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Original article

Genetic and clinical basis for two distinct subtypes of primary Sjögren's syndrome

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Abstract

Objectives. Clinical presentation of primary Sjögren's syndrome (pSS) varies considerably. A shortage of evidence-based objective markers hinders efficient drug development and most clinical trials have failed to reach primary endpoints.

Methods. We performed a multicentre study to identify patient subgroups based on clinical, immunological and genetic features. Targeted DNA sequencing of 1853 autoimmune-related loci was performed. After quality control, 918 patients with pSS, 1264 controls and 107 045 single nucleotide variants remained for analysis. Replication was performed in 177 patients with pSS and 7672 controls.

Results. We found strong signals of association with pSS in the *HLA* region. Principal component analysis of clinical data distinguished two patient subgroups defined by the presence of SSA/SSB antibodies. We observed an unprecedented high risk of pSS for an association in the *HLA-DQA1* locus of odds ratio 6.10 (95% CI: 4.93, 7.54, $P=2.2\times10^{-62}$) in the SSA/SSB-positive subgroup, while absent in the antibody negative group. Three independent signals within the MHC were observed. The two most significant variants in MHC class I and II respectively, identified patients with a higher risk of hypergammaglobulinaemia, leukopenia, anaemia, purpura, major salivary gland swelling and lymphadenopathy. Replication confirmed the association with both MHC class I and II signals confined to SSA/SSB antibody positive pSS.

Conclusion. Two subgroups of patients with pSS with distinct clinical manifestations can be defined by the presence or absence of SSA/SSB antibodies and genetic markers in the *HLA* locus. These subgroups should be considered in clinical follow-up, drug development and trial outcomes, for the benefit of both subgroups.

Key words: Sjögren's syndrome, autoimmunity, gene polymorphism, autoantibodies

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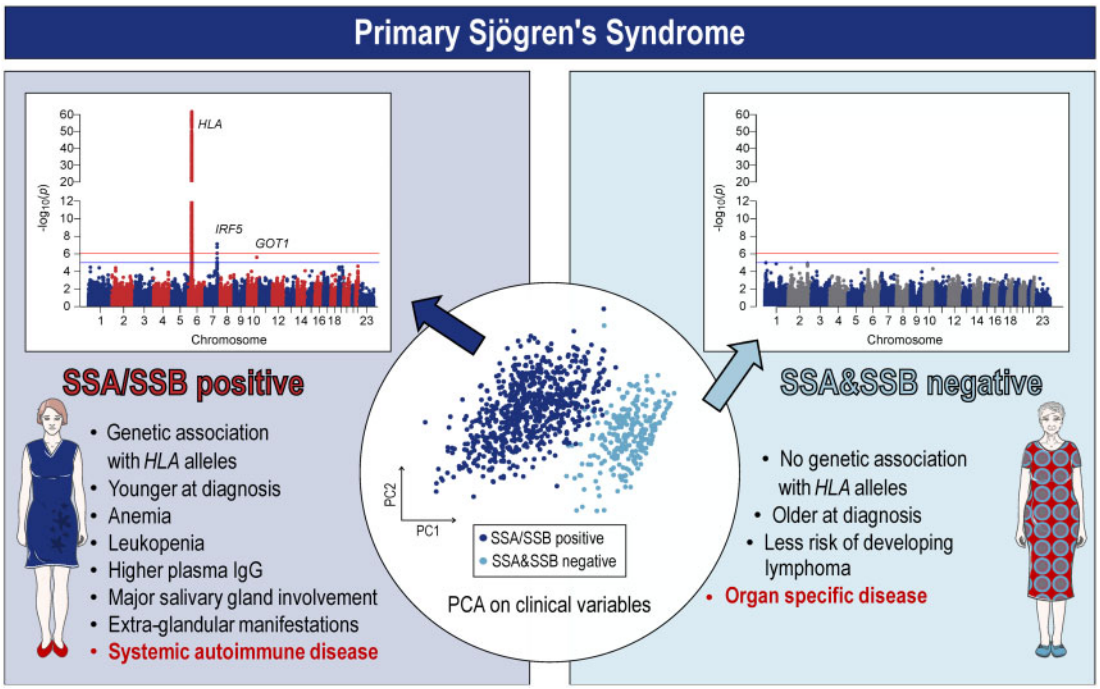
CLINICAL SCIENCE

Graphical Abstract



Rheumatology key messages

- Clinical data analysis provides evidence for two subgroups of primary Sjögren's syndrome best defined by SSA/SSB antibodies.
- Signals within the *HLA* region are unique to patients with SSA/SSB autoantibodies.
- Genetic markers of the *HLA* locus and SSA/SSB autoantibodies define two distinct subgroups of primary Sjögren's syndrome.

Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease that predominantly affects women [1, 2]. Patients are classified as having pSS when fulfilling internationally accepted criteria, but the clinical presentation varies considerably [3, 4]. While sicca symptoms, with dryness of the eyes and mouth, pain, and fatigue are common, some patients present with extra-glandular manifestations such as arthritis, purpura or interstitial lung disease. Additionally, immune-variables differ substantially among the patients. The typical disease-related autoantibodies against SSA and SSB are found in 70% and 45% of patients, respectively, and in some a mild leukopenia or hypergammaglobulinaemia may be detected [5]. Given the heterogeneity in clinical presentation of what is currently referred to as pSS, selecting patients and evaluating outcomes in clinical trials has proven difficult. A recent study suggested patient-reported outcome measures to classify patients with pSS into different subtypes [6]. Variation in clinical manifestations or outcome based on presence or absence of particular biomarkers has also been highlighted [1, 7]. However, patient-reported symptoms and some biomarkers may vary over time and these approaches do not take into account possible underlying genetic predisposition for different clinical subgroups.

During the past decade, genetic association studies have revealed several loci linked to pSS (reviewed in [8, 9]). The most prominent associations are with variants in the *HLA* region, but associations have also been found with single-nucleotide polymorphisms (SNPs) in or around other genes with immunological functions [10–13]. However, the impact of many of these

Table 1 Clinical characteristics of patients with primary Sjögren's syndrome

	All patients ($n = 982$)	Anti-SSA/SSB positive ($n = 717$)	Anti-SSA/SSB negative ($n = 265$)	*P*-value[a]
Females, %	93.1	92.1	95.8	0.037
Age, mean (S.D.), years				
At symptom onset	46.2 (14.7)	45.1 (15.1)	49.0 (13.3)	5.0×10^{-4}
At diagnosis	52.6 (13.7)	51.3 (14.0)	56.1 (12.1)	$<1 \times 10^{-4}$
Laboratory findings, %				
ANA	74.8	85.8	44.9	$<1 \times 10^{-4}$
Anti-SSA	70.5	96.5	0.0	
Anti-SSB	42.8	58.8	0.0	
Anti-SSA and/or anti-SSB	73.0	100	0.0	
Anaemia Hb <120 g/l	22.3	26.5	10.9	$<1 \times 10^{-4}$
Leukopenia $<4.0 \times 10^9$/l	30.5	36.8	13.9	$<1 \times 10^{-4}$
Thrombocytopenia $<100 \times 10^9$/l	3.7	4.3	2.0	0.15
P-IgG >15 g/l	48.8	59.6	14.5	$<1 \times 10^{-4}$
Minor salivary gland biopsies				
Focus score, mean (S.D.)	2.4 (2.4)	2.5 (2.6)	2.2 (1.9)	0.04
Germinal centre formations, %	21.8	25.5	14.2	0.013
Extraglandular manifestations, %				
Raynaud	29.1	29.1	29.0	0.98
Arthritis	19.4	20.6	16.1	0.12
Purpura	10.7	13.8	2.8	$<1 \times 10^{-4}$
Major salivary gland swelling	30.0	32.8	22.6	3.9×10^{-3}
Lymphadenopathy	9.1	10.5	5.3	0.014
Hypothyreoidism	22.5	21.3	25.4	0.21
Myositis	0.9	1.1	0.5	0.38
Interstitial lung disease	6.8	7.6	4.3	0.12
Interstitial nephritis	3.0	3.3	2.2	0.46
Lymphoma	4.6	5.5	2.3	0.036
Age at lymphoma onset, mean (S.D.), years	57.1 (13.7)	54.5 (12.4)	73.8 (9.0)	8.8×10^{-4}

[a]*P*-value for the comparison between anti-SSA/SSB (anti-SSA and/or anti-SSB) positive and anti-SSA/SSB negative patients. Continuous variables compared with Student's unpaired *t*-test, frequencies with χ^2 test.

polymorphisms in pSS pathogenesis has not been studied, nor how differences in the clinical presentation relate to genetic variants.

Using a large set of cases of well-characterized patients with pSS, the aim of this study was to investigate if genetic heterogeneity and variation in clinical phenotypes represent different disease subtypes that may require distinction for both diagnosis and treatment. We sequenced >1800 autoimmunity-related gene loci in nearly 1000 patients from Sweden and Norway and analysed clinical features of the patients focusing on immunological manifestations intersected with genetic associations.

Methods

Patients and controls

A total of 982 patients with pSS from Sweden and Norway, and 1342 healthy blood donors and population controls were included in the study (Supplementary Table S1, available at *Rheumatology* online). All patients fulfilled the American European Consensus Group (AECG) criteria for pSS [4] (Table 1 and Supplementary Table S2, available at *Rheumatology* online). For replication, an independent set of 177 patients with pSS from Sweden and Norway and 7672 controls ($n = 918$ from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) and $n = 6754$ from the Swedish Twin Registry (STR) were included (Supplementary Table S1, available at *Rheumatology* online) [14, 15]). The study was approved by the local ethics committees and patients gave written informed consent.

Targeted sequencing, genotyping and quality control

A sequence capture array was designed to target 1853 genes including their coding and regulatory regions, covering 32.2 Mbp (see Supplementary Materials and methods, Supplementary Table S3 and Supplementary Fig. S1, available at *Rheumatology* online). Genes were selected based on their known role in immunological processes, inflammation and autoimmune diseases. Sequencing libraries were prepared from genomic DNA, hybridized (Roche NimbleGen, Basel, Switzerland) and then sequenced with 100-bp paired-end reads using an Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA, USA). Samples with a mean target coverage of <10× were excluded. A set of bi-allelic single nucleotide variants (SNVs) was generated with call rate 90% for SNVs and 80% for samples. Population

Table 2 Allelic association analysis in patients with primary Sjögren's syndrome compared with healthy controls

Variant	Position	Gene/region	*P*-value	Conditional *P*-value	OR (95% CI)	RAF, cases/controls	Minor/major alleles	snpEff annotation [19]
All cases *vs* controls[a]								
rs6933289	chr6:32604551	*HLA-DQA1*	1.4×10^{-46}	—	3.88 (3.22, 4.66)	0.31/0.13	**T**/C	Upstream
rs3099839	chr6:31430065	*HCP5*	1.6×10^{-43}	2.4×10^{-6b}	3.75 (3.11, 4.52)	0.30/0.11	**T**/C	Upstream
rs4919321	chr10:101230461	*GOT1*	1.1×10^{-6}	—	1.43 (1.23, 1.64)	0.73/0.67	C/**G**	Intergenic
rs6630	chr19:4090422	*MAP2K2*	1.7×10^{-6}	—	1.82 (1.43, 2.33)	0.94/0.88	T/**G**	3′ UTR
rs11761199	chr7:128581835	*IRF5*	1.8×10^{-6}	—	1.39 (1.22, 1.61)	0.55/0.47	A/**G**	Intronic
rs7197	chr6:32412580	*HLA-DRA*	9.2×10^{-5}	1.6×10^{-13c}	1.34 (1.16, 1.55)	0.27/0.23	**T**/C	3′ UTR
SSA/SSB antibody positive cases *vs* controls[a]								
rs6933289	chr6:32604551	*HLA-DQA1*	2.2×10^{-62}	—	6.10 (4.93, 7.54)	0.37/0.13	**T**/C	Upstream
rs2523607	chr6:31322790	*HLA-B*	5.3×10^{-58}	6.8×10^{-07b}	5.27 (4.30, 6.45)	0.36/0.12	**A**/T	Upstream
rs7197	chr6:32412580	*HLA-DRA*	5.2×10^{-8}	2.6×10^{-25c}	1.56 (1.33, 1.84)	0.30/0.23	**T**/C	3′ UTR
rs3823536	chr7:128579666	*IRF5*	7.4×10^{-8}	—	1.52 (1.30, 1.79)	0.57/0.47	G/**A**	Upstream
rs4919321	chr10:101230461	*GOT1*	2.4×10^{-6}	—	1.47 (1.25, 1.72)	0.73/0.67	C/**G**	Intergenic

Independent signals with uncorrected or conditional *P*-values exceeding a suggestive significance threshold of $P < 1 \times 10^{-5}$ are shown. The risk allele is marked in bold. [a]All cases $n = 918$, SSA and/or SSB antibody positive cases $n = 663$, controls $n = 1264$. [b]Analysis passing suggestive significance threshold with rs6933289 and rs7197 as covariates. [c]Analysis passing suggestive significance threshold with rs6933289 as covariate. Chr, chromosome; OR, odds ratio; RAF, risk allele frequency; UTR, untranslated region.

outliers and related samples were excluded and 918 patients with pSS and 1264 controls remained for analysis.

The replication set was genotyped on Illumina OmniExpressExome (cases and PIVUS) and Illumina OmniExpress (STR). Quality control was performed and additional variants imputed based on the Haplotype reference consortium r1.1 (Supplementary Materials and methods, available at *Rheumatology* online) [16].

Statistical analysis

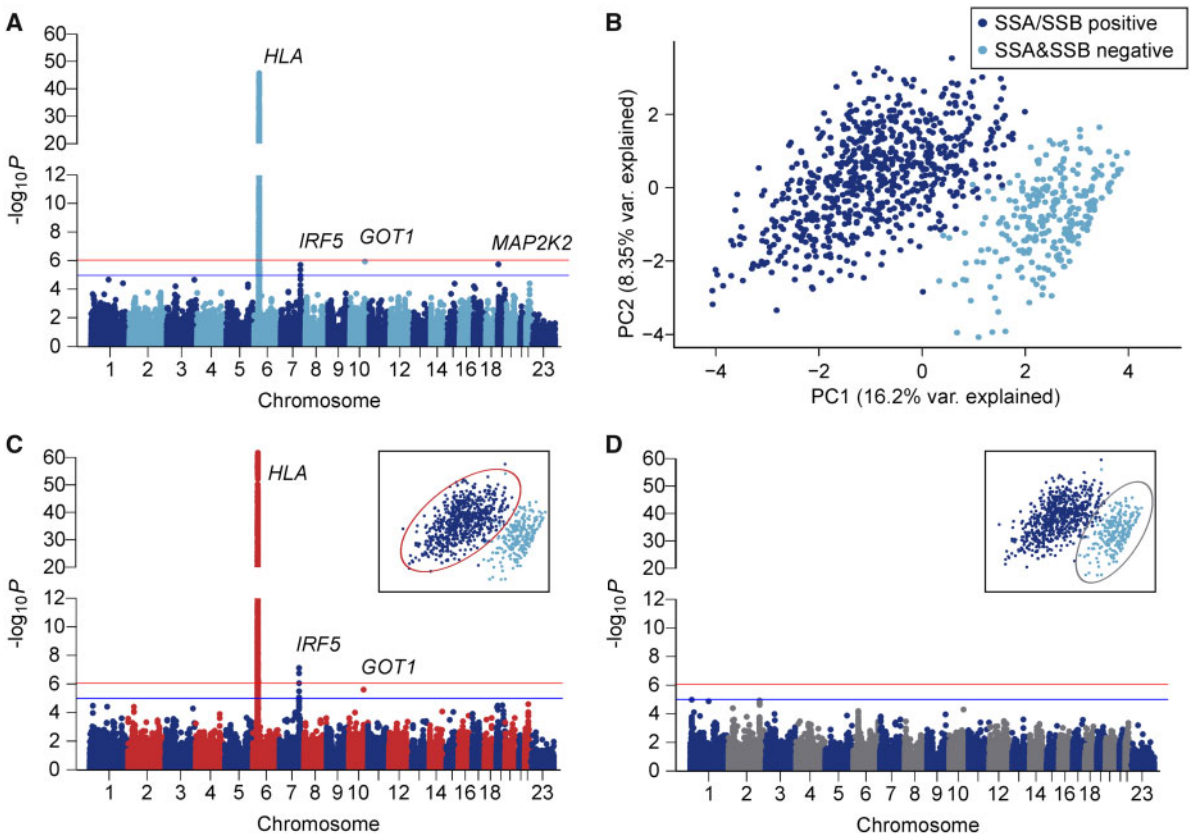
Single variant association analysis in the main set of cases and controls for variants with a minor allele frequency (MAF) of ≥1% ($n = 107\ 045$) was performed in PLINK using a logistic regression model [17]. Experiment-wide Bonferroni corrected significance was set to $P < 8.7 \times 10^{-7}$ after removal of highly correlated SNVs [57 768 SNVs remaining with linkage disequilibrium (LD) $r^2 < 0.8$] and a suggestive significance threshold of $P < 1 \times 10^{-5}$ was applied. For clinical data, continuous variables were analysed using Student's unpaired *t*-test and frequencies were compared with χ^2 unless there were <5 observations, in which case Fisher's exact test was applied (Statistica version 13.4.0.14, TIBCO Software Inc., Palo Alto, CA, USA). Correlations between clinical variables were assessed using Spearman's correlation in GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA) and results plotted in Morpheus (https://software.broadinstitute.org/morpheus/). Principal component analysis of clinical data and plotting of results was carried out in R [18]. Logistic regression analyses of genotypes and clinical variables were performed using a generalized linear model and plotted using R. Association analysis for variants selected for replication was performed in PLINK using a logistic regression model with the first three population stratification principal components as covariates (Supplementary Materials and methods, available at *Rheumatology* online).

Results

Targeted sequencing suggests novel loci and confirms known genetic variants associated with primary Sjögren's syndrome

To map the genetic variability in pSS, targeted sequencing of coding regions of 1853 immune-related genes, including their upstream and downstream regulatory regions, was performed on samples from patients with pSS and controls. Our analysis revealed strong signals of association for pSS in the *HLA* region, with the top variant in the *HLA-DQA1* locus [rs6933289; odds ratio (OR) 3.88; 95% CI: 3.22, 4.66; $P = 1.4 \times 10^{-46}$]. Suggestive associations were also found with variants in the *interferon regulatory factor 5-transportin 3* (*IRF5-TNPO3*) locus (OR 1.39; 95% CI: 1.22, 1.61; $P = 1.8 \times 10^{-6}$), as well as in two novel loci not previously associated with pSS containing the *glutamic-oxaloacetic transaminase 1* (*GOT1*) (OR 1.43;

FIG. 1 Genetic association and subgroup analysis of primary Sjögren's syndrome patients *vs* controls



(**A**) Single variant association analysis between 918 pSS cases and 1264 healthy controls. Logistic regression with minor allele frequency ≥0.01 and three principal components as covariates. A total of 107 045 variants included after quality control. Red line indicates the experiment wide Bonferroni cutoff ($P = 8.7 \times 10^{-7}$); blue line represents the suggestive significance threshold ($P = 1 \times 10^{-5}$). (**B**) PCA of clinical data collected for 982 pSS cases. (**C**) Single variant association analysis between anti-SSA/SSB positive patients (dark blue in PCA plot) *vs* controls. (**D**) Single variant association analysis between anti-SSA and SSB negative patients (light blue in PCA plot) *vs* controls. PCA: principal component analysis.

95% CI: 1.23, 1.64; $P = 1.1 \times 10^{-6}$) and *mitogen-activated protein kinase 2* (*MAP2K2*) (OR 1.82; 95% CI: 1.43, 2.33; $P = 1.7 \times 10^{-6}$) genes (Table 2, Fig. 1A, Supplementary Fig. S2 and Supplementary Table S4, available at *Rheumatology* online).

Clinical features distinguish distinct patient subgroups identifiable by unique *HLA* associations

To identify patient subgroups, we used the clinical information available for all 982 patients and performed a principal component analysis (PCA). Interestingly, this approach distinguished two clinically distinct subgroups of patients. Regression analysis revealed that the clinical variable best corresponding to the first principal component (explaining 16.2% of the overall variability in the clinical data) was SSA and/or SSB (hereafter SSA/SSB) autoantibody status (Fig. 1B and Supplementary Fig. S3A, available at *Rheumatology* online). This was confirmed using *k*-means clustering on the PCA results to reveal two groups that predicted SSA/SSB positivity with 99.3% accuracy (Supplementary Fig. S3B, available at *Rheumatology* online). The clinical variables best associated with the second principal component (explaining 8.35% of the variability in the clinical data) were the two interrelated variables age at symptom onset and age at diagnosis (Supplementary Fig. S3A, C and D, available at *Rheumatology* online).

Since subgroups within the patients were best defined by the presence of SSA/SSB autoantibodies, we stratified the patients based on SSA/SSB autoantibody status and further explored differences in their clinical presentation. Patients positive for SSA/SSB were younger at disease onset and diagnosis, and presented more frequently with anaemia, leukopenia, and hypergammaglobulinaemia. Further, SSA/SSB antibody positive patients displayed an increased prevalence of purpura, major salivary gland swelling, lymphadenopathy and lymphoma, showing an overall more severe disease phenotype compared with patients negative for both SSA and SSB autoantibodies (Table 1).

FIG. 2 *HLA* associations with primary Sjögren's syndrome

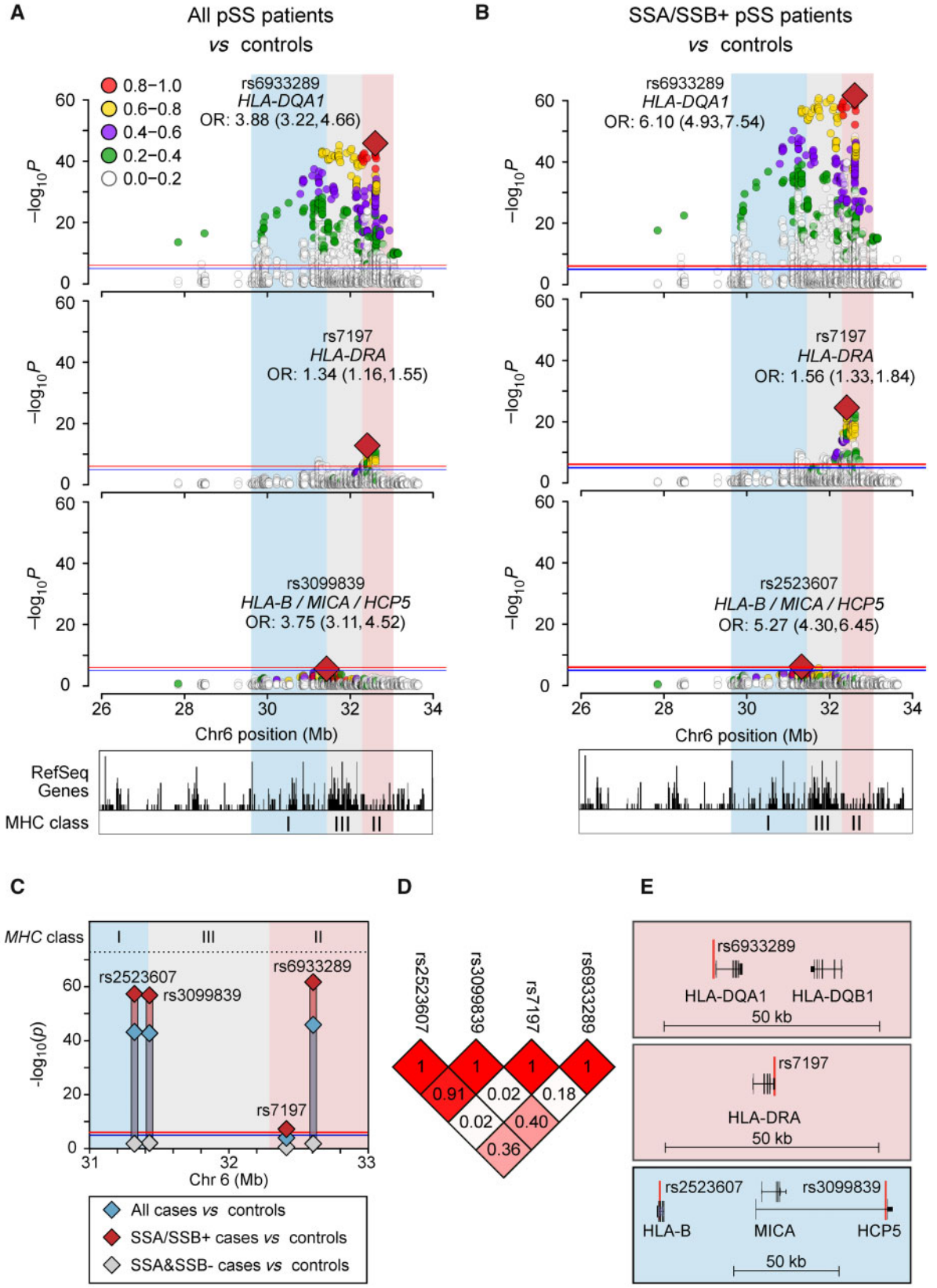
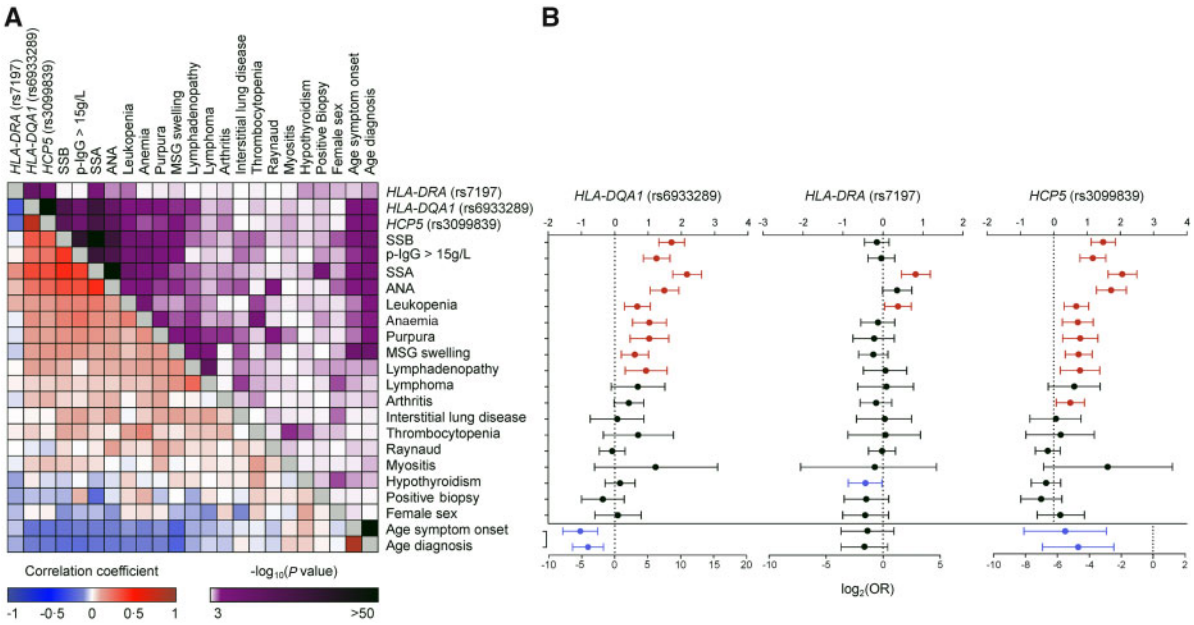


FIG. 3 Correlations between clinical phenotypes and associated variants



(**A**) Non-parametric correlations between associated genetic variants and clinical variables. The purple scale represents *P*-values for the correlation (darker represents more significant), and the blue–red scale represents the correlation coefficient, with darker blue representing stronger negative correlation, and deeper red representing stronger positive correlation. (**B**) Logistic regression of different clinical variables with risk allele count for the top associated independent genetic variants. Red lines represent significant positive associations, blue lines significant negative associations. Whiskers indicate 95% CI. OR: odds ratio.

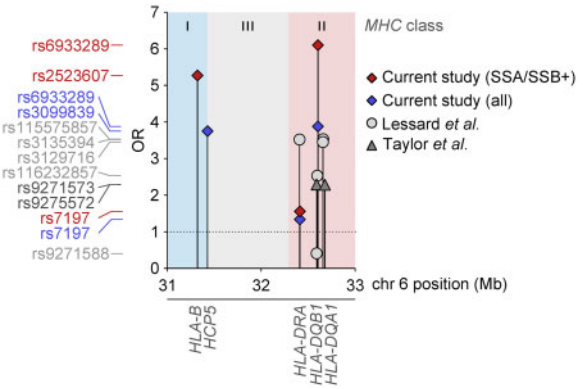
As the two identified pSS patient subgroups differed in their clinical presentation, we explored genetic associations separately for each of the two groups. When only including patients positive for SSA/SSB antibodies in the analysis and comparing them with controls, the association with variants in the *HLA* region was distinctly stronger compared with the analysis of the whole set of cases, with an OR as high as 6.1 for the top associated variant in the *HLA-DQA1* region (rs6933289; OR 6.10; 95% CI: 4.93, 7.54; $P = 2.2 \times 10^{-62}$). The association with variants in the *IRF5-TNPO3* locus was also more prominent (OR 1.52; 95% CI: 1.30, 1.79; $P = 7.4 \times 10^{-8}$), while the association with *GOT1* remained unchanged (OR 1.47; 95% CI: 1.25, 1.72; $P = 2.4 \times 10^{-6}$) and *MAP2K2* did not pass the suggestive significance threshold of $P < 1 \times 10^{-5}$ (OR 1.79; 95% CI: 1.35, 2.33; $P = 4.2 \times 10^{-5}$) (Table 2, Fig. 1C, Supplementary Fig. S4 and Supplementary Table S5, available at *Rheumatology* online).

In contrast, when comparing SSA and SSB autoantibody negative patients with controls, surprisingly no association with *HLA* was observed. In fact, no variant associated with SSA/SSB autoantibody negative pSS exceeded the suggestive significance threshold of $P < 1 \times 10^{-5}$, but two signals near *kinesin family member 1B* (*KIF1B*) on chromosome 1 (rs149524751; OR 4.55; 95% CI: 2.32, 8.92; $P = 1.0 \times 10^{-5}$) and *caspase 8* (*CASP8*) on chromosome 2 (rs17860432; OR 2.75; 95% CI: 1.74, 4.31; $P = 1.2 \times 10^{-5}$) nearly reached the suggestive cutoff (Fig. 1D, Supplementary Fig. S5 and Supplementary Table S6, available at *Rheumatology* online).

A case–case analysis between patients positive for SSA/SSB autoantibodies and patients negative for both autoantibodies identified rs9273058, a variant downstream of *HLA-DQA1* (OR 0.28; 95% CI: 0.22, 0.35; $P = 6.1 \times 10^{-26}$) as the most significantly associated variant (Supplementary Fig. S6 and Supplementary

FIG. 2 Continued
Stepwise adjustment for the top associated variants. (**A**, **B**) Logistic regression analysis of all patients *vs* controls (**A**), or anti-SSA/SSB positive patients *vs* controls (**B**). Second panel after conditioning on rs6933289, bottom panel after conditioning on rs6933289 and rs7197, with rs3099839 top remaining variant in all cases *vs* controls, rs2523607 top remaining variant in anti-SSA/SSB positive *vs* controls. (**C**) Unadjusted *P*-values for all cases *vs* controls (blue), SSA/SSB positive *vs* controls (red), and SSA and SSB negative *vs* controls (grey). (**D**) Linkage disequilibrium (r^2) between the variants. (**E**) Gene regions of the independent *HLA* variants: *HLA-DQA1*, *HLA-DRA* and the *HLA-B/MICA/HCP5* locus.

Fig. 4 Comparison of effect sizes for associated variants in the HLA region



Top variants from Lessard *et al*. [10] are shown as light grey circles and variants from Taylor *et al*. [13] are depicted as dark grey triangles. Red diamonds represent SSA and/or SSB positive primary Sjögren's syndrome associations and blue diamonds represent the full primary Sjögren's syndrome associations from the current study. Position on chromosome 6 is shown on the *x*-axis and ORs on the *y*-axis. The nearest genes are labelled below. OR: odds ratio.

Table S7, available at *Rheumatology* online). The *HLA-DQA1* variant most strongly associated with SSA/SSB positive pSS (rs6933289) remained significantly associated in the case–case comparison of the two patients subgroups (OR 4.03; 95% CI: 2.97, 5.47; $P = 3.0 \times 10^{-19}$), despite the lower power. This demonstrates that the association with the *HLA* region in pSS is unique to the SSA/SSB autoantibody positive patient subgroup, providing evidence for distinct genetic aetiologies in the two groups.

Independent genetic signals within the *HLA* region are unique to patients with SSA/SSB autoantibodies

Considering that the associations with variation in the *HLA* domain differ between the two pSS subgroups, we examined the *HLA* region in more detail. When conditioning on the top variant in the *HLA-DQA1* locus (rs6933289) in the analysis of all patients with pSS *vs* controls and SSA/SSB positive cases *vs* controls, independent signals with the highest peak in *HLA-DRA* (rs7179) remained (for all patients *vs* controls, OR 1.85; 95% CI: 1.57, 2.17; $P = 1.6 \times 10^{-13}$; and for SSA/SSB positive cases *vs* controls, OR 2.85; 95% CI: 2.34, 3.47; $P = 2.6 \times 10^{-25}$), passing the experiment-wide Bonferroni correction cutoff of $P < 8.7 \times 10^{-7}$ (Table 2, Supplementary Tables S8 and S9, available at *Rheumatology* online). Further, conditioning on the top SNVs from both independent signals revealed a third association signal in the *HLA-B/MICA/HCP5* locus exceeding the suggestive significance threshold of $P < 1 \times 10^{-5}$ (rs3099839 in *HCP5* for all cases *vs* controls, OR 2.07; 95% CI: 1.53, 2.80; $P = 2.4 \times 10^{-6}$; and rs2523607 in *HLA-B* for SSA/SSB positive cases *vs* controls, OR 2.14; 95% CI: 1.59, 2.89; $P = 6.8 \times 10^{-7}$). These signals are in high LD ($r^2 = 0.91$) (Table 2, Fig. 2A–D, Supplementary Tables S10 and S11, available at *Rheumatology* online). Each of these three independent HLA signals were more significant in the SSA/SSB positive cases compared with the analysis of all patients (Fig. 2C). The LD between the independent signals and the positions of the nearest genes is depicted in Fig. 2D and E. Together, these data suggest several independent *HLA* associations in pSS, which all are unique to patients with SSA/SSB autoantibodies.

Genetic variants identify patients with extraglandular manifestations

To examine the interplay between the three independently associated HLA genetic variants and clinical presentation, we performed a non-parametric correlation analysis between risk allele counts and clinical variables (Fig. 3A and Supplementary Table S12, available at *Rheumatology* online). This analysis revealed significant correlations between *HLA-DQA1* (rs6933289) and *HCP5* (rs3099839) and a number of clinical variables including younger age at symptom onset and diagnosis, ANA, SSA and SSB autoantibodies, hypergammaglobulinaemia, leukopenia, anaemia, purpura, major salivary gland swelling and lymphadenopathy ($-0.10 < \rho > 0.10$, $P < 0.05$). These correlations mirror the grouping according to SSA/SSB antibody status previously identified in the clinical data.

To further examine the associations between genotypes and phenotypes, we also performed a logistic regression analysis between the top associated variants and the clinical variables (Fig. 3B). The *HLA-DQA1* and *HCP5* risk variants significantly increased the OR of the clinical variables previously shown to co-occur with SSA/SSB autoantibodies, emphasizing the phenotype identified in the SSA/SSB positive patient subgroup. In contrast, the *HLA-DRA* risk variant rs7197 was only associated with an increased OR for SSA and leukopenia, and with a significantly reduced OR for hypothyroidism. In all, we conclude that carrying the risk alleles at *HLA-DQA1* and *HCP5* predicts distinct clinical manifestations of pSS and the presence of SSA/SSB autoantibodies.

Replication analysis in additional Scandinavian patients and controls

To replicate the MHC class I association at *HLA-B/HCP5*, and the suggestive associations with *GOT1* and *MAP2K2*, an independent set of 177 Scandinavian pSS cases ($n = 153$ SSA/SSB antibody positive) and 7672 controls were included. We confirmed the association between *HLA* and pSS being confined to the SSA/SSB autoantibody positive subgroup. The MHC class I association *HLA-B* (rs2523607)/*HCP5* (rs3099839) replicated in all patients *vs*

controls (rs3099839; OR 3.47; 95% CI: 2.74, 4.41; $P = 1.6 \times 10^{-24}$) and SSA/SSB positive patients *vs* controls (rs2523607; OR 3.83; 95% CI: 2.98, 4.92; $P = 5.3 \times 10^{-26}$). There were no significant associations with *GOT1* (rs49193219) or *MAP2K2* (rs6630), in the analyses of all patients with pSS or SSA/SSB antibody positive patients *vs* controls. The results are presented in Supplementary Table S13, available at *Rheumatology* online.

Discussion

pSS is a heterogeneous disease with an apparent need for biomarkers to identify patient subgroups for monitoring, prognosis, treatment and inclusion in clinical trials. Using a combination of genetic information and analysis of extensive clinical information for nearly a thousand Scandinavian patients, we detected clear signs of grouping in the patient data, evident in both clinical manifestations and genetic associations. We found that pSS with associated variants in the *HLA* region is unique to a subgroup of patients, best identified by the presence of the hallmark SSA/SSB autoantibodies, and that the top variant is associated with a six times increased risk of pSS in this group. Further, to the best of our knowledge, this is the first time a direct correlation between *HLA* risk variants and specific clinical features in patients with pSS has been described.

Notably, we detected clear grouping of the patients, with SSA/SSB antibodies and age at disease onset and diagnosis being the factors explaining most of the variability in the data. Earlier reports confirm the validity of the observation as patients positive for SSA/SSB antibodies are known to have an earlier disease onset and present with more systemic extraglandular manifestations, such as leukopenia, hypergammaglobulinaemia, purpura, and major salivary gland swelling [1]. Furthermore, in a recent epidemiological study we found that patients positive for SSA/SSB antibodies are particularly at increased risk for cardiovascular disease, highlighting the importance of subgroup stratification in clinical monitoring and risk assessment [20].

Combining high resolution variant information, together with the observation of clinical clustering of patients, we could demonstrate an unprecedented high odds ratio of 6.10 for developing pSS compared with the general population in carriers of the associated variant rs6933289 in the *HLA-DQA1* region, when restricting the analysis to the identified SSA/SSB antibody positive subgroup. Notably, there was no risk of SSA/SSB antibody negative pSS associated with the same genetic variant. Previous studies in Caucasians have described OR of different genetic variants of the *HLA* locus in the range of 2–3.5, depicted in Fig. 4 [10, 13].

Different strategies in subgrouping patients with pSS can be applied. A recent study defined four different subgroups based on patient-reported outcome measures of levels of pain, dryness, fatigue, depression and anxiety [6]. The subgroups differed in laboratory parameters and gene expression as well as response in clinical trials in retrospective analysis of pain, dryness and fatigue scores, and salivary flow measurements. Our approach of letting clinical data guide subgrouping of pSS clearly identifies distinct groups also at the genetic level. We believe these two subgroups defined by the presence or absence of SSA/SSB antibodies, demarking autoimmune disease processes, need to be considered before, or in addition to, applying additional markers for further subgrouping. The genetic differences are likely to relate to different pathogenic mechanisms, and this knowledge will be valuable both for continued efforts in understanding the mechanisms driving development of the respective subtypes and for designing treatment strategies for each group. Further, with an OR higher than 6 detectable by a single variant, the precision approaches clinical usability, at least in the population studied (Caucasian).

The hitherto few genome-wide association studies performed in pSS have not stratified patients according to autoantibodies or clinical manifestations [10, 11, 13]. The association of *HLA* variants with the presence of SSA/SSB autoantibodies has previously been described in several smaller studies including patients of different ethnicities [21–23]. However, none of these studies described associations between *HLA* and clinical features, either because the question was not addressed or possibly because of lack of power [21]. Here we extend our knowledge by establishing a direct link between specific *HLA* risk variants and clinical manifestations. This implicates that *HLA* risk variants not only drive the autoantibody response, but also predict the various disease manifestations seen in patients with pSS.

In our study we confirmed the association between variants in *IRF5* and pSS, previously established in multiple ethnicities (reviewed in [8, 9]). Additionally, we found associations with *GOT1* and *MAP2K2* passing our suggestive significance threshold. The variants near *GOT1* are located in the intergenic region between *GOT1* and *NKX2-3*, a gene encoding a NKX2 homeobox protein, necessary for marginal-zone B cell development and implicated in lymphomagenesis [24]. This is intriguing given the increased risk for B cell lymphoma in pSS. Gene variants in *GOT1-NKX2-3* have previously been associated with inflammatory bowel disease [25]. *MAP2K2* encodes a protein kinase not previously associated with autoimmune diseases. However, we were not able to confirm these associations in a small independent Scandinavian pSS set of cases. To clarify the role of *GOT1* and *MAP2K2* variants in pSS susceptibility, these results need confirmation in additional samples.

The mechanisms that explain the high risk of developing pSS with SSA/SSB autoantibodies in carriers of specific *HLA* variants remain to be understood. While the HLA proteins have their main role in antigen presentation, many additional genes are encoded within the locus and understanding which genes, or combination of genes and their respective variants, drive the autoimmune reaction will require detailed mechanistic investigations. The SSA and SSB antigens are RNA-binding

proteins which together with SSA/SSB autoantibodies form immune complexes that induce type I IFN production, eliciting an immune response [26, 27]. The upregulated expression of IFN-induced genes, i.e. the IFN signature, is mainly seen in SSA/SSB positive patients, which is also supported by observations from studies on epigenetic regulation [28, 29]. These autoantibodies appear before pSS is clinically apparent [30]. Notably, an association with *HLA* class II alleles in patients with different systemic autoimmune diseases and an IFN signature has been described [31]. Consequently, activation of the type I IFN system as an aetiopathogenic mechanism for clinical disease in SSA/SSB antibody positive patients has been proposed, and variants of *IRF5* may amplify this process, while it is likely that other immune mechanisms operate in the antibody negative subgroup of patients with pSS [27, 32, 33]. Further studies into the pathogenic mechanisms behind antibody negative pSS are warranted, to discover potential therapeutic targets for this patient subgroup.

Limitations of our study include the low frequency of certain clinical manifestations and missing data, possibly precluding some additional associations with SSA/SSB antibodies or *HLA* risk variants. Unfortunately, data on the severity of dryness, pain, fatigue, anxiety or depression, which are common manifestations in patients with pSS, were not available. Antibody data were retrieved from the medical records and analysed according to routine clinical immunology methods that may have varied over time, but SSA/SSB antibody status in pSS is stable through the disease course [34]. The targeted sequencing approach precludes identification of novel risk genes not included in the panel. Future studies should aim at whole genome sequencing to fully elucidate the genetic background to pSS and its subphenotypes. Strengths of our study are the large number of patients included, the homogeneous genetic background, meticulous evaluation of diagnostic criteria and detailed clinical data collected in a similar manner by the participating clinicians.

In conclusion, we define two subgroups of patients with pSS based on *HLA* association, SSA/SSB antibodies and clinical manifestations, and demonstrate a direct correlation between *HLA* risk variants, age of onset and several clinical features. The SSA/SSB antibody positive subgroup clearly presents with a systemic autoimmune disease. In contrast, the patients negative for these antibodies and lacking the *HLA*-associated genetic features could be defined as having an organ-specific disease with less obvious autoimmune features and potentially a different underlying pathogenesis, not least in terms of the predisposing genetic makeup. These differences need to be considered not only during clinical follow-up, but also in drug development and when designing clinical trials and determining trial outcomes, for the benefit of all patients with pSS.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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