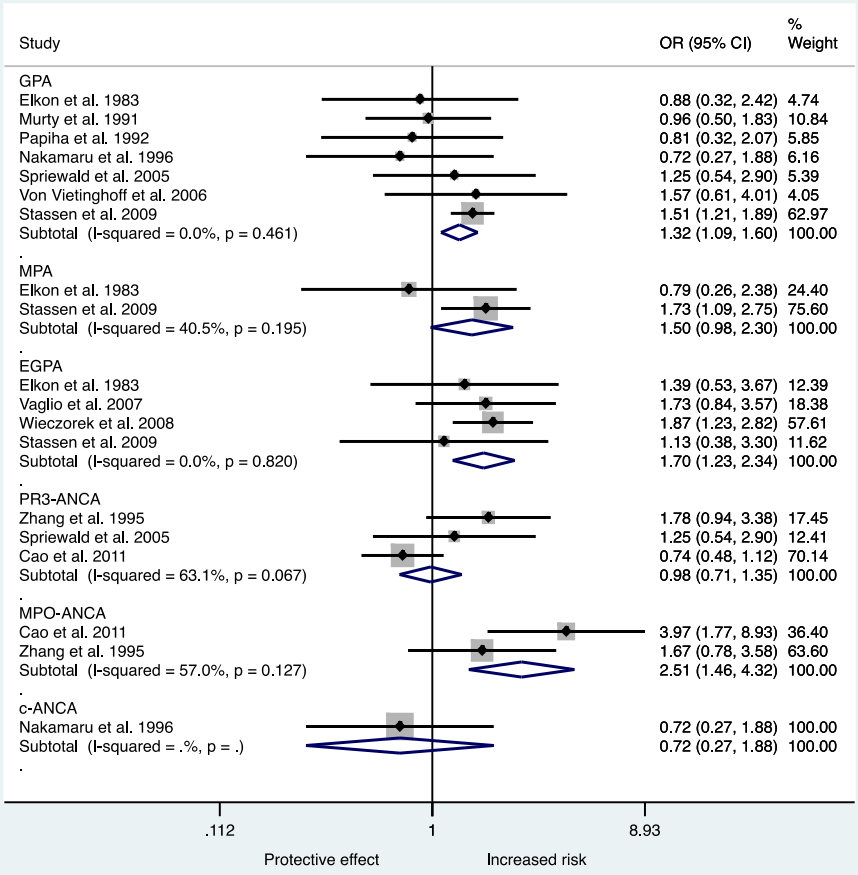


References: [23], [32], [31], [18], [19], [34], [5], [29], [26], [33]



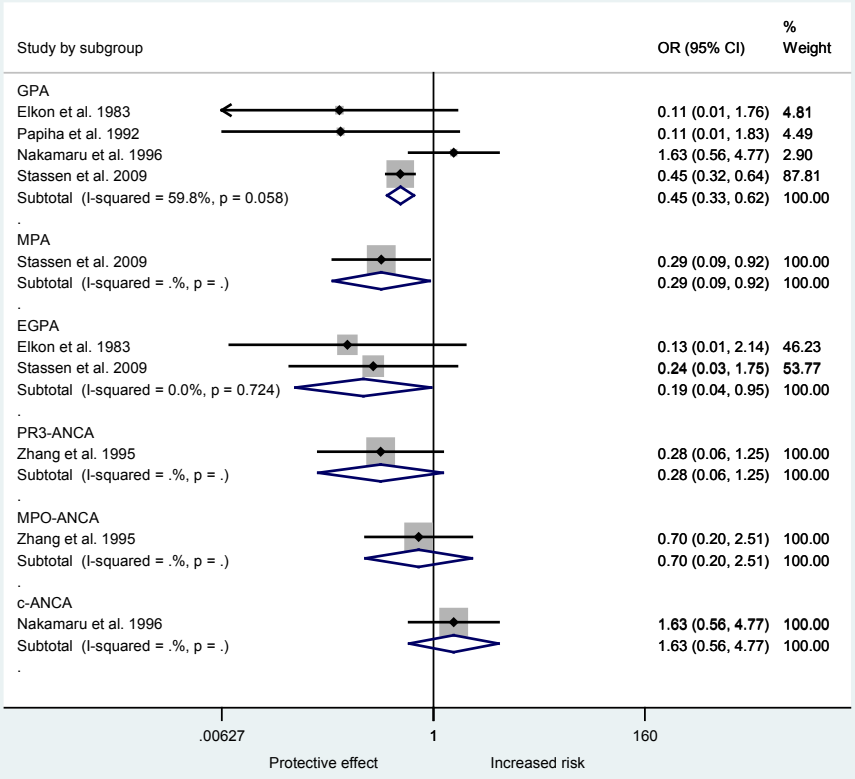
References: [23], [32], [26], [20], [19]

Subgroup analysis HLA-DR4



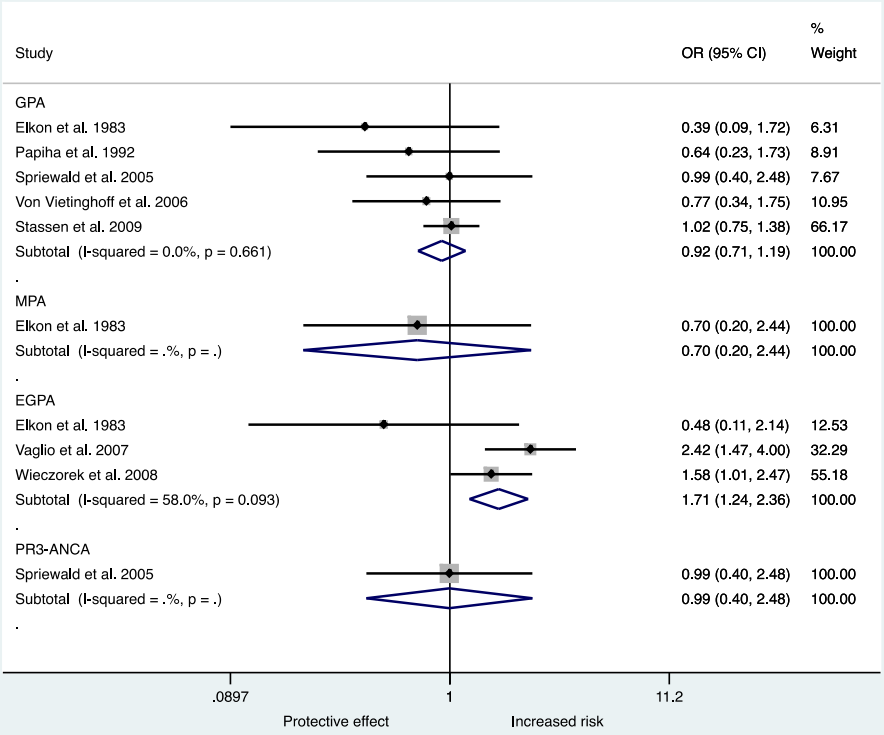
References: [23], [17], [32], [20], [31], [18], [19], [35], [29], [26], [33]

Subgroup analysis HLA-DR6



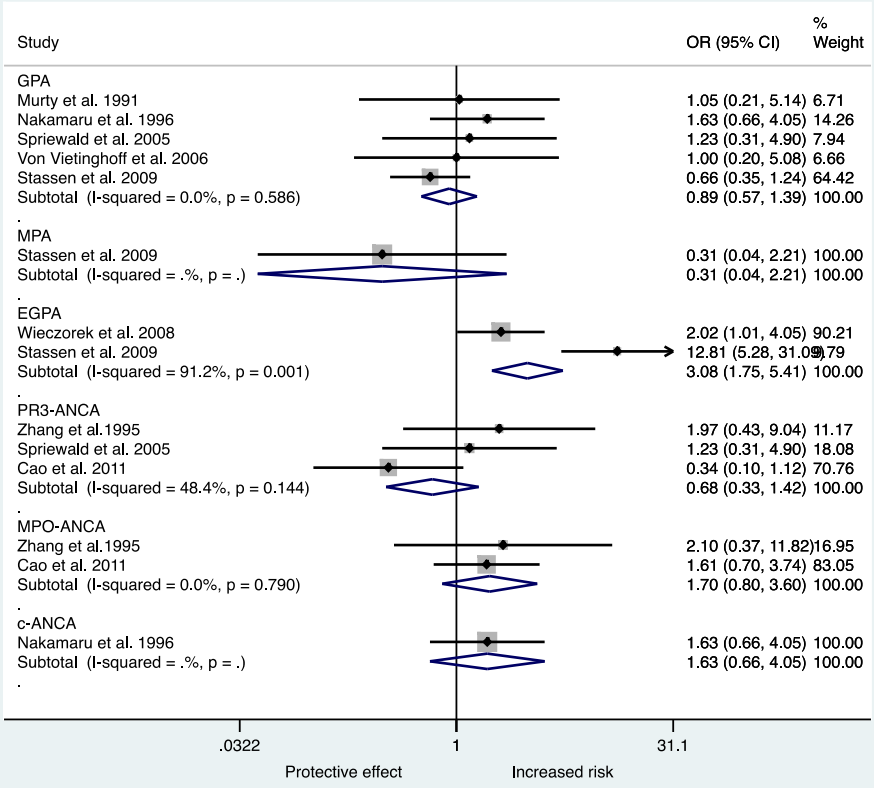
References: [23], [32], [26], [20], [19]

Subgroup analysis HLA-DR7

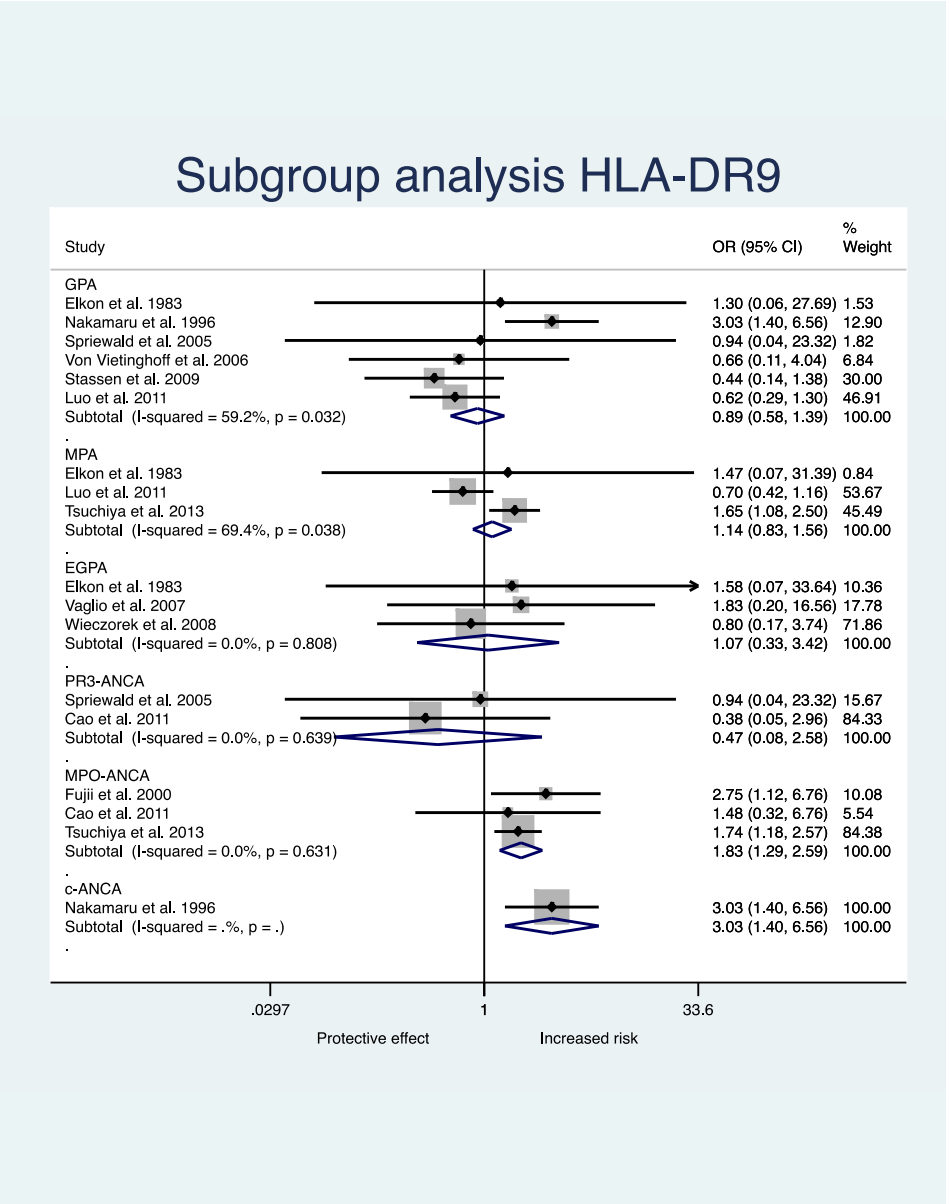


References: [23], [32], [31], [18], [19], [35], [29]

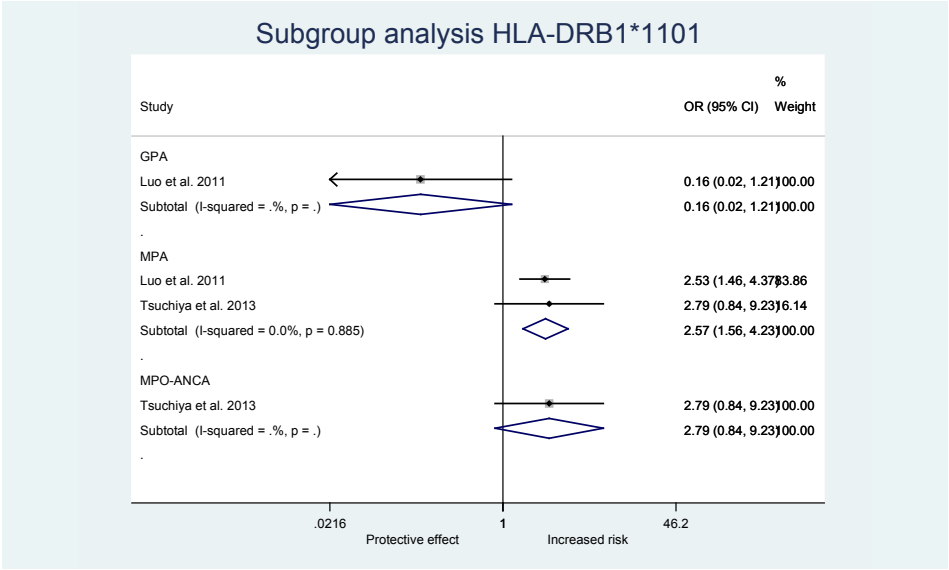
Subgroup analysis HLA-DR8



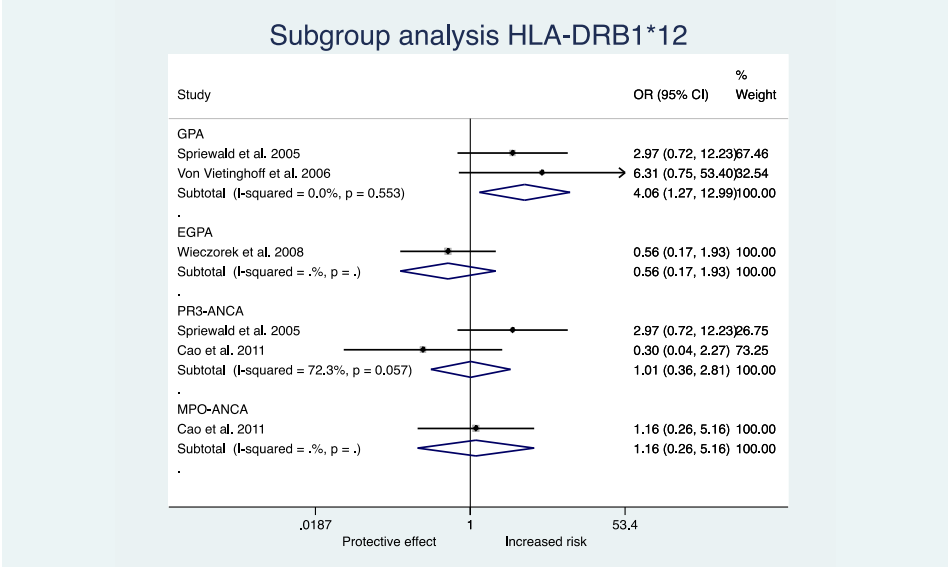
References: [17], [20], [31], [18], [19], [29], [26], [33]



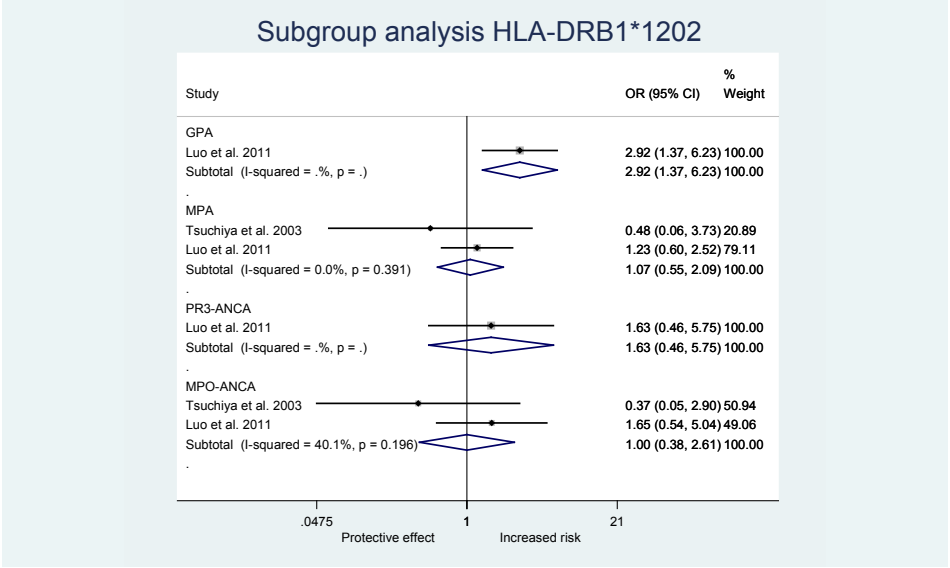
References: [23], [20], [31], [18], [19], [34], [37], [35], [29], [33], [36]



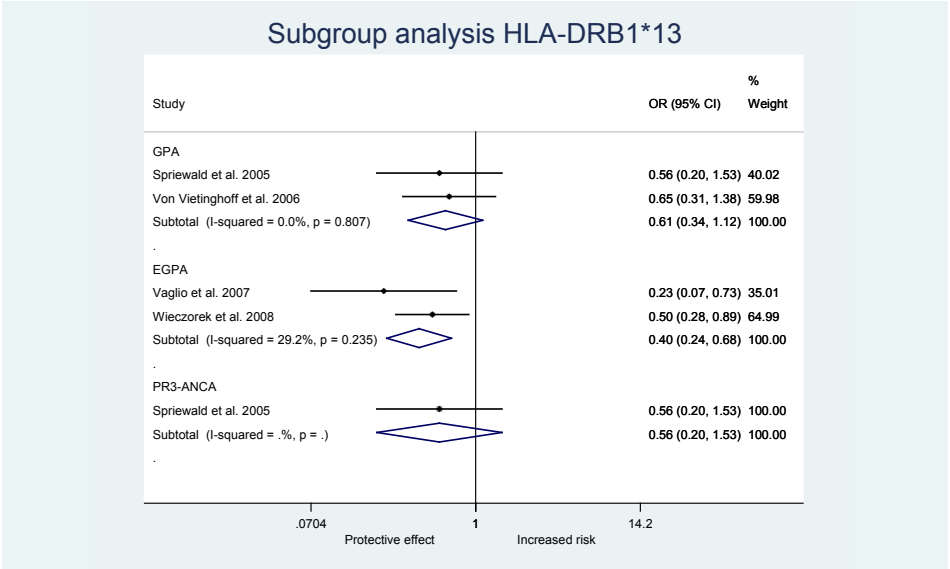
References: [34], [37]



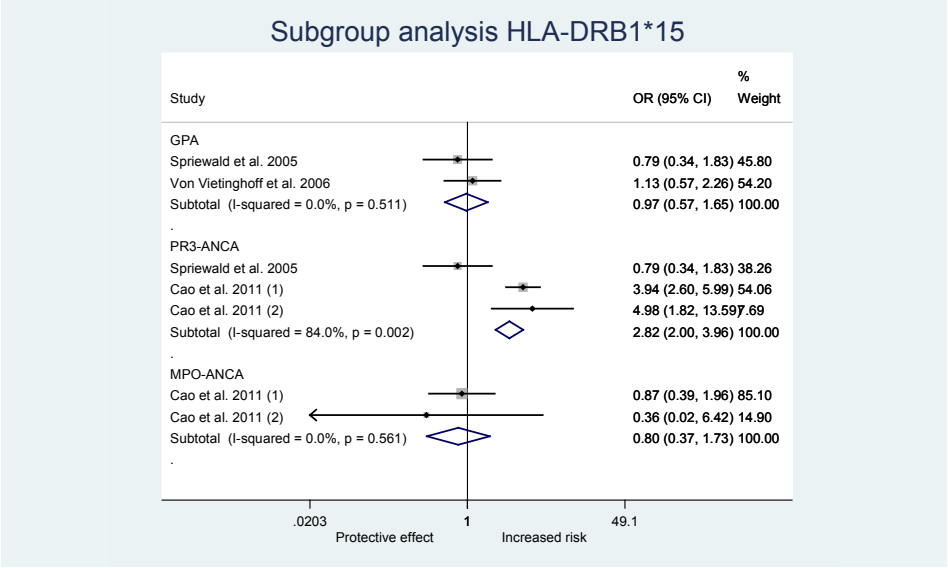
References: [31], [18], [29], [33]



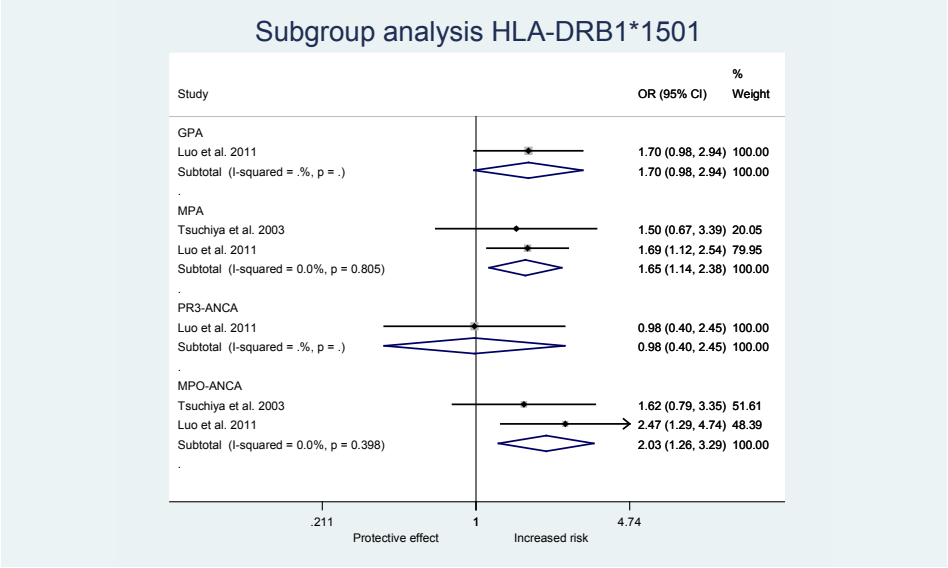
References: [5], [34]



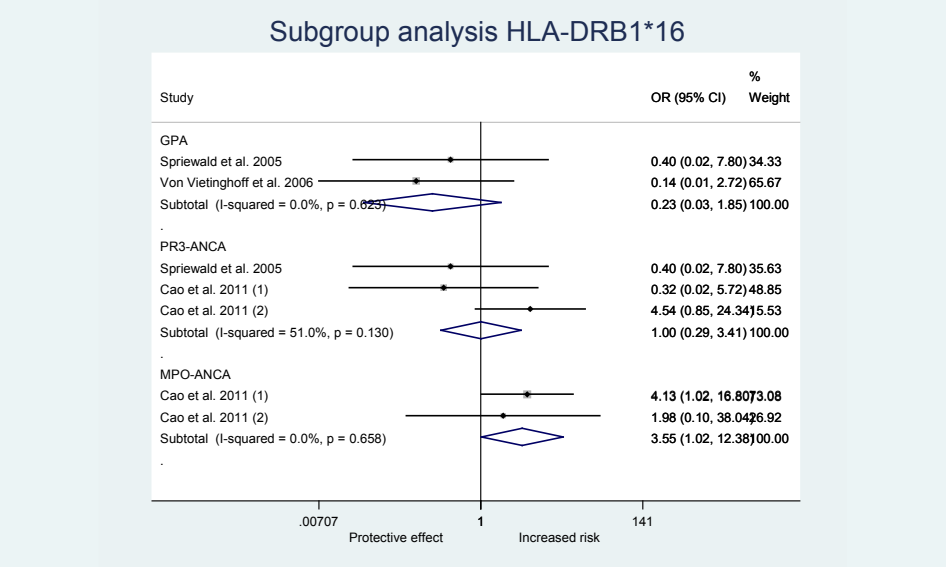
References: [31], [18], [35], [29]



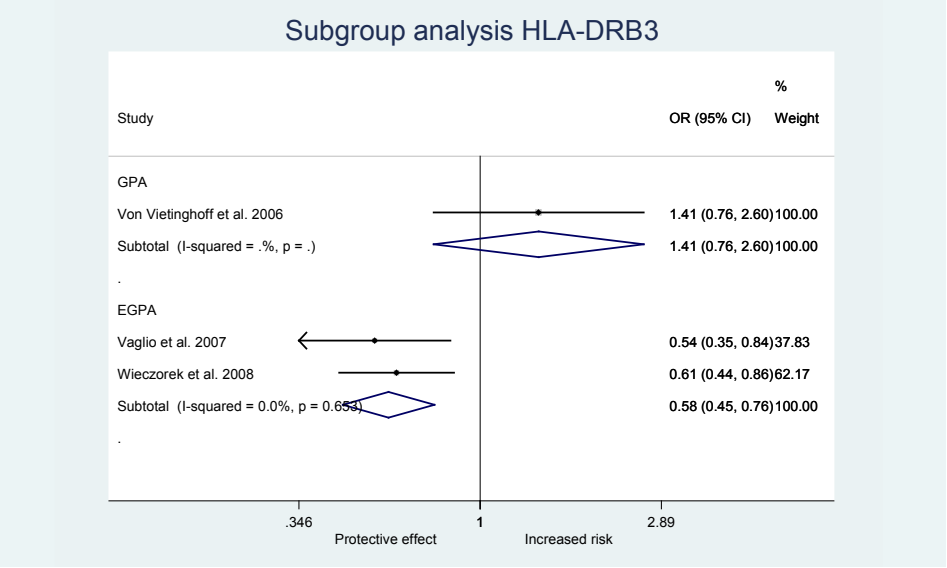
References: [31], [18], [33]**



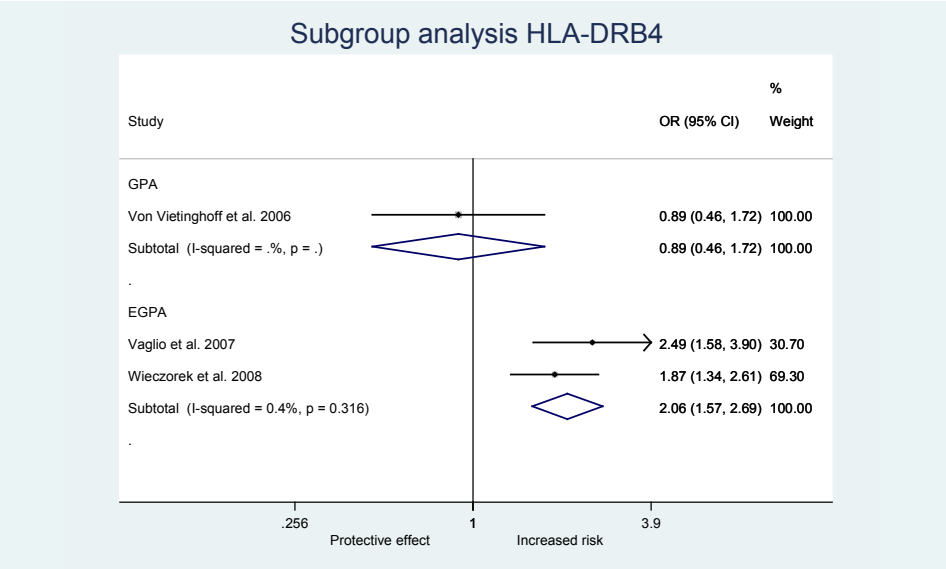
References: [5], [34]



References: [31], [18], [33]**

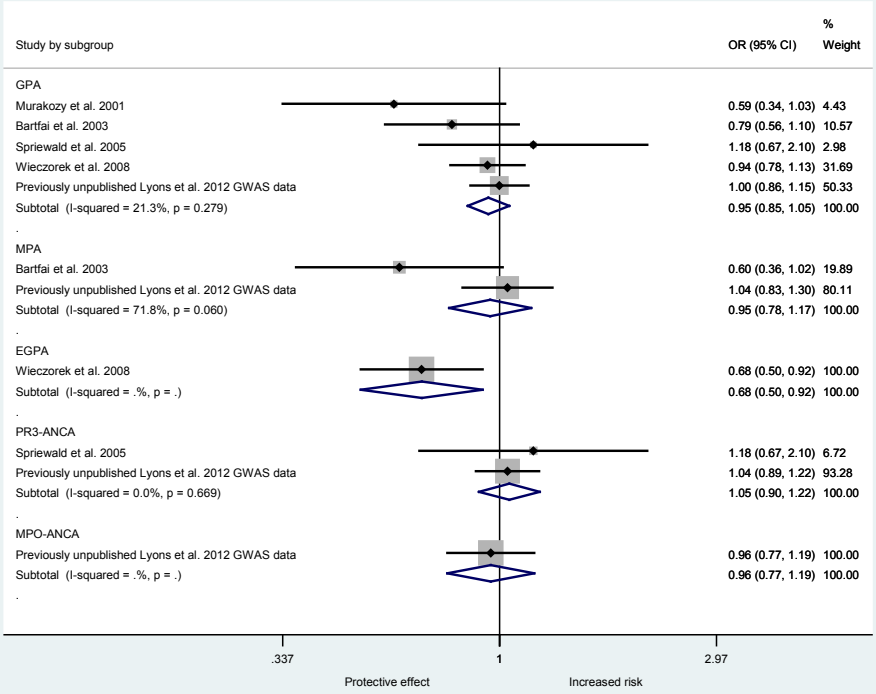


References: [18], [35], [29]

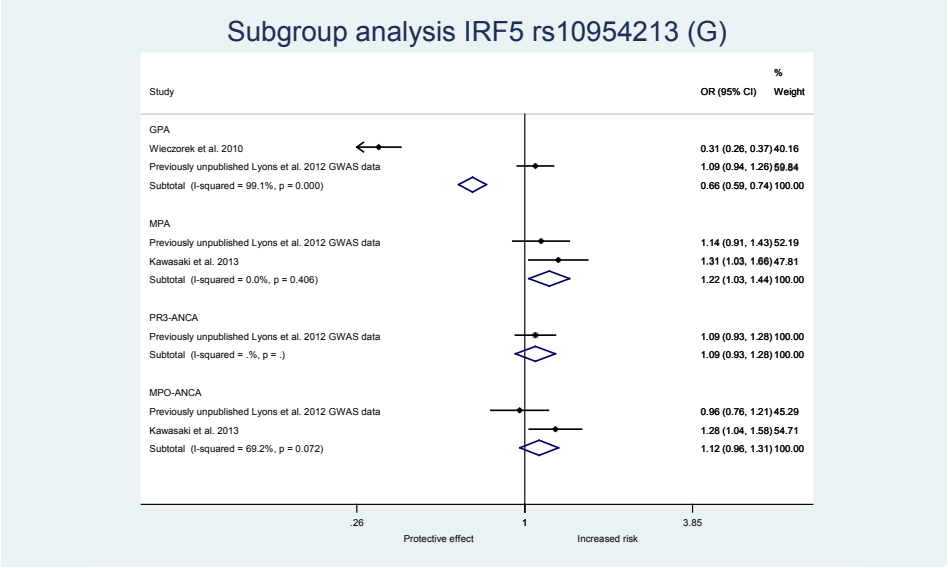


References: [18], [35], [29]

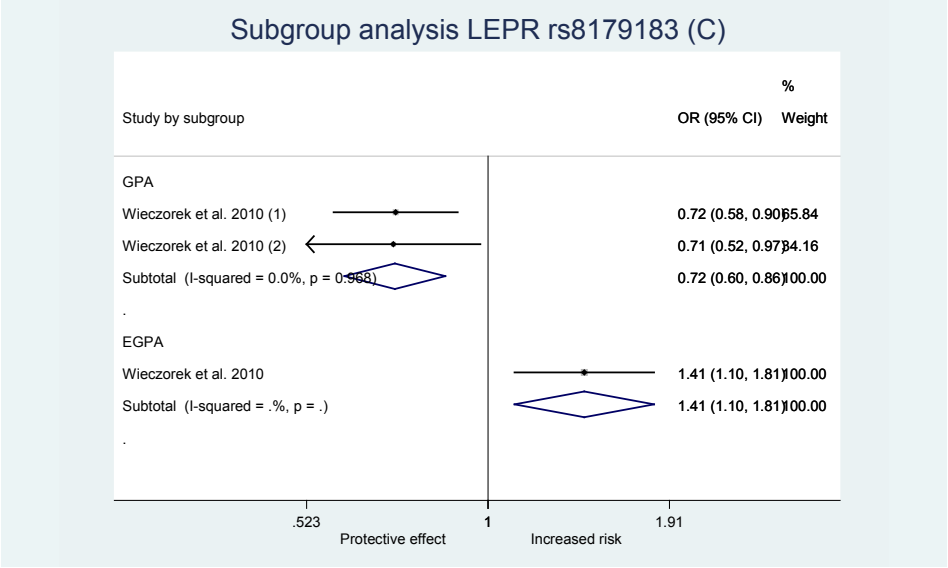
Subgroup analysis IL-10 rs1800896 (G)



References: [42], [43], [31], [41], [3]

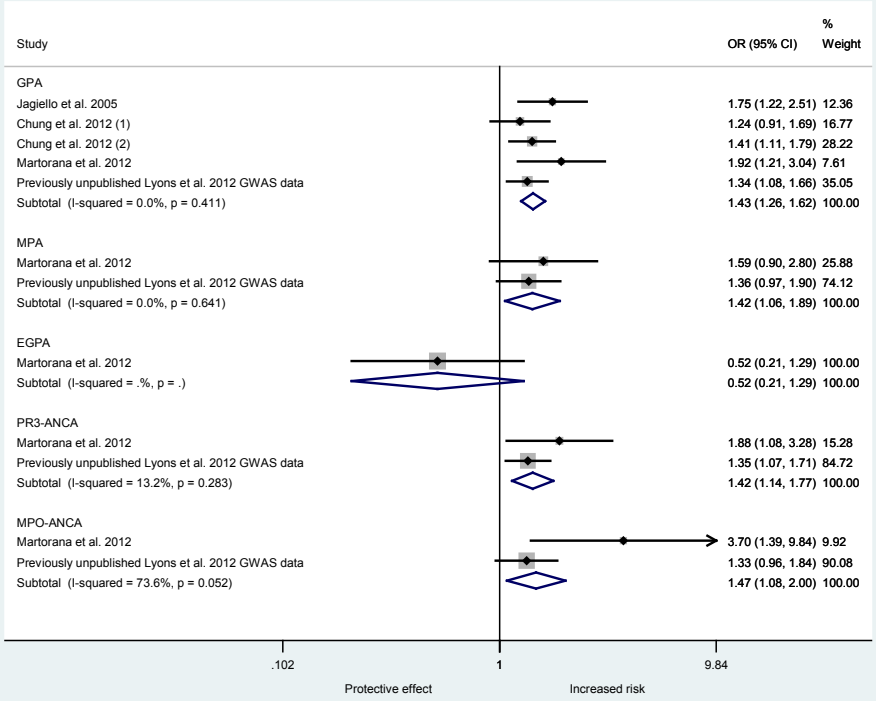


References: [44], [3], [45]



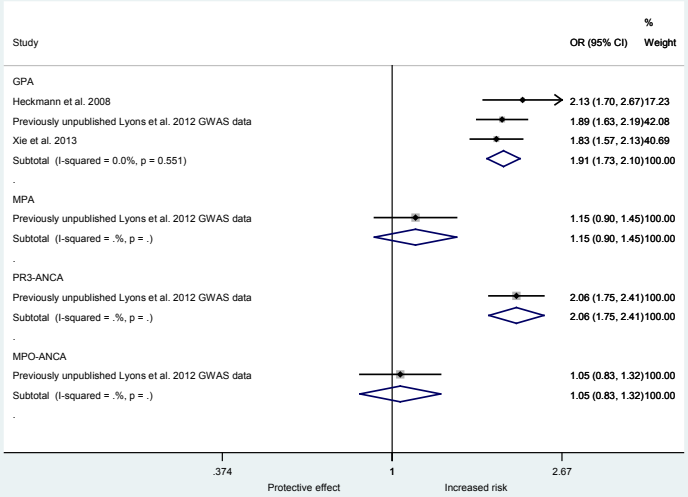
References: [15]**

Subgroup analysis PTPN22 rs2476601 (A)



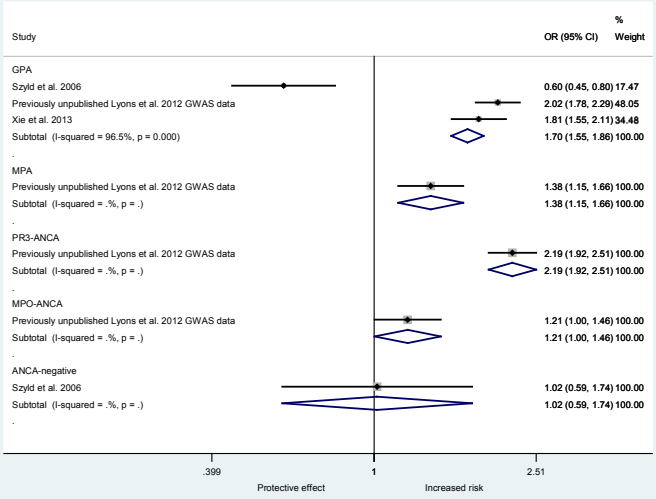
References: [50], [2]**, [51], [3]

Subgroup analysis RING1/RXRB rs213213 (A)

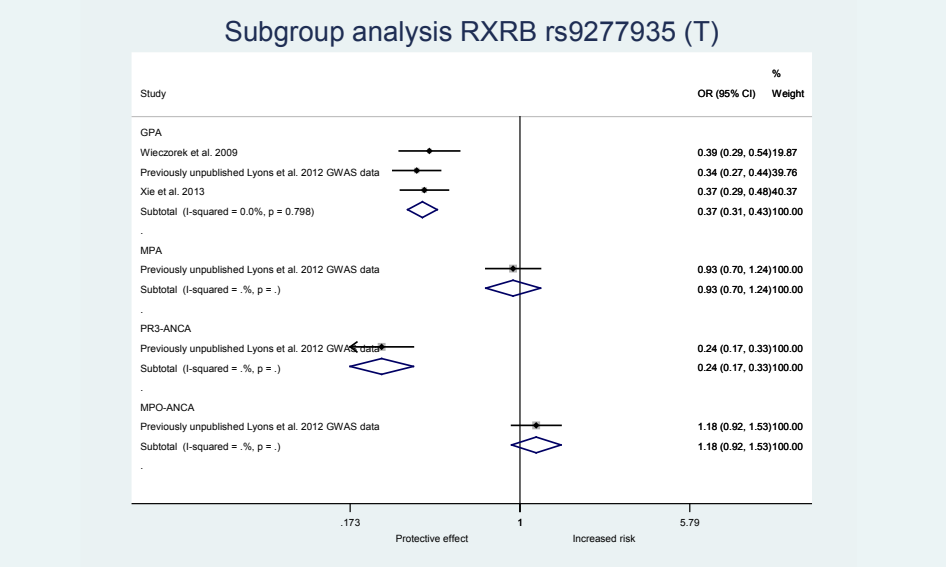


References: [24], [3], [25]

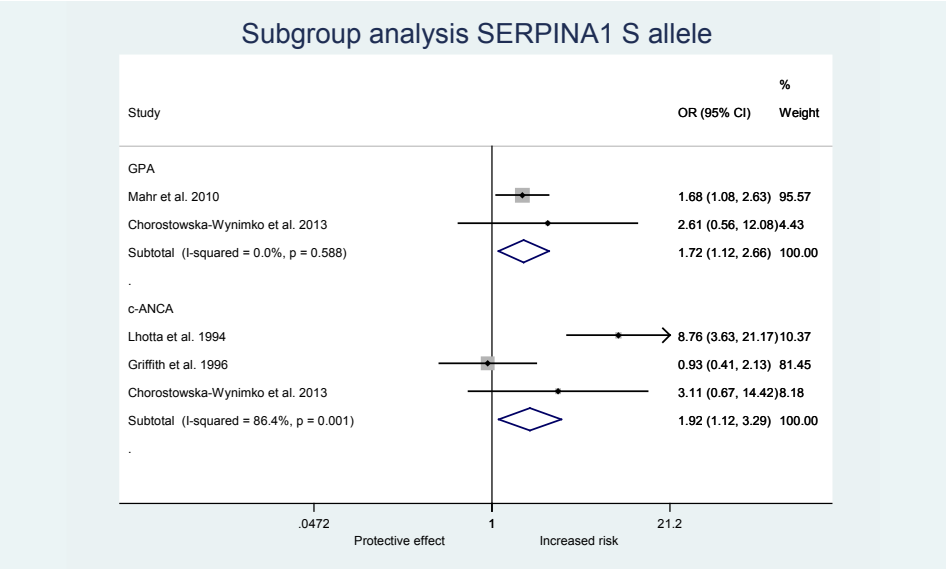
Subgroup analysis RXRB rs6531 (C)



References: [52], [3], [25]

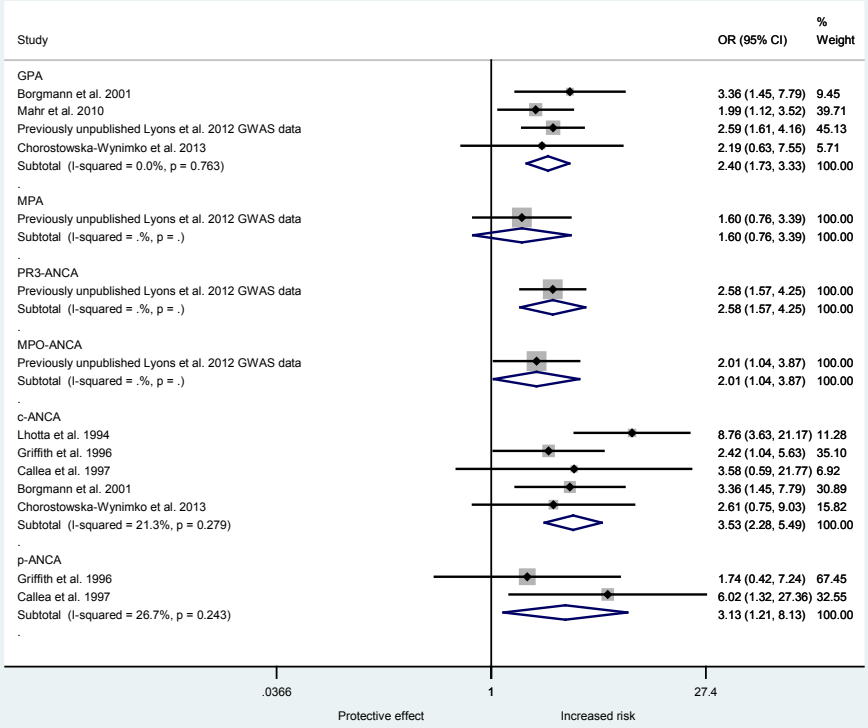


References: [53], [3], [25]

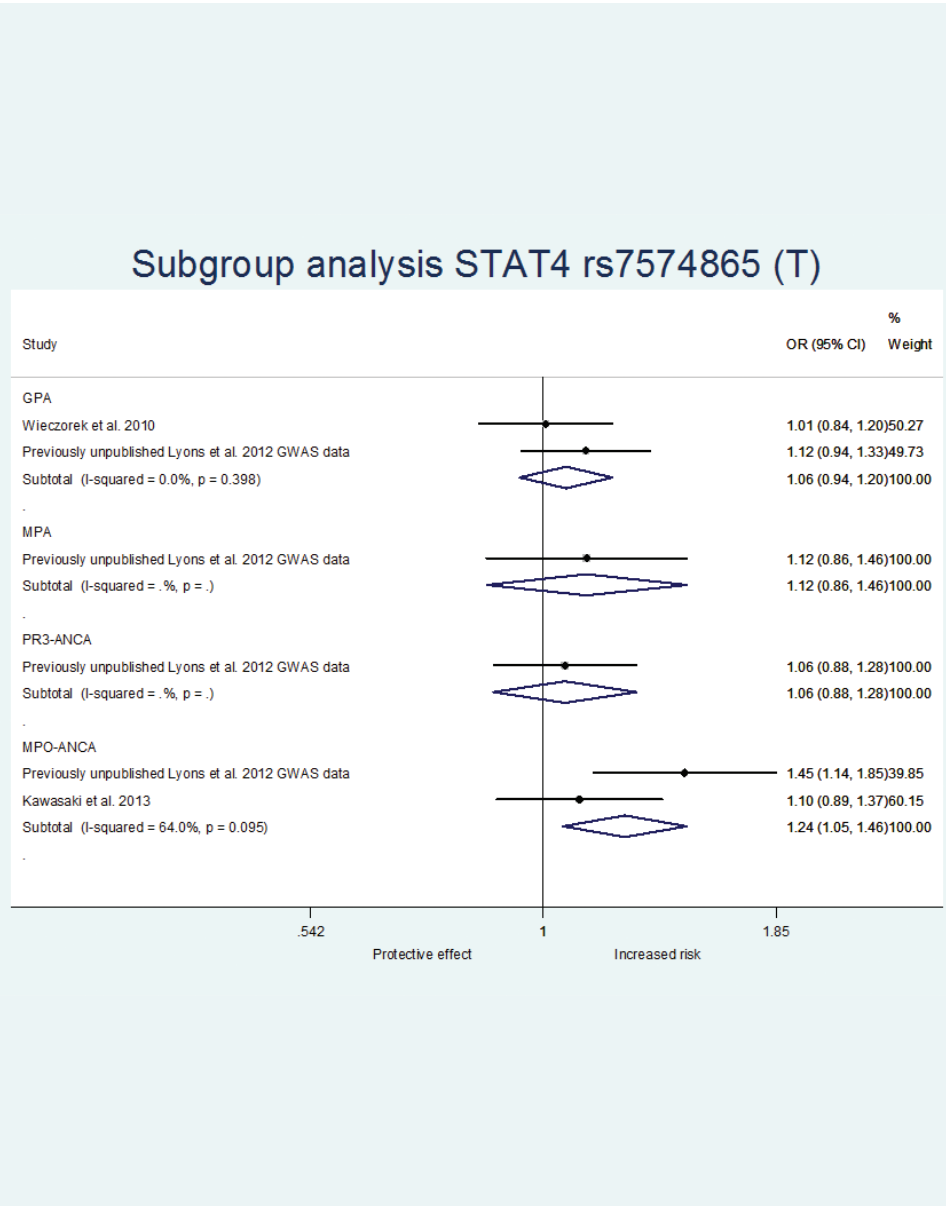


References: [54], [55], [56], [58]

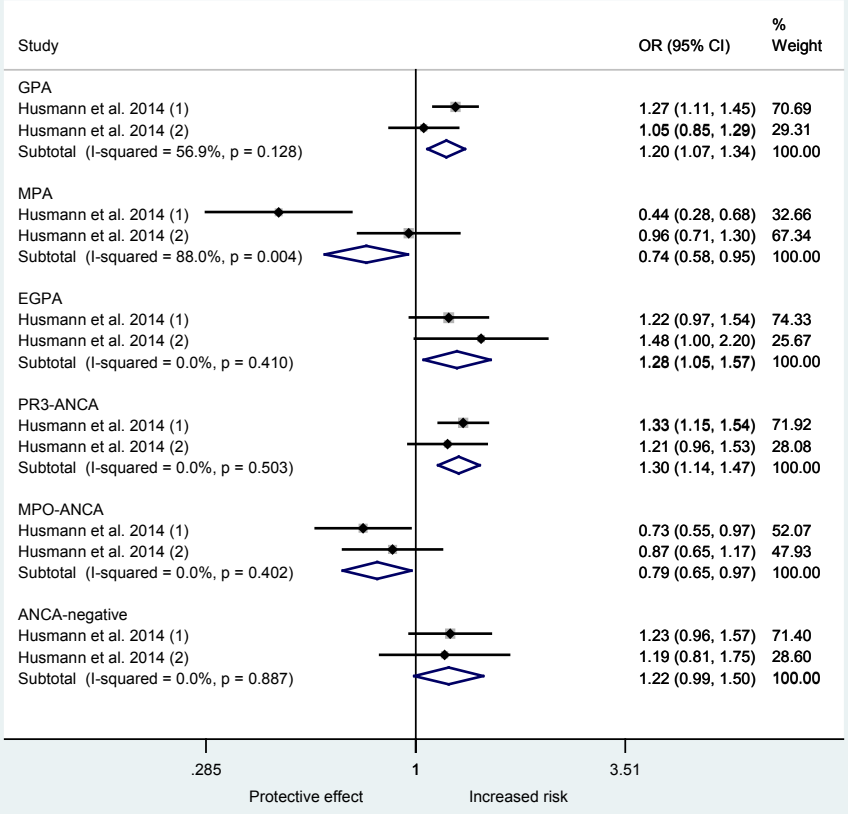
Subgroup analysis SERPINA1 Z allele



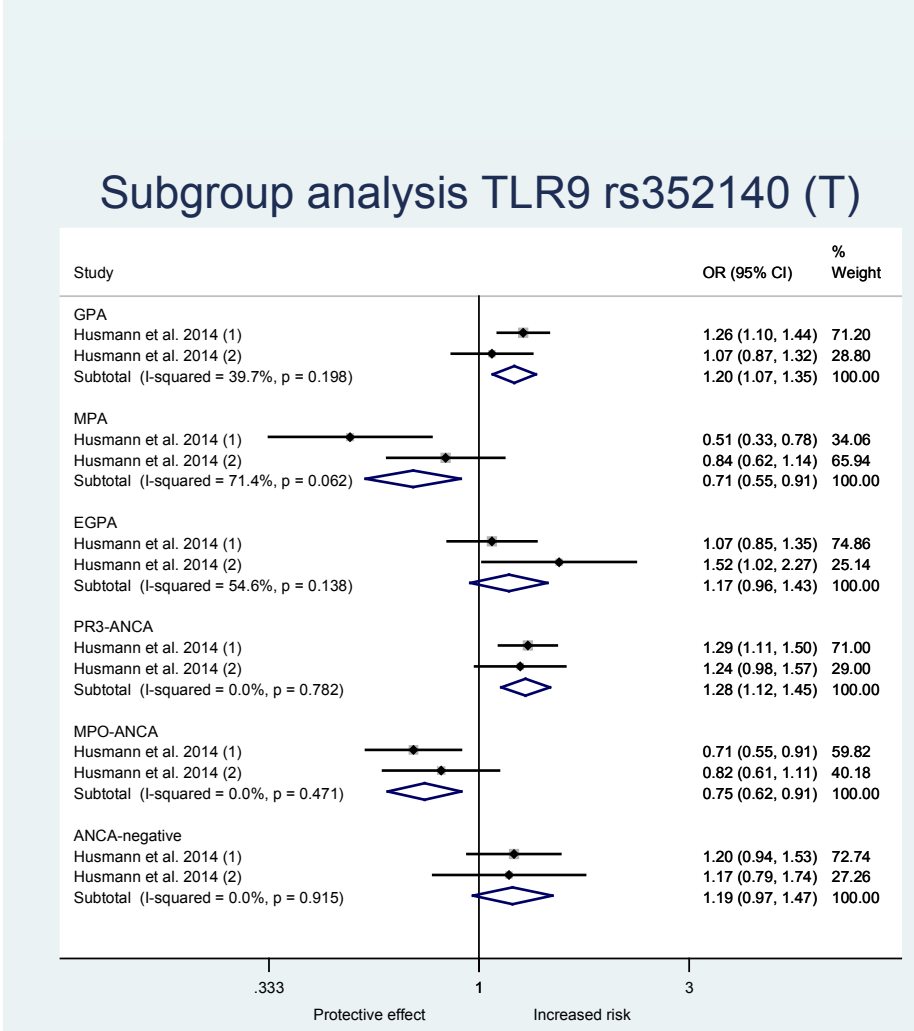
References: [54], [55], [59], [60], [56], [3], [58]



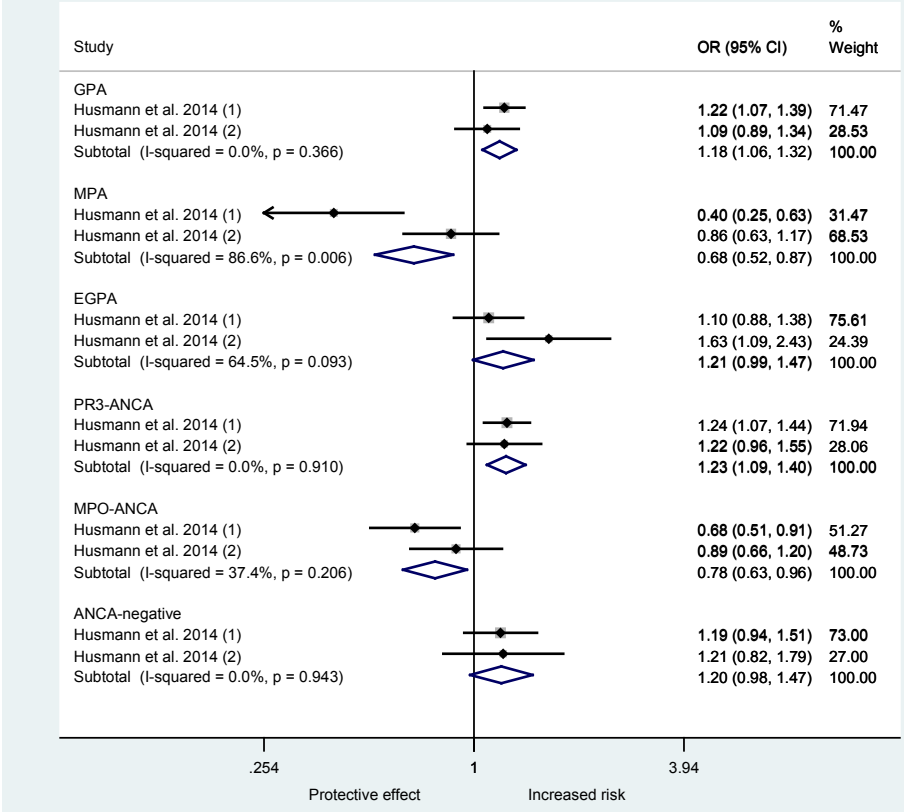
References: [44], [45]



References: [40]**



References: [40]**



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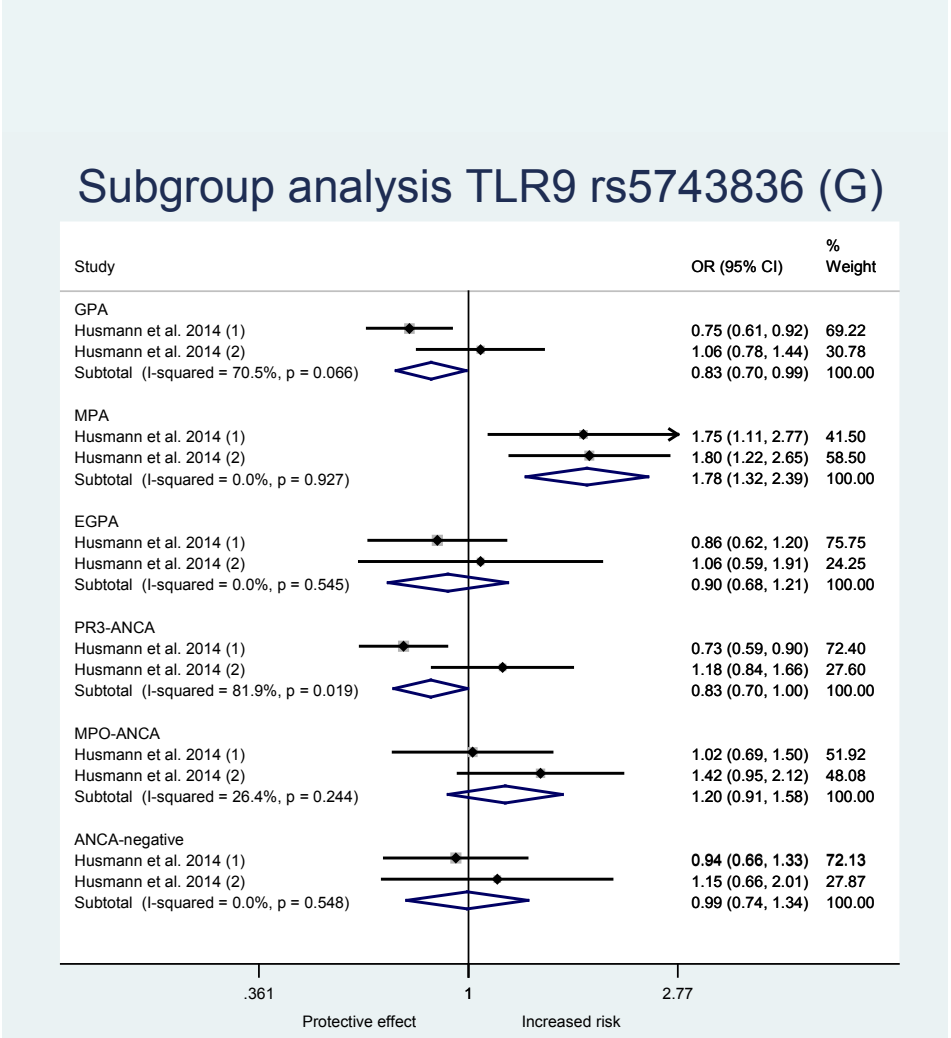
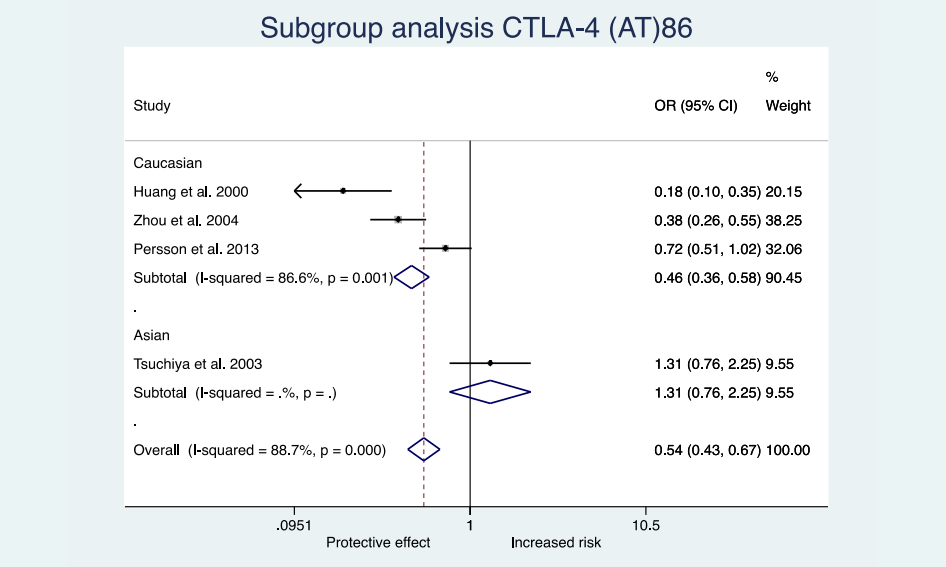


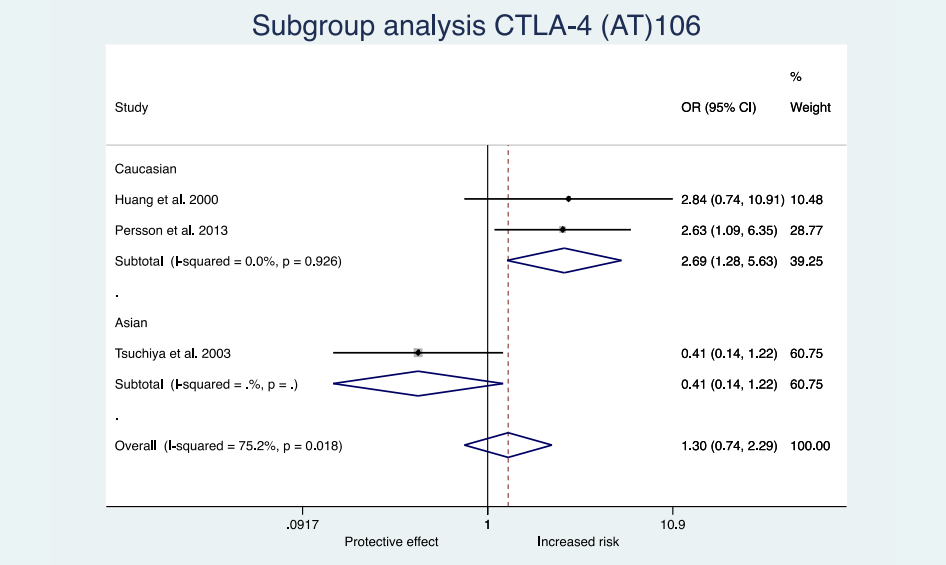
References: [40]**

**Two cohorts described in the same publication.
***Three cohorts described in the same publication.

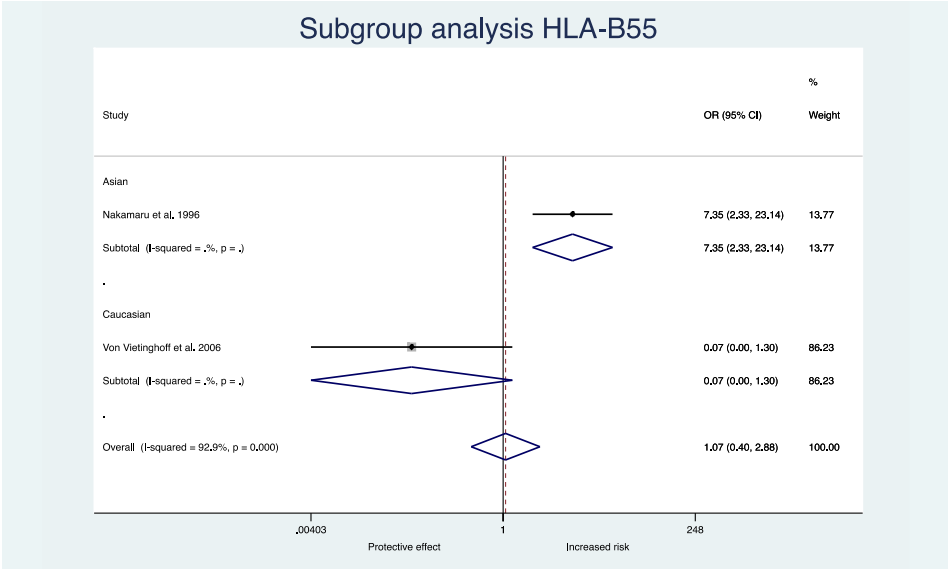
Supplementary figure 3. Forest plots by ethnic subgroups



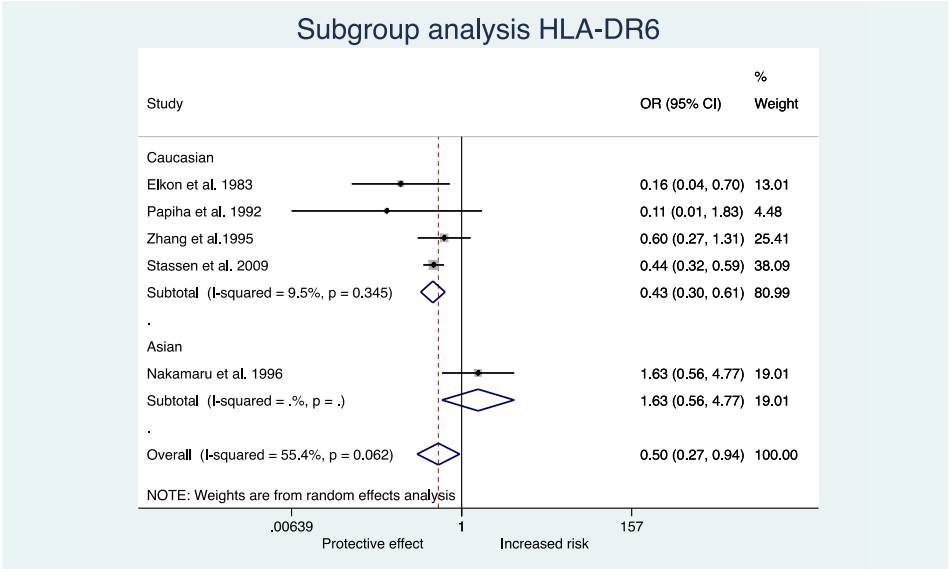
References: [4], [5], [6], [7]



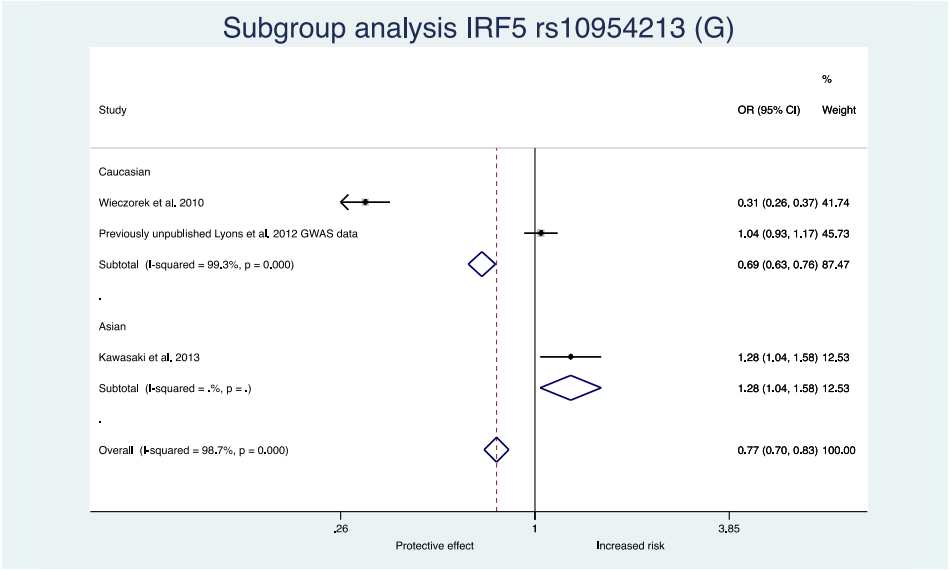
References: [4], [5], [7]



References: [20], [18]



References: [23], [32], [26], [20], [19]



References: [44], [3], [45]

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II. Supplementary data Chapter III

Renal function and ear, nose, throat involvement in ANCA associated vasculitis: prospective data from the European Vasculitis Society clinical trials

Supplementary table 1. ENT manifestations

ENT symptom	Patients
Nasal obstruction	76 (43)
Bloody nasal discharge	70 (40)
Nasal crusting	60 (34)
Sinus involvement	45 (25)
Hearing loss	46 (26)
Hoarseness/stridor	12 (7)
Otorhinolaryngologist's opinion	
Granulomatous sinusitis	28 (16)
Conductive hearing loss	20 (11)
Sensorineural hearing loss	9 (5)
Significant subglottic inflammation	4 (2)

All data are presented as n (%). ENT symptoms scored using the Birmingham Vasculitis Activity Score are shown. Data were available for 177 of the 185 patients with ENT involvement. Percentages are expressed relative to the number of patients with ENT involvement. All items below 'otorhinolaryngologist's opinion' were only scored by the otorhinolaryngologist. ENT, ear-, nose-, and throat**.**

Supplementary table 2. Associations between ENT symptoms and ANCA-subtype in patients with ENT involvement

ENT symptom	PR3-ANCA patients (n=116)	MPO-ANCA patients (n=46)	P Value
Nasal obstruction	57 (49)	14 (30)	0.03
Bloody nasal discharge	45 (39)	19 (41)	0.77
Nasal crusting	43 (37)	13 (28)	0.29
Sinus involvement	30 (26)	13 (28)	0.76
Hearing loss	34 (29)	7 (15)	0.06
Hoarseness/stridor	7 (6)	5 (11)	0.30
Otorhinolaryngologist's opinion			
Granulomatous sinusitis	25 (22)	2 (4)	0.008
Conductive hearing loss	16 (14)	3 (7)	0.20
Sensorineural hearing loss	5 (4)	2 (4)	0.99
Significant subglottic inflammation	2 (2)	2 (4)	0.33

Data are presented as n (%). The distribution of ANCA-subtype in patients with ENT involvement is shown. Data regarding the specific ENT symptoms were available for 177 of the 185 patients with ENT involvement. All 8 patients with missing ENT symptoms data were PR3-ANCA positive. All items listed under 'otorhinolaryngologist's opinion' were scored by the otorhinolaryngologist alone. ENT, ear-, nose-, and throat; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; MPO-ANCA, anti-myeloperoxidase anti-neutrophil cytoplasm antibody.

Supplementary table 3. Associations between ENT symptoms and baseline eGFR

ENT symptom	eGFR in symptom+ patients (mL/min/1.73 m^2)	eGFR in symptom- patients (mL/min/1.73 m^2)	95% CI	P Value
Nasal obstruction	43.09	26.59	9.91 – 23.10	<0.001
Bloody nasal discharge	37.81	28.05	2.82 – 16.71	0.006
Nasal crusting	43.25	27.36	8.60 – 23.19	<0.001
Sinus involvement	36.38	28.93	-0.95 – 15.84	0.08
Hearing loss	36.78	28.86	-0.40 – 16.23	0.06
Hoarseness/stridor	28.70	29.82	-16.72 – 14.47	0.89
Otorhinolaryngologist's opinion				
Granulomatous sinusitis	41.56	28.88	2.32 – 23.03	0.02
Conductive hearing loss	44.89	29.98	3.80 – 28.02	0.01
Sensorineural hearing loss	26.88	29.86	-20.91 – 14.96	0.74
Significant subglottic inflammation	45.53	29.63	-10.78 – 42.60	0.24

ENT symptoms were scored using the Birmingham Vasculitis Activity Score. Data regarding the specific ENT symptoms were available for 177 of the 185 patients with ENT involvement. All items listed under 'otorhinolaryngologist's opinion' were scored by the otorhinolaryngologist alone. ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate.

Supplementary table 4. Correlations of clinical and histological parameters with baseline and 5-year follow-up eGFR

	Baseline eGFR		5-year follow-up eGFR	
	r	p Value	r	P Value
ENT involvement	0,274	< 0.001	0,224	0.005
Age	-0,379	< 0.001	-0,395	< 0.001
PR3-ANCA	0,240	< 0.001	0,097	<0.24
Tubulitis	-0,445	< 0.001	-0,266	0.12
Interstitial infiltrate	-0,477	< 0.001	-0,396	< 0.001
IFTA	-0,436	< 0.001	-0,477	< 0.001
AAGN classification	-0,424	< 0.001	-0,511	< 0.001

ENT, ear-, nose-, and throat; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis; eGFR, estimated glomerular filtration rate.

Supplementary table 5. Three multivariable regression models investigating the association between ENT involvement and baseline eGFR

Model 1	**β (95% CI)**	**P Value**
ENT involvement	10.40 (5.24 – 15.55)	< 0.001
Age	-0.65 (-0.82 – -0.47)	< 0.001
PR3-ANCA	6.21 (1.03 – 11.38)	0.02

Model 2	**β (95% CI)**	**P Value**
ENT involvement	10.32 (4.05 – 16.59)	0.001
Age	-0.46 (-0.68 – -0.24)	< 0.001
PR3-ANCA	-1.43 (-7.73 – 4.88)	0.66
Tubulitis	-13.93 (-20.87 – -6.99)	< 0.001
Interstitial infiltrate	-8.56 (-12.64 – -4.47)	< 0.001
IFTA	-10.54 (-15.61 – -5.47)	< 0.001

Model 3	**β (95% CI)**	**P Value**
ENT involvement	9.14 (2.30 – 15.98)	0.009
Age	-0.60 (-0.83 – -0.36)	< 0.001
PR3-ANCA	-1.26 (-8.28 – 5.75)	0.71
Tubulitis	-17.82 (-25.78 – -9.86)	< 0.001
Interstitial infiltrate	-6.86 (-11.20 – -2.52)	0.002
IFTA	-8.72 (-14.67 – -2.77)	0.004
AAGN classification	-6.20 (-9.84 – -2.57)	0.001

Since renal biopsies were not available for all patients, including the histopathological parameters limited the number of patients included in the analysis. To be able to include all patients we therefore created three models. The first model included only clinical parameters (n=412). In the second model, we added tubulointerstitial parameters (n=195). The third model, we added the histopathological classification (n=149). In all models, age is included per year unit. ENT involvement was significantly associated with higher baseline eGFR. 95% CI, 95% confidence interval; ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis.

Supplementary table 6. Multivariable regression analyses investigating the relationship between ENT involvement and eGFR in GPA patients

	β (95% CI)	**P Value**
ENT involvement	12.44 (0.08 – 24.80)	0.04
Age	-1.26 (-8.28 – 5.75)	0.02
PR3-ANCA	-5.17 (-17.02 – 6.68)	0.39
Tubulitis	-9.47 (-23.10 – 4.15)	0.17
Interstitial infiltrate	-16.29 (-24.93 – -7.64)	< 0.001
IFTA	-9.35 (-18.46 – -0.24)	0.01
AAGN classification	-9.79 (-15.58 – -4.00)	0.001

ENT involvement is associated with higher baseline eGFR in GPA patients. Age is included per year unit in the model. 95% CI, 95% confidence interval; ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis.

Supplementary table 7. Multivariable regression analyses investigating the relationship between ENT involvement and 5-year follow-up eGFR

	Model investigating 5-year follow-up eGFR		**Model investigating 5-year follow-up eGFR in PR3-ANCA positive patients**	
	β (95% CI)	P Value	β (95% CI)	P Value
ENT involvement	2.12 (-4.90 – 9.14)	0.55	8.10 (-1.73 – 17.93)	0.10
Age	-0.27 (-0.52 – -0.03)	0.03	-0.03 (-0.37 – 0.30)	0.84
PR3-ANCA	-6.28 (-13.33 – 0.77)	0.08	N/A	N/A
Baseline eGFR	0.33 (0.17 – 0.48)	< 0.001	0.43 (0.24 – 0.61)	< 0.001
Tubulitis	4.25 (-4.43 – 12.92)	0.33	-1.92 (-13.49 – 9.64)	0.74
Interstitial infiltrate	-2.42 (-8.13 – 3.29)	0.40	-0.86 (-9.05 – 7.32)	0.83
IFTA	-1.65 (-8.23 – 4.92)	0.62	-2.73 (-11.14 – 5.69)	0.52
AAGN classification	-5.44 (-9.71 – -1.17)	0.01	N/A	N/A

ENT involvement is no longer associated with 5-year follow-up eGFR when baseline eGFR is included in the model. Both models are adjusted for within-trial therapy. Age is included per year unit in both models. 95% CI, 95% confidence interval; ENT, ear-, nose-, and throat; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis; N/A, not applicable.

Supplementary table 8. Three multivariable regression models investigating the association between baseline eGFR and other early disease manifestations

	Cutaneous model		Arthralgia/arthritis model		Lung model	
Model 1	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**
Cutaneous involvement	5.61 (-0.27 – 11.49)	0.06	N/A	N/A	N/A	N/A
Arthralgia/arthritis	N/A	N/A	3.40 (-1.67 – 8.48)	0.19	N/A	N/A
Lung involvement	N/A	N/A	N/A	N/A	-2.63 (-7.56 – 2.30)	0.30
Age	-0.66 (-0.84 – -0.48)	< 0.001	-0.63 (-0.81 – -0.45)	< 0.001	-0.69 (-0.87 – -0.51)	< 0.001
PR3-ANCA	8.78 (3.74 – 13.82)	0.001	8.73 (3.65 – 13.80)	0.001	9.58 (4.56 – 14.60)	< 0.001
Model 2	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**
Cutaneous involvement	-0.76 (-8.47 – 6.95)	0.85	N/A	N/A	N/A	N/A
Arthralgia/arthritis	N/A	N/A	-1.74 (-7.94 – 4.46)	0.58	N/A	N/A
Lung involvement	N/A	N/A	N/A	N/A	-5.46 (-11.46 – 0.53)	0.07
Age	-0.48 (-0.71 – -0.25)	< 0.001	-0.40 (-0.63 – -0.16)	0.001	-0.48 (-0.71 – -0.25)	< 0.001
PR3-ANCA	1.45 (-4.81 – 7.70)	0.65	0.67 (-5.65 – 6.99)	0.83	2.57 (-3.74 – 8.89)	0.42
Tubulitis	-13.12 (-20.27 – -5.97)	< 0.001	-11.02 (-18.30 – -3.74)	0.003	-13.20 (-20.25 – -6.14)	< 0.001
Interstitial infiltrate	-8.86 (-13.06 – -4.66)	< 0.001	-9.21 (-13.46 – -4.95)	< 0.001	-8.75 (-12.91 – -4.59)	< 0.001
IFTA	-11.97 (-17.11 – -6.84)	< 0.001	-11.89 (-17.16 – -6.62)	< 0.001	-12.07 (-17.16 – -6.98)	< 0.001
Model 3	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**
Cutaneous involvement	-2.98 (-11.58 – 5.62)	0.49	N/A	N/A	N/A	N/A
Arthralgia/arthritis	N/A	N/A	-4.85 (-11.40 – 1.71)	0.15	N/A	N/A
Lung involvement	N/A	N/A	N/A	N/A	-4.74 (-11.19 – 1.70)	0.15
Age	-0.63 (-0.87 – -0.38)	<0.001	-0.54 (-0.78 – -0.29)	<0.001	-0.61 (-0.85 – -0.37)	<0.001
PR3-ANCA	1.29 (-5.62 – 8.21)	0.71	0.41 (-6.52 – 7.33)	0.91	1.86 (-5.06 – 8.78)	0.60

Supplementary table 8. Three multivariable regression models investigating the association between baseline eGFR and other early disease manifestations (Continued)

	Cutaneous model		Arthralgia/arthritis model		Lung model	
Model 3	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**
Tubulitis	-16.54 (-24.62 – -8.47)	<0.001	-14.71 (-22.91 – -6.50)	0.01	-16.83 (-24.87 – -8.79)	<0.001
Interstitial infiltrate	-7.48 (-11.96 – -3.00)	0.001	-7.38 (-11.83 – -2.93)	0.001	-7.12 (-11.53 – -2.71)	0.002
IFTA	-9.57 (-15.62 – -3.51)	0.002	-9.92 (-16.01 – -3.83)	0.002	-9.97 (-15.97 – -3.97)	0.001
AAGN classification	-6.82 (-10.61 – -3.03)	0.001	-6.98 (-10.79 – -3.17)	<0.001	-6.79 (-10.49 – -3.09)	<0.001

Since renal biopsies were not available for all patients, including the histopathological parameters limited the number of patients included in the analysis. To be able to include all patients we therefore created three models. The first model included only clinical parameters (n=412). In the second model, we added tubulointerstitial parameters (n=195). The third model, we added the histopathological classification (n=149). In all models, age is included per year unit. 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis.

Supplementary table 9. Multivariable regression analyses investigating the relationships between 5-year follow-up eGFR and other early disease manifestations

	Cutaneous model		Arthralgia/arthritis model		Lung model	
	β (95% CI)	P Value	β (95% CI)	P Value	β (95% CI)	P Value
Cutaneous involvement	0.78 (-8.80 – 10.35)	0.87	N/A	N/A	N/A	N/A
Arthralgia/arthritis	N/A	N/A	0.00 (-8.62 – 8.62)	0.99	N/A	N/A
Lung involvement	N/A	N/A	N/A	N/A	6.99 (-1.19 – 15.17)	0.09
Age	-0.37 (-0.67 – -0.07)	0.02	-0.32 (-0.63 – -0.01)	0.04	-0.32 (-0.61 – -0.03)	0.03
PR3-ANCA	-4.19 (-12.48 – 4.09)	0.32	-5.39 (-13.67 – 2.89)	0.20	-5.66 (-13.97 – 2.65)	0.18
Tubulitis	-3.68 (-12.87 – 5.50)	0.43	-0.96 (-10.45 – 8.53)	0.84	-2.70 (-11.76 – 6.36)	0.56
Interstitial infiltrate	-4.84 (-11.66 – 1.99)	0.16	-6.89 (-13.77 – -0.02)	0.05	-4.74 (-11.42 – 1.94)	0.16
IFTA	-10.07 (-16.84 – -3.29)	0.004	-10.74 (-17.91 – -3.57)	0.004	-10.12 (-16.75 – -3.48)	0.003

To investigate whether our associations are specific to ENT involvement, analyses between eGFR at 5-year follow-up and other early disease manifestations were performed. There are no associations between eGFR at 5-year follow-up and cutaneous involvement, arthralgia/arthritis, or lung involvement. All models are adjusted for within-trial therapy. In all models, age is included per year unit. 95% CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; PR3-ANCA, anti-proteinase 3 anti-neutrophil cytoplasm antibody; IFTA, interstitial fibrosis and tubular atrophy; AAGN, anti-neutrophil cytoplasm antibody associated glomerulonephritis; N/A, not applicable

III. Supplementary data Chapter VI

Effect of rituximab on malignancy risk in patients with ANCA-associated vasculitis

Supplementary table 1. Subgroup analysis

	N patients	N observed malignancies	SIR (95% CI)*	SIR p value*	RR (95% CI)*	RR p value*
Gender						
Male	149	20	1.58 (0.96–2.44)	0.07	1 (reference)	
Female	174	25	2.25 (1.45–3.32)	<0.001	1.43 (0.76–2.71)	0.30
Age at diagnosis						
≥59 years	159	29	1.60 (1.07–2.29)	0.02	1 (reference)	
<59 years	164	16	2.84 (1.62–4.61)	<0.001	1.78 (0.90–3.38)	0.10
Clinical diagnosis						
Microscopic polyangiitis	160	23	1.59 (1.01–2.38)	0.05	1 (reference)	
Granulomatosis with polyangiitis	109	14	2.20 (1.20–3.68)	0.01	1.39 (0.66–2.81)	0.43
Eosinophilic granulomatosis with polyangiitis	54	8	2.75 (1.19–5.41)	0.02	1.73 (0.67–4.02)	0.27
ANCA serotype†						
MPO-ANCA	110	15	1.56 (0.87–2.58)	0.13	1 (reference)	
PR3-ANCA	152	24	2.18 (1.40–3.25)	<0.001	1.40 (0.70–2.87)	0.39
Renal transplantation						
No	311	41	1.79 (1.29–2.43)	<0.001	1 (reference)	
Yes	12	4	4.31 (1.17–11.04)	0.03	2.40 (0.62–6.62)	0.20
Follow-up						
0–5 years	156	16	2.38 (1.36–3.86)	<0.001	1 (reference)	
5–10 years	135	23	1.81 (1.15–2.72)	0.01	0.76 (0.39–1.54)	0.50
>10 years	32	6	1.38 (0.51 – 3.00)	0.55	0.58 (0.19–1.56)	0.35

* The standard incidence ratio (SIR) is the ratio of observed to expected malignancies and represents the malignancy risk compared to the general population, and the relative risk (RR) represents the malignancy risk compared to the reference group. Calculated by exact Poisson regression analysis.

† Unknown for 61 patients.

MPO-ANCA, myeloperoxidase ANCA; PR3-ANCA, proteinase 3 ANCA.

Supplementary table 2. Cyclophosphamide and rituximab treatment in the subgroups

	N patients treated with cyclophosphamide	Duration of cyclophosphamide treatment, months (SD)	Mean cumulative cyclophosphamide dose, g (SD)	N patients treated with rituximab	Duration of rituximab treatment, months (SD)	Mean cumulative rituximab dose, g (SD)
Gender						
Male	107	5.5 (5.5)	9.9 (6.5)	68	22.1 (14.9)	5.8 (3.4)
Female	116	6.4 (14.4)	8.4 (10.8)	85	20.9 (14.4)	5.9 (3.4)
Age at diagnosis						
≥59 years	114	6.5 (13.8)	6.8 (3.9)	63	20.2 (14.2)	5.2 (2.9)
<59 years	109	5.1 (4.2)	11.5 (11.8)	90	22.5 (14.9)	6.3 (3.6)
Clinical diagnosis						
Microscopic polyangiitis	116	6.3 (12.6)	8.1 (10.5)	65	18.7 (13.3)	5.2 (2.6)
Granulomatosis with polyangiitis	88	5.4 (4.4)	10.7 (7.3)	66	27.7 (15.0)	6.7 (4.0)
Eosinophilic granulomatosis with polyangiitis	19	4.5 (2.8)	7.8 (4.2)	22	22.6 (15.7)	5.3 (2.9)
ANCA serotype*						
MPO-ANCA	72	7.4 (16.1)	7.2 (4.7)	43	20.7 (16.9)	5.1 (2.9)
PR3-ANCA	121	5.0 (4.2)	9.2 (6.5)	82	20.9 (13.0)	6.1 (3.3)
Renal transplantation						
No	213	5.2 (4.9)	9.1 (9.0)	149	21.6 (14.4)	5.9 (3.4)
Yes	10	15.6 (37.3)	8.5 (8.6)	4	16.7 (19.4)	4.5 (2.4)
Follow-up						
0–5 years	102	4.7 (3.5)	7.0 (4.6)	52	17.5 (10.9)	4.6 (2.8)
5–10 years	101	7.2 (15.5)	10.8 (11.9)	82	23.5 (15.2)	6.3 (3.6)
>10 years	20	5.4 (5.1)	10.9 (6.8)	19	24.6 (19.6)	7.4 (3.1)

* Unknown for 61 patients.

MPO-ANCA, myeloperoxidase ANCA; PR3-ANCA, proteinase 3 ANCA.

Supplementary table 3. SIR for non-melanoma skin cancer according to cumulative cyclophosphamide and rituximab dose*

Cumulative dose (g)	N patients	N observed non-melanoma skin cancer	SIR (95% CI)†	SIR p value†
Cyclophosphamide				
0	89	3	2.17 (0.45– 6.34)	0.16
0.1–20	207	18	4.89 (2.90 – 7.72)	<0.001
20–108	16	3	11.72 (2.42 – 34.25)	0.002
Rituximab				
0	167	23	8.47 (5.37 – 12.71)	<0.001
0.1–6	70	1	0.83 (0.02 – 4.64)	0.66
6–18	83	0	0 (0 – 2.47)	0.23

* SIR, standardised incidence ratio; the SIR is the ratio of the observed to expected malignancies adjusted for sex, age (per 5-year age group), and calendar time period (per 1-year calendar time period).
† Calculated by exact Poisson regression analysis.

Supplementary table 4. Cumulative cyclophosphamide and rituximab dose of each patient with a malignancy

Malignancy or malignancy site	N observed malignancies	Cumulative cyclophosphamide dose (g)*	Cumulative rituximab dose (g)*	Time to malignancy (years)†
Lung	4	36.0; 21.1; 4.9; 0.0	0.0; 7.0; 0.0; 5.0	3.7; 4.8; 0.5; 8.5
Breast	3	18.0; 6.0; 3.4	0; 4.0; 8.0	0.9; 1.1; 4.0
Colon or rectum	3	13.8; 9.5; 4.0	0.0; 0.0; 0.0	2.4; 4.5; 4.0
Prostate	2	10.0; 0.0	0.0; 0.0	7.4; 1.2
Bladder	1	0.0	1.0	2.4
Pancreas	1	0.0	0.0	1.4
Testis	1	7.0	5.0	4.6
Ovary	1	3.0	6.6	2.4
Melanoma	1	3.3	0.0	1.8
Tongue	1	0.0	0.0	4.5
Central nervous system	1	2.0	5.6	3.2
Kidney	1	7.0	4.0	2.4

* The cumulative doses of each patient with a malignancy is given. Patients with a cumulative dose of 0.0 did not receive the treatment. When more cases of the malignancy were observed, the first reported cumulative cyclophosphamide dose corresponds to the first reported cumulative rituximab dose, and to the first reported time to malignancy.
† This is the time between the date of diagnosis of ANCA-associated vasculitis and the date of diagnosis of the malignancy.

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Chinar Rahmattulla werd geboren op 16 oktober 1989 te Tuz, Kirkuk (Irak). Drie maanden later verhuisde haar gezin in het kader het promotieonderzoek van haar vader naar Frankrijk. Hierna was het gezin woonachtig in Libië en Irak voordat het in 1997 naar Nederland verhuisde.

In 2009 behaalde Chinar haar VWO-diploma *cum laude* (Natuur & Gezondheid en Natuur & Techniek). In datzelfde jaar begon zij met de opleiding Geneeskunde aan de Universiteit Leiden. Daarnaast begon zij in 2012 met de studie Biomedische Wetenschappen aan dezelfde universiteit. Nadat zij in 2011 werd toegelaten tot het MD/PhD traject van het Honours College (Universiteit Leiden) ontving zij in het kader van dit traject in 2013 een beurs voor een tweejarige promotieaanstelling. Onder begeleiding van prof. dr. J.A. Bruijn, dr. I.M. Bajema en dr. A.E. Berden startte zij op de afdeling Pathologie van het Leids Universitair Medisch Centrum (LUMC) met onderzoek naar ANCA-geassocieerde vasculitis. In het kader van haar promotieonderzoek deed zij in 2015 een half jaar onderzoek aan de Universiteit van Cambridge (Verenigd Koningrijk) onder begeleiding van prof. dr. D.R. Jayne. Voor de publicatie die hieruit voortkwam – waarvan zij de laatste auteur was – won zij de KNMG Dick Held juniorprijs en de Stichting Hippocrates Studiefonds Prijs. Zij heeft tevens onderzoek verricht op de afdelingen Cardiologie (2010 – 2011; begeleider: dr. ir. C.A. Swenne) en Neonatologie (2017 – 2018; begeleider: prof. dr. E. Lopriore) van het LUMC. Sinds 2013 is zij vrijwilligster bij de Vasculitis Stichting (landelijke vereniging vasculitispatiënten) en redacteur van het tijdschrift Vascuzine.

Tijdens haar studie Geneeskunde volgde zij klinische verdiepingsstages aan het Neurenberg Ziekenhuis (Duitsland) en het Academisch Ziekenhuis Innsbruck (Oostenrijk). In 2017 behaalde zij – als eerste student aan de Universiteit Leiden – het masterdiploma Geneeskunde *summa cum laude* en in 2018 behaalde zij het masterdiploma Biomedische Wetenschappen *cum laude*.

Chinar Rahmattulla was born on the 16th of October in Tuz, Kirkuk (Iraq). Three months later she moved with her parents to France for her father to work on his PhD thesis. Hereafter, the family lived in Libya and Iraq before moving to the Netherlands in 1997.

In 2009, she graduated from secondary school *cum laude*. That same year she started studying Medicine at Leiden University. In addition, she started studying Biomedical Sciences at the same university in 2012. She was accepted to participate in the MD/PhD Honours Program of Leiden University in 2011. As part of this program she received a PhD grant for two years in 2013. Under the guidance of prof. dr. J.A. Bruijn, dr. I.M. Bajema and dr. A.E. Berden, she started research into the ANCA-associated vasculitides at the Department of Pathology of the Leiden University Medical Center (LUMC). She was a visiting PhD researcher at the University of Cambridge (United Kingdom) for six months in 2015 (supervisor: prof. dr. D.R. Jayne). For the publication that resulted from that research – of which she was the senior author – she won the KNMG Dick Held Junior Prize and the Hippocrates Scholarship Prize. In addition to the Department of Pathology, she conducted research at the Departments of Cardiology (2010 – 2011; supervisor: dr. C.A. Swenne) and Neonatology (2017-2018; supervisor: prof. dr. E. Lopriore) of the LUMC. From 2013 onwards she has been volunteering at the Vasculitis Foundation (the Dutch vasculitis patient foundation), and in the same year she became part of the editorial board of the Vascuzine.

During her medical studies, she followed clinical internships at the Nuremberg Hospital (Germany) and the Academic Hospital Innsbruck (Austria). In 2017, she became the first student to graduate *summa cum laude* from Leiden University with a Master of Sciences in Medicine, and in 2018 she graduated with a Master of Sciences in Biomedical Sciences *cum laude*.

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