Bacterial fingerprints across Europe

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CHAPTER 8

Molecular fingerprints of nasal *Staphylococcus aureus* isolates from ANCA-associated vasculitis patients in the Netherlands

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ABSTRACT

Staphylococcus aureus **is a microorganism of prominent versatility. While the nasal colonization by *S. aureus* is fairly widespread and occurs asymptomatically, *S. aureus* can also cause diverse infections ranging from mild skin infections to life-threatening conditions. Patients suffering from anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) positive for proteinase 3 (PR3)-ANCA are in 60-70% of the cases chronic nasal *S. aureus* carriers (compared to 20-30% in the general population) and nasal carriage of *S. aureus* is considered a risk factor for disease relapse. Intriguingly, studies on *S. aureus* from patients suffering from myeloperoxidase (MPO)-AAV are so far lacking. Previous genotyping already revealed the diversity of a large *S. aureus* sample from PR3-ANCA-positive patients that mirrored the diversity of the general *S. aureus* population. The objective of this study was therefore to assess the overall gene repertoire of *S. aureus* isolates from PR3-ANCA- and MPO-ANCA-positive patients to gain more knowledge on the *S. aureus* populations colonizing these two AAV patient groups. This was achieved by profiling 61 *S. aureus* isolates from PR3-ANCA-positive, 27 *S. aureus* isolates from MPO-ANCA-positive patients and 18 *S. aureus* isolates from healthy controls with a DNA microarray comprising 336 probes of *S. aureus* genes. The present data reveal for the first time the genomic diversity of AAV-associated *S. aureus* isolates, for both PR3-ANCA- and MPO-ANCA-positive patients. Interestingly, principal component analysis determined a clustering of isolates based on their assigned multilocus sequence type clonal complexes and revealed that differences between *S. aureus* colonizing these two patient groups are reflected by a limited set of loci. Specifically, different distributions in the staphylococcal superantigen and virulence gene repertoire were discovered, including the genes *lukXY*, *isaB*, *mprF*, Q2YUB3, *set4*/*set7*, *cap*-1, *cap*-5, *cap*-8, *cna* and *sasG*. In conclusion, the definition of the virulence gene repertoire of the *S. aureus* population of patients with PR3-ANCA- and MPO-ANCA-AAV has paved the way for further studies on the molecular traits that define the possible roles of *S. aureus* in these diseases.**

INTRODUCTION

Unravelling the genomic diversity of different *Staphylococcus aureus* types is key to identify genetic determinants that are specific for the commensal or pathogenic lifestyles of this opportunistic pathogen. *S. aureus* is a chronic inhabitant of the human skin and nasopharynx, in approximately 20-30% of the healthy population [1]. From previous studies, it has become clear that the human nasopharynx represents a favourable niche and that the perfect fit between *S. aureus* and its host has probably evolved through long-term co-existence [1,2]. Several studies have addressed the significance of *S. aureus* nasal carriage indicating a central role in the subsequent epidemiology and pathogenesis of infection [1,3,4]. Induced by unknown and probably multifactorial stimuli originating from the bacterium and/or the host, *S. aureus* can transform into a dangerous pathogen that is capable of causing a diverse array of infections. Specifically, it has been shown that the majority of nosocomial infections (~80%) are caused by the endogenous strains of the carrier. However, persistent *S. aureus* nasal carriers have the lowest risk of death caused by *S. aureus* bacteraemia [1,5].

Epidemiological and molecular studies have shown that certain *S. aureus* lineages prevail in a geo-spatial distribution and that some lineages show a restricted dominance in either healthcare or community environments [6-9]. However, a clear association between certain *S. aureus* types and particular diseases has so far not been uncovered in studies addressing selected *S. aureus* populations of patients with diseases, such as cystic fibrosis, epidermolysis bullosa, HIV/AIDS and the systemic autoimmune disease granulomatosis with polyangiitis (GPA). The latter belongs to the anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitides (AAVs), that represent one subgroup of systemic vasculitides [10-13]. AAVs are characterized by the presence of circulating ANCAs, the predominant involvement of small and medium-sized vessels, and a high risk of glomerulonephritis [14]. The main targets of ANCAs in AAV patients are either proteinase 3 (PR3) or myeloperoxidase (MPO), which are both present in granules of neutrophils and lysosomes of monocytes. The presence of ANCAs targeting PR3 is a hallmark of GPA patients. Interestingly, different geographical distributions of the PR3- and MPO-AAV types have been described. PR3-AAV occurs most frequently in Europe and the United States of America (USA), whereas MPO-AAV is predominantly found on the Southern Hemisphere and in East Asia and Japan [14]. The reasons for this different distribution are unknown but, clearly, AAVs are multifactorial diseases with numerous contributing genetic and environmental factors [15-19]. Among the latter, microbial upper airway infections have been associated with PR3-ANCA-GPA. In particular, 60-70% of PR3-ANCA-GPA patients are chronic nasal carriers of *S. aureus*, in contrast to 20-30% of healthy individuals [1,15,20]. Moreover, PR3-ANCA-GPA patients carrying *S. aureus* are at risk for disease relapses while antibiotic therapy with co-trimoxazole reduces the incidence of disease relapses [20,21]. Accordingly, particular virulence factors of *S. aureus*, such as the staphylococcal superantigen (SAg) toxic shock syndrome toxin-1 (TSST-1), have been implicated in disease relapse of PR3-ANCA-GPA [22,23]. In this context, it is remarkable that no studies have so far addressed the *S. aureus* population colonizing MPO-ANCA-positive patients.

We have recently demonstrated a high genetic diversity in a large *S. aureus* sample from PR3-ANCA-GPA patients, indicating that the *S. aureus* population of these patients mirrors the general *S. aureus* population in the Netherlands as judged by highly discriminatory DNA typing methods [Glasner *et al.*, submitted]. Yet, this finding does not rule out the possibility that *S. aureus* carried by PR3-ANCA-positive patients possesses a particular gene repertoire responsible for disease onset, progression and/or relapses. This idea formed the grounding for our present study, which was aimed at determining potential differences in the overall gene repertoire of *S. aureus* nasal isolates from both PR3-ANCA- and MPO-ANCA-positive patients suffering from any form of AAV. For this purpose, DNA microarrays containing 336 probes for a large variety of staphylococcal genes, including virulence, adhesion, resistance and other genes, were employed. This uncovered molecular fingerprints of the studied *S. aureus* samples, which highlight a limited number of potentially disease-associated genetic determinants.

MATERIALS AND METHODS

Bacterial isolates

The characteristics of the *S. aureus* isolates used in this study are summarized in Table 1. The 61 isolates from 32 PR3-ANCA-positive patients (referred to as PR3-ANCA isolates) and 18 isolates from 10 healthy control (HC) individuals (referred to as HC isolates) have previously been described in more detail [24,25,Glasner *et al.*, submitted]. The isolates from MPO-ANCA-positive patients (referred to as MPO-ANCA isolates) were not previously described.

Table 1. *S. aureus* isolate characteristics grouped by PR3-ANCA, MPO-ANCA and HC.

	PR3-ANCA isolates (n = 61)	MPO-ANCA isolates (n = 27)	HC isolates (n = 18)
No. of patients	32	27	10
No. of males	22	11	4
Age at time of swab, mean±SD	55±13	60±13	33.6±11.8
Collection period	2006 - 2012	2010 - 2013	2007 - 2010
No. of different *spa*-types (most dominant (%))	18 (*spa*-type t091 (21), *spa*-type t064 (38))	17 (*spa*-type t012 (15), *spa*-type t064 (22))	12 (*spa*-type t012 (17))
CC strain assignment and frequency (%)	CC1 (3.3) CC5 (14.8) CC7 (21.3) CC8 (45.9) CC22 (1.6) CC25 (3.3) CC30 (4.9) CC45 (1.6) CC97 (1.6) None (1.6)	CC5 (11.1) CC7 (22.2) CC8 (14.8) CC12 (3.7) CC22 (7.4) CC30 (18.5) CC45 (14.8) CC361 (3.7) CC101 (3.7)	CC5 (5.5) CC7 (11.1) CC8 (11.1) CC9 (5.5) CC30 (38.8) CC20 (11.1) CC22 (5.5) CC45 (5.5) CC97 (5.5)
agr type and frequency (%)	I (75.4) II (14.8) III (8.2) None (1.6)	I (66.7) II (14.8) III (18.5)	I (50) II (11.1) III (38.9)
mecA-positive	0	1	0
tst-1-positive	2	5	8
PVL-positive	0	0	0

*CC; clonal complexes

spa-typing

spa-typing was performed as previously described [26].

DNA microarray

DNA isolation was carried out with the UltraClean Microbial DNA Isolation Kit (MoBio, Carlsbad, USA) following the manufacturer's instruction. The Clondiag *S. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH, Jena, Germany) was used for DNA microarray analysis. The *S. aureus* microarray contains 336 DNA probes to detect genes for species-specific markers, antibiotic resistance, SCC*mec* elements, adhesion and virulence factors, capsule and *agr* group markers. The analysis was performed according to the manufacturer's instructions and as previously described [27,28]. The affiliations of isolates to specific multilocus sequence type clonal complexes (MLST CC) were determined by synchronization of the hybridisation profiles to the available reference database [27].

Statistical analysis

Principal component analysis (PCA) was performed on the genes that were not universally present or absent from the three sampled *S. aureus* isolate groups (i.e. PR3-ANCA, MPO-ANCA and HC) to identify clusters of similar or different gene profiles. PCA is an ordination method based on multivariate statistical analysis that maps the samples in different dimensions. All statistical analyses were performed with SPSS Statistics 20 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

To compare *S. aureus* carried by MPO-ANCA-positive patients with *S. aureus* carried by PR3-ANCA-positive patients or HC, we collected 27 isolates from MPO-ANCA-positive patients regularly visiting our outpatient clinic. These isolates were characterized by *spa*-typing, which revealed that the *spa*-types t012 and t064 were the most dominant *spa*-types, similar to previously collected PR3-ANCA and HC isolates (Table 1). The lower number of MPO-ANCA isolates that were included in the present study reflects the lower prevalence of this AAV type in the Netherlands compared to PR3-AAV. Next, we employed DNA microarrays to determine the genetic profiles of the 27 MPO-ANCA isolates, 61 PR3-ANCA isolates, and 18 HC isolates. The results of these 106 array analyses underscored the previously reported high genetic diversity of PR3-ANCA isolates and revealed a comparably high genetic diversity for MPO-ANCA isolates, consistent with the *spa*-typing results [Glasner *et al.*, submitted]. Table 1 summarizes the basic molecular characteristics of the three *S. aureus* samples and a complete overview of the DNA microarray data is presented in the Supplementary Material Table 1 (available on request). Importantly, clear trends in the genetic profiles of the two disease-associated *S. aureus* samples were discovered as presented and discussed in the following paragraphs.

PCA identifies a limited number of potentially disease-associated genetic determinants

A PCA was done on all genes that were not universally present or absent from all three *S. aureus* samples representing the PR3-ANCA, MPO-ANCA and HC isolates, respectively. Genes that showed no variation between the three *S. aureus* samples included the majority of genes for adhesion factors, microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and antibiotic resistance (Supplementary Material Table 1, available upon request). The location of all 106 study isolates on the first two principal components (PC), which together already explained 62% of the variation within the entire combined data set, revealed several interesting patterns (Figure 1). The distribution of the 106 *S. aureus* isolates on PC1 (x-axis) and PC2 (y-axis) showed that a majority of the MPO-ANCA isolates are scattered more to the upper left of the plot while the PR3-ANCA isolates are scattered more to the lower right corner. On first sight, the isolates from the HC sample appeared to scatter along the hypothetical axis between the PR3-ANCA and MPO-ANCA isolates with a predilection towards the MPO-ANCA isolates. Figure 1 also indicates particular genes or gene groups (blue and red vectors/boxes) that were identified after a correlation analysis to identify particular associations with either of the two PCs.

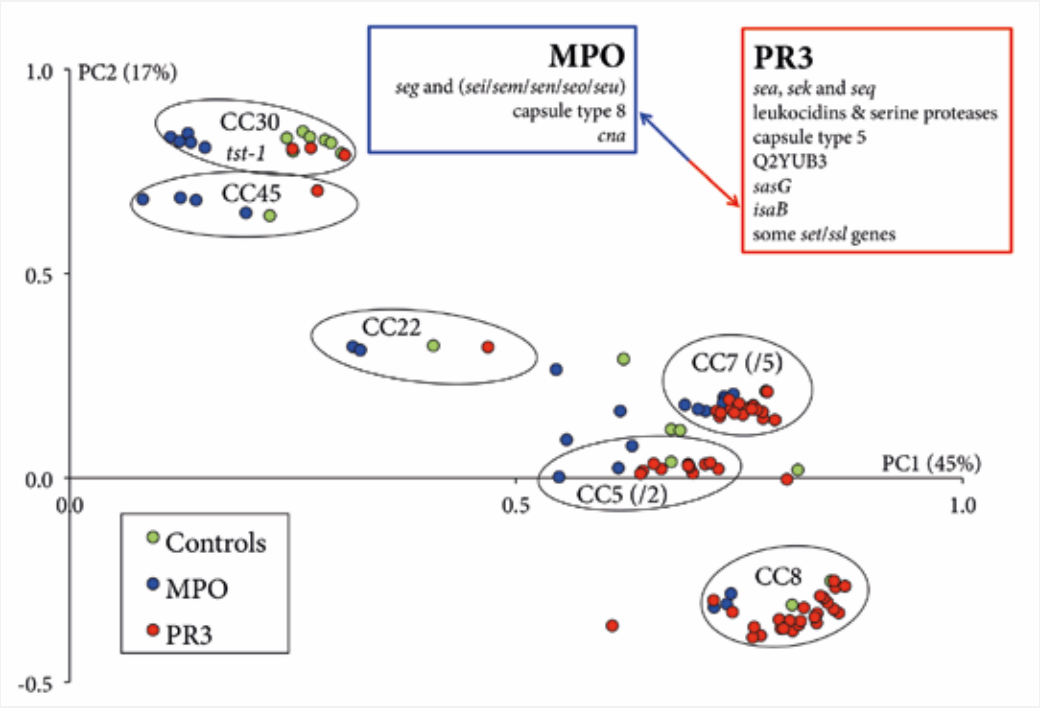

Figure 1. PCA of the ClonDiag microarray data. Only loci that differed between the three investigated *S. aureus* samples (PR3-ANCA, MPO-ANCA and HC) were included and displayed in a two-dimensional manner. The x-axis represents PC1 and the y-axis PC2. Note that the circle indicating CC7 also includes five isolates that belong to a different CC. The same is true for the CC5 circle that includes two isolates assigned to a different CC.

All but one study isolate could be assigned unambiguously to CCs by synchronizing the hybridisation patterns with the available reference database (Table 1). Importantly, the hypothetically assigned CCs coincided with the experimentally determined *spa*-types of all study isolates and were in line with previously detected associations between CCs and *spa*-types/*spa*-CCs (http://www.ridom.de). PCA clearly illustrated the repeatedly reported lineage-specificity of genetic determinants in *S. aureus*, as isolates assigned to the same CC based on their gene repertoire also clustered together on the PCA plot (Figure 1) [29-31]. As the distribution of CCs amongst the isolates was far from equal between the three *S. aureus* samples, it is of no surprise that they were also distributed differently on the PCA plot (Table 1 and Figure 1). CC8 was predominant amongst PR3-ANCA isolates (46%) while CC30 and CC45 were more common amongst MPO-ANCA isolates (19% and 15%, respectively). CC5 and CC7 were both common amongst the MPO-ANCA and GPA-ANCA isolates. HC isolates belonged mainly to CC30 (39%) and the remaining 11 (61%) isolates were assigned to nine different CCs, comprising only one to two isolates each. Besides the overall detected diversity in the three investigated *S. aureus* samples, it is important to note that the assigned CCs in the present collection belong to the most common CCs observed for both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) across Europe with different epidemiological backgrounds and geographical dominance [7,32-37].

At first view, particular CCs might indeed be associated with PR3-ANCA (CC8) or with MPO-ANCA (CC45), but this could also be due to a possible sampling bias of the respective isolates. Hence, some genes might solely be associated with either of the two disease types because of the affiliated CC, leading to an indirect association with the respective disease type. A more detailed examination of the PCA plot, with particular focus on PC1, revealed that the MPO-ANCA isolates were always more shifted to the left, whereas the PR3-ANCA and HC isolates were always more shifted to the right within each of the indicated six CC circles (Figure 1). This observation led us to examine each of the

indicated CC clusters in detail, in order to identify loci associated with the shift to either direction along the PC1 axis. The PC1 value of each clonal complex was used to calculate the shift to the left or right of each isolate within a particular clonal complex. The shift to the left or right (ΔPC1) was subsequently correlated with the presence or absence of genes within all of the isolates that were part of these six indicated CCs (CC30, CC45, CC22, CC7 (/5), CC5 (/2), CC8). Correlation analysis of the indicated six clusters revealed that MPO-ANCA isolates are strongly negatively correlated with ΔPC1, meaning that these isolates were always shifted more to the left within each CC circle. PR3-ANCA and HC isolates on the other hand were equally correlated positively with a shift to the right. This implies that HC isolates have apparently more in common with PR3-ANCA isolates than with MPO-ANCA isolates, contrary to what was initially observed in the non-clustered PCA analyses (without the CC indications) of Figure 1. Correlation analyses with ΔPC1 furthermore resulted in the identification of several genes that are more associated with both the PR3-ANCA and HC isolates, but not with the MPO-ANCA isolates ($p < 0.001$). These genes include *lukX*, *isaB*, *mprF*, Q2YUB3, *set4* and *set7*. Figure 2 displays the calculated percentages of these six genes and four additional genes (*cap*-5, *cap*-8, *cna* and *sasG*) that were also identified as being associated with either PR3-ANCA or MPO-ANCA before the correlation analyses of each CC circle (Figure 1). Interestingly, the *cap*-5, *sasG, lukX*, *isaB*, *mprF*, Q2YUB3, *set4* and *set7* genes appeared to be more abundant in the PR3-ANCA sample than in the MPO-ANCA or HC samples, while the *cap*-8 and *cna* genes appeared to be less abundant in the PR3-ANCA sample. Whether the higher abundance of the *cap*-5 and *sasG* genes in the PR3-ANCA sample and the lower abundance of these and other genes in the PR3-ANCA or MPO-ANCA samples could be related to the onset and/or progression of any of these two types of AAV requires further investigations, especially since the observed differences could relate to a sample bias. To test this idea, a correlation analysis of the indicated CC clusters was performed. According to this analysis, only the reduced abundance of *lukX* was unambiguously demonstrated for the MPO-ANCA isolates. This gene encodes the LukX component of the newly identified two-component leukocidin LukXY (designated LukAB in [38] and Lukgh in [39]). Notably, the LukXY leukotoxin has a high cytolytic activity and is capable to kill neutrophils, macrophages and dendritic cells. Whether the reduced abundance of *lukX* in the MPO-ANCA isolates could have an effect on the disease pathology still needs to be investigated.

Altogether, the PCA suggests associations of several groups of genes or single genes with either the PR3-ANCA or MPO-ANCA samples, although a possible sample bias cannot be excluded. In particular, these associations concerned SAgs, leukocidins, hemolysins and other virulence genes that are discussed in more detail in the following paragraphs.

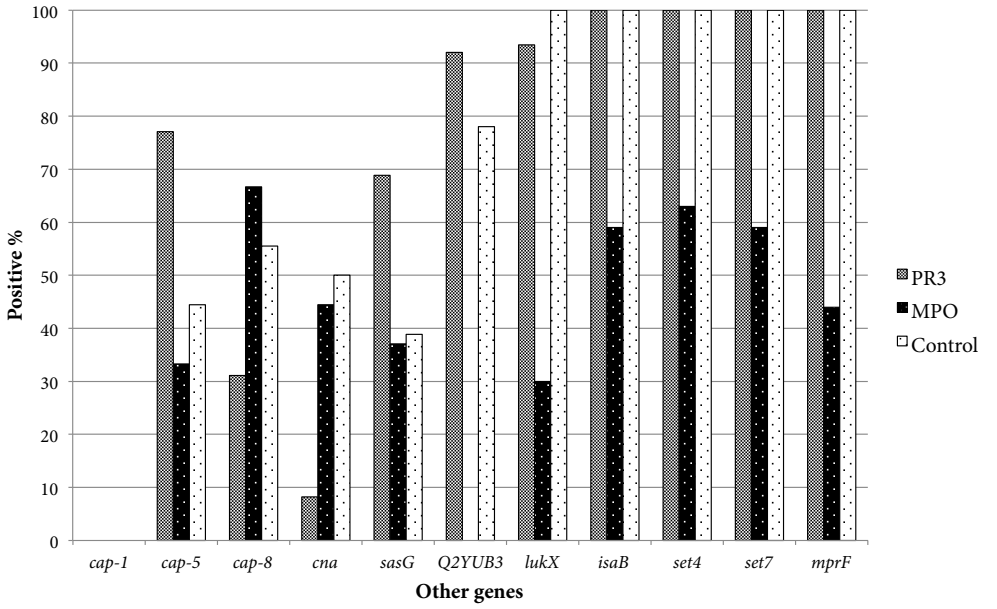

Figure 2. Percentages of 11 genes with a different prevalence in PR3-ANCA, MPO-ANCA and HC *S. aureus* isolates.

Low prevalence of *egc*– and non-*egc* genes for SAgs in the two disease-associated samples

In view of the infectious lifestyle of *S. aureus*, SAgs have been in the centre of numerous previously published research studies [40-42]. SAgs are a large group of antigens that cause non-specific activation of T-cells resulting in polyclonal T-cell activation and massive cytokine release. Several studies have investigated potential implications of SAgs in staphylococcal disease [23,41,43,44]. In particular, Popa *et al.* reported that *S. aureus* isolates from PR3-GPA patients contain a specific subset of SAgs with a high prevalence of *sea* and *tst-1*, and that the risk for disease relapse increases with the carriage of *tst-1*-positive *S. aureus* [23]. In this context, the presently discovered difference in the *tst-1* prevalence in the three *S. aureus* sample groups is remarkable (Figures 1 and 3; Table 1). Although *tst-1* has been reported to be associated with PR3- GPA, in the present study only 3% of the PR3-ANCA isolates carry this gene. Conversely, 19% of the MPO-ANCA isolates and even 44% of the HC isolates were *tst-1*-positive (Figure 3). Importantly, the previously reported association between CC30 and *tst-1* was unambiguously confirmed in the present dataset (Figure 1) [29,30,45]. It should be noted, however, that the high prevalence of *tst-1*-positive *S. aureus* in the HC sample compared to previously reported numbers could be explained through sample bias and the overall low number of HC isolates compared to the disease-associated isolates [24,25]. In general, approx. 15-25% of *S. aureus* isolates have been reported *tst-1*-positive for different *S. aureus* samples [30,32,46,47]virulence factors, agr groups (alleles, suggesting that *tst-1* is underrepresented in PR3-ANCA isolates. Furthermore, with respect to the overall SAg gene repertoire of the three *S. aureus* sample groups, we observed an interesting distribution for *egc* and non-*egc* genes. Both the *egc* cluster, consisting of the five SAg genes *seg*, *sei*, *sem*, *sen* and *seo* and the non-egc gene *seu* were found in only 30-31% of the PR3-ANCA isolates, but in 56-59% of the MPO-ANCA and even in 67-72% of the HC isolates (Figure 3). The remaining non-*egc* SAg genes *sec*, *sed*, *see*, *seh*, *sej*, *sek*, *sel*, *seq* and *ser* were only identified in a very limited number of isolates in either of the three samples (Figure 3). Only the non-*egc* SAg gene *sea* was abundantly detected in the PR3-ANCA isolates, which is in accordance with the results reported by Popa *et al.* (Figure 1 and 3) [23].

The *egc* cluster of *S. aureus* has been studied in several molecular analyses of different *S. aureus* samples [48-50]. Interestingly, it was shown that the *egc*-encoded SAgs are less efficiently neutralized by human sera

than the classical SAgs or the TSST-1, resulting in an increased pathogenic potential within the human host [50]. Moreover, complementary superantigenic activity of different SAgs has been shown, indicating that analysing the diversity of SAgs in certain *S. aureus* isolates or lineages may enhance our knowledge of their virulence. Nevertheless, when comparing the overall numbers and diversity of the SAg genes in the PR3-ANCA and MPO-ANCA *S. aureus* samples, no major difference could be detected with other studies [47,51]. The overall low prevalence of *egc*– and non-*egc* SAg genes in the two disease-associated samples compared to the HC sample and the literature questions the true involvement of SAgs in the onset and/or progression of AAV. In addition, several other groups have reported remarkable variations in the SAg gene profiles within *S. aureus* populations possessing the same CC or *spa*-type [43,44]. All these observations are in agreement with the results from our present study, especially in view of the surprisingly high prevalence of SAg genes in the HC isolates. Together, these results suggest that SAgs and *tst-1* might not play the significant pathogenic role in AAV as previously proposed and that the prevalence of SAg genes in *S. aureus* isolates from these patients simply reflects the prevalence of SAg genes in the general *S. aureus* population. Hence, SAgs could fulfil the same function in both healthy carriers and patients, namely to trigger the apoptosis of leukocytes, helping the bacteria to persist and survive under any circumstances. This might also explain why SAgs are so abundant and found in different combinations in many different *S. aureus* lineages, while SAg-mediated diseases are very rare. The latter could then be attributed to host factors such as the immune system, which might play a more dominant role in AAV patients than recently considered. In this respect it is noteworthy that the low abundance of the *tst-1*, *sen* and *seo* genes in the PR3-ANCA isolates is in perfect agreement with the results from our recent study on the GPA patient-specific immune responses against a range of *S. aureus* antigens. By determining serum antibody levels, it was shown that GPA patients have lower levels of antibodies against TSST-1, SEN and SEO than HC [Glasner *et al.*, submitted]. In fact, GPA patients displayed overall lower anti-staphylococcal immunoglobulin G (IgG) responses than HC, indicating that they have a lower ability to mount potentially protective antibody responses to *S. aureus*, despite their long-term exposure to this pathogen.

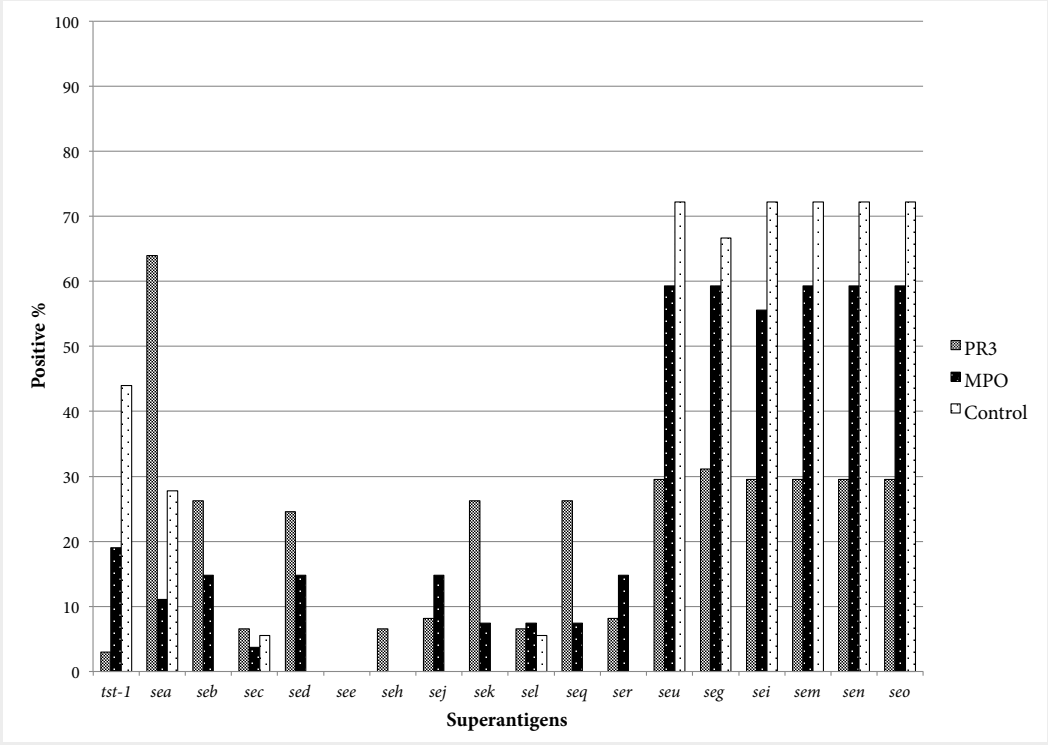

Figure 3. Prevalence of superantigen-encoding genes in PR3-ANCA, MPO-ANCA and HC *S. aureus* isolates.

Differential distribution of leukocidins between PR3-ANCA and MPO-ANCA isolates

Previous studies have shown that *S. aureus* has both a variant and an invariant virulence gene repertoire [52-54]. In accordance with this notion, major *S. aureus* virulence genes, such as *lukF*, *hlgA*, *hl*, *hla*, *hld*, *hlIII* and *hlb* were identified in almost all study isolates (Figure 4). In contrast, all *S. aureus* isolates tested negative for the Panton-Valentin leukocidin (PVL) and almost all isolates lacked the genes for the exfoliative toxins (*etABD*) and the epidermal cell differentiation inhibitors (*edinABC*) (Supplementary Table 1, available upon request). Intriguingly, differences in the prevalence between the three *S. aureus* samples were observed for several leucocidin genes, including *lukS*, *lukD*, *lukE*, *lukX* (already discussed above) and *lukY*. While *lukS* and *lukX* appeared less abundant only in the MPO-ANCA sample, the *lukD*, *lukE* and *lukY* genes showed a general trend to be more common in the PR3-ANCA sample than in the MPO-ANCA sample (Figures 1 and 4). Noteworthy appears to be the imbalance in the distribution of the *lukX* and *lukY* genes in the MPO-ANCA and HC samples, while these genes are present at comparable numbers in the PR3-ANCA sample. This suggests an association between the PR3-ANCA isolates colonizing GPA patients and the possible production of the bi-component LukXY leucocidin. This may be a relevant association as LukXY has a strong cytolytic activity that is able to kill neutrophils, macrophages and dendritic cells [38,39], which could account for the apparent inability of GPA patients to mount an appropriate immune response against *S. aureus* [Glasner *et al.*, submitted].

For the immune evasion cluster (IEC), which consists of the *chp*, *scn* and *sak* genes on the so-called *hlb*-converting phage, it was noted that many isolates in the PR3-ANCA and MPO-ANCA samples lacked the *chp* gene, while the *scn* and *sak* genes were abundantly detected in all samples (Figure 4). With regard to the proteolytic potential of the investigated *S. aureus* isolates, the repertoire of protease genes was generally comparable for the three sample groups, although the prevalence of the two protease genes *splA* and *splB* was higher in PR3-ANCA isolates than in MPO-ANCA and HC isolates (Figures 1 and 4). However, as pointed out above, it should be noted that some of the observed differences in the presence or absence of virulence genes between the two disease-associated samples may relate to sample bias.

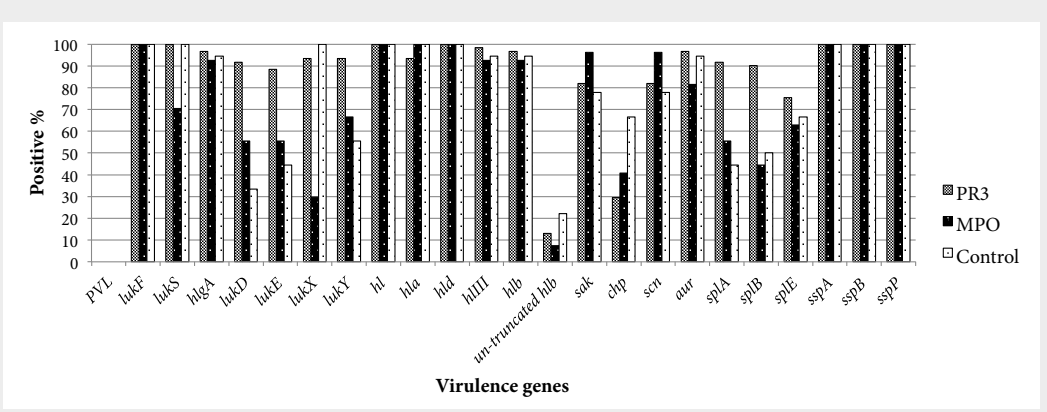

Figure 4. Prevalence of leukocidin, hemolysin, immune evasion and protease genes in PR3-ANCA, MPO-ANCA and HC *S. aureus* isolates.

CONCLUSIONS

The present study provides for the first time insights into the gene repertoire of nasal *S. aureus* isolates from patients suffering from the autoimmune disease AAV, in particular PR3- and MPO-AAV. The results underscore our previous observation that these patients mainly carry *S. aureus* types that are also carried in the general population, as judged by two highly discriminatory DNA typing methods. However, the present application of DNA microarrays generated a more detailed portrait of the gene repertoire of the PR3-ANCA and MPO-ANCA *S. aureus* samples. In the first place, a different CC distribution between these two disease types was unveiled. Secondly and more importantly, several loci were found to be associated with either PR3- or MPO-AAV. Target genes for the initiation of further studies could be *cap-5*, *sasG* and *lukX-lukY* that are positively correlated with the PR3-ANCA isolates. However, the determined association of these loci should first be confirmed in the larger *S. aureus* population of patients with PR3- and MPO-AAV, preferably from different geographical locations. Crucially, the present study provided no evidence for a specific SAg profile related to the investigated *S. aureus* samples, which is in marked contrast to the results from previous studies on PR3-ANCA isolates. This observation also questions a true involvement of SAgs in the onset, progression and/or relapse of AAV [22,41]. In conclusion, the definition of the virulence gene repertoire of the *S. aureus* population of patients with PR3- and MPO-AAV has paved the way for further studies on the molecular traits that define the possible roles of *S. aureus* in disease onset, progression and relapse.

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‘It is important to fight, and fight again, and keep fighting, for only then can evil be kept at bay, though never quite eradicated.’
Albus Dumbledore