Editorial

Genetic association studies in ANCA-associated vasculitides: what we have learnt so far and what needs to be done in the future

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Genetic association studies have provided significant evidence for the underlying pathophysiological processes in various and rare autoimmune diseases, *e.g.* in chronic inflammatory bowel disease (1). In recent years, a growing effort has been undertaken to study the genetic background of even rarer diseases such as ANCA-associated vasculitides (AAV). So far, genetic risk factors in AAV have only been studied by a candidate gene approach; furthermore, many of the genetic association studies in AAV have been performed with small sample sizes producing inconsistent results. Currently, large nation-wide and pan-European genome-wide association studies investigating genetic risk factors in AAV are underway in Europe and the US that pose the advantage of a larger sample size and a hypothesis-free approach. These studies will provide us with a large set of possible genetic risk factors that will need careful reviewing and functional studies to fully elucidate the pathophysiological meaning of suspected genetic risk factors. Here, we highlight what needs to be taken into consideration in the analysis of genetic risk factors in AAV and give an impression of what we know about genetic risk factors so far.

Disease entities and phenotypes need to be considered

In the context of complex disease entities such as AAV, it becomes even more important to recognise common, but also different pathophysiological processes, phenotypes and disease courses in a group of well-known disease entities. AAV share *per definitionem* the common immunological feature of a positive ANCA status (presence of Anti-neutrophil cytoplasm antibodies directed against proteinase 3 or myeloperoxidase) that is associated with clinical signs of predominantly small-vessel vasculitis, but are otherwise very heterogeneous and have unique hallmarks (*e.g.* asthma in Churg-Strauss syndrome, CSS). Apart from Microscopic polyangiitis (MPA) that is associated with a relatively homogenous pattern of disease ("pure ANCA-associated vasculitis"), within Wegener's granulomatosis (WG) and CSS different phenotypes can be distinguished: In WG, a localised disease phenotype can occur that is associated with granulomatous disease manifestations (*e.g.* granulomatous sinusitis, orbital and pulmonary granuloma or masses etc.), but has no obvious clinical signs of vasculitis and ANCA is detected in less than 50% (2, 3). In contrast, generalised disease is associated with classic systemic vasculitis that is associated with ANCA in nearly 100% (4). Likewise, in CSS two phenotypes can be distinguished. One is characterised by eosinohpil organ infiltration with no detectable ANCA, the other one corresponds to vasculitis manifestations associated with ANCA (5). Additionally, some AAV patients develop severe or refractory disease stages while others respond to standard therapy immediately. These disease courses may also be conveyed by a genetic predisposition.
As pointed out below, in spite of the very limited knowledge on the genetic background of AAV we have gained so far, evidence suggests that there are common and different genetic risk factors that may be associated with one or several AAV and/or certain disease subgroups or phenotypes.

A consensus on disease and phenotype classification is required

With regard to disease subgroups and phenotypes, it is important to mention

that among the genetic studies that have been undertaken so far, different approaches to classify AAV have been applied. While some authors consider AAV as one disease entity, others distinguish AAV on the basis of ANCA status and ANCA target antigen (Proteinase 3 or myeloperoxidase); in turn other researchers classify AAV according to the American College of Rheumatology (ACR) classification criteria (6) and/or Chapel Hill Consensus Conference (CHC) Definitions (7). Clearly, the different approaches may give rise to inconsistent results and point to the necessity of diagnostic criteria for AAV or at least a consensus definition for AAV and AAV subgroups/phenotypes to apply to genetic studies.

Major findings of the candidate gene approach in AAV

In WG, the most remarkable genetic associations have been described for the *HLA-DPB1*0401* allele (OR [odds ratio] 3.91 and 3.01, respectively) (8, 9), the 620W allele of *PTPN22* (OR 1.76 in all WG, OR 2.01 in ANCA-positive WG) (10), that is associated with various autoimmune disorders and the deficiency allele *PI*Z* of the alpha1-antitrypsin gene (11, 12). Various other genes and alleles have been studied, in part with small sample numbers or inconsistent results and therefore need confirmation (13-15).

*HLA DPB1*0401 seems to represent a unique risk factor for WG in the group of AAV.*

HLA-DPB1*0401 allele seems to convey a unique risk for WG, but not for CSS (16) and MPA (unpublished observation). Interestingly, the *HLA-DPB1*0401* allele is only associated with ANCA-positive, but not ANCA-negative WG.

*PTPN22*620W conveys a genetic risk for several autoimmune disease and may be associated with a positive autoantibody status.*

Similar to *HLA DPB1*0401* allele, the *PTPN22**620W allele has been identified as risk factor for ANCA-positive WG and is linked to classic vasculitic manifestations such as kidney involvement. The *PTPN22* risk allele has been linked to a positive autoantibody status in other autoimmune diseases such as rheumatoid arthritis (Anti-CCP positive) (17) and may therefore confer not only an increased risk for autoimmune disease but also autoantibody production. This hypothesis is further underlined by the findings that *PTPN22*620W* is associated with MPA (OR 2.55) (18), which is usually ANCA-positive, but not with CSS (unpublished observation), that is ANCA negative in the majority of cases. It will be intriguing to investigate the association of the *PTPN22*620W* allele with WG patients who remain in the localised disease phase, as in this stage, patients have no signs of systemic vasculitis by definition and are ANCA positive in less than 50% of cases.

IRF5 may be one example of differences in genetic risk factors of disease phenotypes

Recently, a protective haplotype of *IRF5* (interferon regulatory factor 5) gene was identified in a large WG study population (n=601) (OR 0.73) (19). Interestingly, when compared to controls, the protective effect was stronger in patients with systemic disease compared to localised disease (OR 0.68, p=0.0000641 *vs.* OR 0.8, p=n.s.). Although the number of localised patients in this study is very small and needs confirmation, this finding may point to differences in genetic background of two phenotypes belonging to one disease.

HLA genes/alleles other than HLA-DPB may play role in AAV.

Many other genes have been investigated in WG, in particular HLA genes. Recently, an association with the *HLA-DR4* serotype was found in a relatively large study (20); all other *HLA* associations described so far have included only small sample sizes and should be considered preliminary; furthermore, a lack of association was described for other genes which may at least to some extent result from a limited number of samples (see 21 for review).

HLA genes have also been extensively studied in CSS. Interestingly, as mentioned above, no association was found with the WG risk allele *HLA-DPB1*0401*, but several other associations with *HLA* genes such as with HLA-*DRB1, -DRB3, -DRB4* have been described (22).

The IL10.2 haplotype is so far the most convincing genetic risk factor in CSS.

A recent study found a strong association of the *IL10.2* haplotype with CSS (23), but not with WG, which is consistent with the finding of elevated serum levels of IL-10 in CSS, but not in WG. Moreover, this association was in particular significant in ANCA-negative CSS patients (OR for all patients: 1.73, OR for ANCA-negative patients: 2.16), suggesting again that behind the different clinical phenotypes of one disease there is indeed a different genetic background.

Only few studies are available regarding genetic association in MPA, suggesting certain *HLA* genes, *CD18* alleles and the *PTPN22* risk allele as risk factors (see 21 for review).

Major findings of genetic association studies analysing MPA and WG together

Recent studies with large sample numbers focused on identifying common genetic risk factors in AAV by analyzing WG and MPA together (18, 24): Here, the *PTPN22* risk allele was confirmed as risk factor for both WG and MPA. No association was found for various *IL2RA* polymorphisms (24) and the 307 Ser polymorphism of *CD226*, which had previously been described as a risk factor for WG in a German study population (25). Furthermore, associations of the *CTLA4* gene have been identified but these are hard to evaluate as various polymorphisms have been analysed in different disease subgroups (26-30) and the number of included cases was quite low in each individual study, thus excluding sound conclusions *a priori*.

In summary, the evidence from genetic studies in AAV suggests that the genetic make-up consists of risk factors that are common for several autoimmune diseases including AAV (such as *PTPN22*620W*), but that there are

also unique genetic risk factors that are characteristic for one disease entity within the AAV-group (such as *HLA-DPB1*0401*) or even for a phenotype within one AAV (such as the *IL10.2* haplotype being associated mainly with ANCA-negative CSS). Disease groups and phenotypes need to be considered in genetic association studies in order to dissect the underlying genotype and disease mechanisms.

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