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THE CHALLENGES AND COMPLICATIONS OF LIVING WITH SJÖGRENS SYNDROME

by

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The Challenges and Complications of Living with Sjögren's Syndrome

Annie Chou

This work was aimed at understanding the challenges and complications of living with Sjögren's Syndrome (SS). We examined health-related quality of life (HRQoL) and utilization of dental care among individuals with SS. Additionally, we sought to identify genetic risk variants associated with germinal center-like structures in labial salivary glands among individuals with SS. In these studies, we utilized the well-characterized Sjögren's International Collaborative Clinical Alliance (SICCA) registry and applied the 2012 American College of Rheumatology classification criteria for SS. Various statistical tools were used in these studies including multivariate linear and logistical regression models, Mann-Whitney U test, and chi-square test. Statistical interaction, stratification, and genetic association analyses were also explored in our analyses. We found that individuals with SS had better physical and mental HRQoL and less depression when compared to SICCA participants without SS. Although individuals with SS had more dental needs than individuals without SS, they had less dental care utilization. And finally, we showed that individuals with SS and GC-like structures in their labial salivary glands were more likely to harbor genetic risk variants within *B-cell activating factor (BAFF)* and *tumor necrosis factor, alpha-induced protein 3 (TNFAIP3)*. Therefore, individuals with SS experience significant health care challenges and complications. Fortunately, these can be managed, and even prevented, with early intervention by a multidisciplinary team of health care professionals consisting of rheumatologists, ophthalmologists, dentists, and oral medicine specialists.

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Introduction

Understanding the health care needs of individuals living with Sjögren's Syndrome (SS) is essential due to their growing numbers amidst an aging worldwide demographic. SS is a chronic autoimmune disorder characterized by symptoms of oral and ocular dryness due to inflammation and decreased function of the salivary and lacrimal glands. Previously, SS was subdivided into primary SS, which occurs alone, while secondary SS occurs in the presence of another autoimmune connective tissues disease, most commonly rheumatoid arthritis (RA) or systemic lupus erythemaotosus (SLE). However, this distinction became irrelevant with discovery of the overlapping nature of autoimmune diseases components and inadequate diagnostic criteria for primary SS.

Individuals with SS typically develop a serological profile of autoantibodies against ribonucleoproteins Ro/SSA (and sometimes La/SSB)[1], antinuclear antibodies (ANA), and rheumatoid factor (RF), although ANA and/or RF positivity are not specific for SS and are observed among many individuals with other autoimmune diseases. There is a strong female propensity for SS. The female to male ratio can be as high as 20:1 with the age of onset usually in the 4th and 5th decades. SS prevalence ranges from 0.5 to 3.0 percent in adult women, making it one of the more common autoimmune disorders affecting women in the US (2, 3). Oral and ocular dryness are hallmark symptoms of SS; however, with disease progression, 5-10% of individuals with SS can develop extraglandular manifestations, which can include severe fatigue, cutaneous vasculitis, musculoskeletal pain, and neuropathy (4-6). Due to the progressive nature of this autoimmune disease, SS is associated with some morbidity; however, most sequelae of SS can be managed, and even prevented, with early intervention by a

[1] Baer *et al.* found that the presence of anti-SSB/La serology had no significant association with key phenotypic features of SS (1).

multidisciplinary team of health care providers consisting of rheumatologists, ophthalmologists, dentists, and oral medicine specialists.

As one of the early and more common manifestations, salivary gland hypofunction interferes with basic daily functions, such as eating and speaking. Quantitative and qualitative reductions in salivary flow increase susceptibility to dental caries and oral yeast infections among individuals with SS (5-9). Similarly, decreased and altered tear secretion in SS can lead to ocular symptoms of foreign body sensation, pain, and inability to tear. These changes are associated with significant complications such as visual impairment, corneal ulceration, and infection (10). Most therapeutic options for SS are palliative in nature and are insufficient to prevent or halt exocrine and extraglandular damage. In fact, several studies have shown significant reductions in overall quality of life in SS patients due to poor physical functioning (11, 12) and emotional well-being (13). Thus, improving health-related quality of life has emerged as an important clinical outcome in patient-centered care in SS. Health-related quality of life (HRQoL) is a multi-dimensional concept that describes the aspects of quality of life, including self-perceived well-being and functionality, that relates specifically to a person's health. Recently, new classification criteria for SS were endorsed by the American College of Rheumatology (ACR) requiring fulfillment of at least 2 objective measures of oral, ocular, and systemic/autoimmune features for a diagnosis of SS (14). With recent advances in SS treatment modalities, including intravenous anti-CD20 therapies (15) and potential newer modalities, it becomes imperative to identify individuals with reduced HRQoL so they may benefit from current and future therapies while minimizing health complications from such treatment due to misclassification of symptomatic individuals.

Access to oral health care is essential in this population. More than one-third of SS individuals report feelings of dryness in their mouth, or xerostomia, as one of their initial symptoms and is one of the most frequent manifestations of SS (8). The predominant effects of SS on the oral cavity are a consequence of hyposalivation. The loss of oral lubrication from

salivary hypofunction causes many SS patients to complain of difficulty speaking, swallowing, and chewing foods. In addition, the lack of saliva causes fissuring and erythema of the mucous membranes from overgrowth of oral yeast, change/loss in taste, and halitosis. Most dental complications, such as tooth decay, are exacerbated by the lack of lubrication and barrier provided by saliva, which reduces acidity of the oral environment and stimulates dental remineralization (16, 17). Since caries prevention is preferable to restorative dental treatment, it is important to emphasize bi-annual dental cleaning and examination, high concentration fluoride toothpastes, and optimal daily oral hygiene among this high-risk group. As needed, these can be supplemented by daily fluoride rinses. However, the availability and access to preventative health care is often limited, and individuals with SS must rely on dental restorations and extractions as treatment, which are often not fully covered by insurance companies. Since SS is an international phenomenon, significant differences in health care systems among countries poses a significant barrier to affordable and uniform access to dental care for SS patients worldwide. Much of the differences in access to care may be attributed to differences in health care system organization since the range of services covered and amount of out-of-pocket expenses applies to those services can vary across countries. Thus, the lack of access to dental care among this high-risk group exposes the burden of SS such that improved access to care can be a major goal of future legislative action.

Another complication associated with SS, and by far the most serious, is development of lymphoma. As states earlier, individuals with SS typically develop a serologic profile of autoantibodies directed against Ro/SSA and La/SSB, ANA, and RF (18). This B-cell hyperactivity places individuals with SS at increased risk for the development of non-Hodgkin's lymphoma, particularly of the mucosa-associated lymphoid tissue (MALT) subtype, for which individuals with SS have an estimated 9-44 fold increased risk compared to healthy controls (19-23). Compared to other rheumatologic diseases, individuals with SS have the greatest risk for development of lymphoma (24), highlighting the need to better understand biologic

mechanisms responsible for this serious complication. Recent work provides evidence that germinal center (GC)-like structures in labial salivary glands (LSG) may serve as precursors to lymphoma in SS. GC are aggregates of B-cell generation and have also been implicated as sites of immune dysregulation in autoimmune disorders (25, 26). Ectopic GC-like structures within LSG have been described in approximately 25% of individuals with SS, and their formation is usually preceded by the presence of focal lymphocytic sialadenitis (27, 28). The precise mechanisms that contribute to development of these ectopic structures in SS remain unclear. However, given the association of GC-like formation with serologic markers (29) and genetic risk factors of lymphoma (30, 31), GC-like formation is an attractive target for further investigation in SS given its location within a target tissue and their development years before the transformation to lymphoma, facilitating earlier detection and intervention for high-risk patients.

In the following chapters, we take advantage of the large cohort provided by the Sjögren's International Collaborative Clinical Alliance (SICCA) registry to explore several research questions. The primary goals of the registry were to: 1) develop standardized diagnostic criteria for SS and 2) establish an international SS data registry and bio-repository for clinical data and biospecimens (tears, whole and parotid saliva, serum, plasma, and minor salivary glands) collected from individuals with suspected or diagnosed SS. Enrollment occurred from 2004 through 2012 within 9 academically based research sites located in 7 countries. These sites were located at the University of Buenos Aires and German Hospitals, Buenos Aires, Argentina; Peking Union Medical College Hospital, Beijing, China; Copenhagen University Hospital Glostrip, Denmark; Aravind Eye Hospital, Madurai, India; Kanazawa Medical University, Ishikawa, Japan; Kings College London, United Kingdom; John Hopkins University (JHU), Maryland, USA; University of Pennsylvania (UPenn), Philadelphia, USA; and University of California, San Francisco (UCSF), USA, where both the data coordinating center and

biorepository are currently located. Early characteristics of this cohort were previously described (32)

To be eligible for the SICCA registry, an individual had to be at least 21 years of age and must have met at least one of the following conditions: complaint of dry eyes or dry mouth; previous diagnosis or suspicion of SS (primary or secondary); elevated ANA titer, positive RF, or anti-SSA (Ro) and/or anti-SSB (La); bilateral parotid enlargement in a clinical setting of SS; a recent increase in dental caries without the presence of other risk factors; or diagnosis of RA or SLE. Individuals were excluded if they have a known diagnosis of Hepatitis C, human immunodeficiency virus (HIV), sarcoidosis, amyloidosis, active tuberculosis, graft versus host disease, or autoimmune connective tissue diseases other than RA or SLE; past head and neck radiation treatment; current treatment with daily eye drops for glaucoma; corneal surgery in the last 5 years to correct vision; cosmetic eyelid surgery in the last 5 years; or a physical or mental condition that may interfere with participation in the study. Contact lens wearers were asked to discontinue wear for 7 days before the SICCA examination. Individuals taking prescription drugs that may affect salivary or lacrimal secretion were not excluded but their use and all other current medications were recorded.

Using this unique resource, we had 3 primary thesis objectives: 1) To assess the relationship between SS and health-related quality of life; 2) To assess the relationship between SS and utilization of dental care; and 3) To assess the relationships between GC-like formation in minor salivary glands with serologic markers, clinical manifestations, and genetic associations with lymphoma among individuals with SS.

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Health-Related Quality of Life and Depression Among Individuals Participating in the Sjögrens International Collaborative Clinical Alliance (SICCA) Registry

Introduction

Individuals with Sjögren's syndrome (SS) have poor health-related quality of life (HRQoL), a term reflecting self-perceived well-being and functionality (1-10). Many studies have shown that individuals with SS experience significantly poorer physical capacity (1, 11), reduced productivity (12), and more cognitive impairments (13) compared to healthy controls. These characteristics are often correlated with diminished HRQoL and higher depression levels (9, 10, 13-16) in SS and comparable to those observed in fibromyalgia, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) (8, 10). There is no curative treatment for SS; however, current therapies for the symptomatic relief of dry eyes and dry mouth are available and reasonably effective in improving symptoms and overall HRQoL (17, 18). Early trials with biological agents, such as anti-CD20 therapies (19), in SS have shown some potential in reducing disease damage and improving patient symptoms.

Recently, EULAR (European Union League Against Rheumatism) developed a SS disease activity index, ESSDAI (EULAR Sjögrens Syndrome Disease Activity Index), to grade disease activity in patients with primary SS based on physician-assessment of 12 domains (20). Early studies showed that high ESSDAI scores (i.e., greater disease activity) were associated with poor health status based on a quality of life measure developed by the EuroQol Group, the EQ-5D (21). Concomitantly, new classification criteria for SS were developed by the Sjögren's International Collaborative Clinical Alliance (SICCA) investigators and endorsed by the American College of Rheumatology (ACR) in 2012 to establish tests that could objectively measure systemic/autoimmune, oral, and ocular characteristics (22). Fulfilling at least 2 of the 3 objective tests satisfies the ACR criteria for SS. Given the importance of HRQoL and depression

as an outcome, there is need to determine whether having SS is associated with lower HRQoL and higher measures of depression using the new ACR classification criteria. With the emergence of biological agents as treatment for SS, it becomes important to identify individuals with SS whose HRQoL since altered HRQoL should be a consideration when initiating a drug with potential toxic side effects.

Our study utilized the large well-characterized cohort provided by the *Sjögren's International Collaborative Clinical Alliance* (SICCA) registry to better assess the burden of SS on HRQoL using the *12-item Short Form, version 2* (SF-12v2) health survey and of depression using the *9-item Patient Health Questionnaire* (PHQ-9). The SF-12v2, a validated alternative to its longer predecessor the *36-item Short Form* (SF-36) health survey, was created for large sample-size studies that seek to provide overall assessment of physical and mental health. The SF-12v2 measures HRQoL by assessing 8 health sub-domains to produce a *physical component summary (PCS)* and *mental component summary (MCS)* score. The PHQ-9 survey is a concise, self-administered screening and diagnostic instrument used by clinicians to measure and monitor severity of depression. Thus, our primary study objective was to determine whether individuals with SS have lower HRQoL and higher depression scores than those without SS.

Methods

Study sample

Data was obtained from the SICCA registry. The cohort (23) and eligibility criteria are described in more detail in the Introduction. This analysis was based on women enrolled in the SICCA registry and for whom data was available for analysis as of September 30, 2013 after all data collected as part of the registry had undergone comprehensive clean-up.

HRQoL: SF-12v2 Health Survey

SICCA participants completed the *12-item Short Form, version 2* (SF-12v2) survey, which is a self-report health survey covering the previous 4 weeks (24, 25). The SF-12v2 survey is adapted from its parent survey, SF-36, to meet the demand of a brief health survey for inclusion in large-scale studies. This general health survey comprises of 12 items representing 8 sub-domains of functional health and well-being: physical functioning (2 items), role limitations due to physical problems (2 items), bodily pain (1 item), general health (1 item), vitality (1 item), social functioning (1 item), role limitations due to emotional problems (2 items), and mental health (2 items). From these 8 health sub-domains, two summary scores can be calculated, the *Physical Component Summary* (PCS) and *Mental Composite Summary* (MCS). Sub-scale and summary scores range from 0 to 100, with higher scores indicating better health. PCS and MCS are standardized to a mean of 50 and standard deviation of 10 and compared to a 2009 US general population normative sample provided by QualityMetric (24, 25). For this analysis, we examined changes in PCS and MCS values between chosen groups and did not compare values to the US general population normative sample since it is not an adequate comparison group for this international study. SS is predominantly a disease of women whereas the US general population normative sample for the SF-12v2 consists of an equal representation of men and women living in the US (24, 25).

Depression: 9-item Patient Health Questionnaire (PHQ-9)

Depressive symptoms were assessed using the 9-item Patient Health Questionnaire (PHQ-9) (26), which was also featured on the SICCA baseline questionnaire. The PHQ-9 is used by health professionals to screen for major depressive disorders and to monitor treatment covering the previous 2 weeks. The PHQ-9 is brief and often used in a clinical setting since it can be completed by the patient in minutes and rapidly scored by the clinician to determine degree and changes in depressive symptoms. A cumulative PHQ-9 score is obtained through

the collection of points amassed from each of the nine questions, ranging from 0-3 points, for a summed score within the range of 0 to 27. The PHQ-9 yields an index of depressive symptom severity with scores of 0-4 indicating no depression, 5-9 mild depression, 10-14 moderate depression, 15-19 moderately severe depression, and 20-27 severe depression.

SS Classification

Basic demographic information, frequency and severity of symptoms, HRQoL, and depression data were collected from the SICCA baseline questionnaire. All data was self-reported. In addition, trained SICCA team clinicians (including oral medicine specialists, ophthalmologists, and rheumatologists) performed focused protocol-driven clinical evaluations, which assessed for oral, ocular, and rheumatological features. All serological test results were obtained from the same Quest laboratory. SICCA questionnaires, data collection forms, and protocols are available for review at http://sicca.ucsf.edu.

The SS classification used in this study was developed by the SICCA investigative team and endorsed by ACR (22), which requires fulfillment of at least two of the following requirements: 1) positive serum anti-SSA (Ro) and/or anti-SSB (La) or [positive RF and ANA titer ≥ 1:320]; 2) ocular staining score ≥ 3 using lissamine green or fluorescein to diagnose keratoconjuncitivitis sicca (27); or 3) presence of focal lymphocytic sialadenitis with a focus score ≥ 1 focus/4mm^2 in their labial salivary gland biopsy (28). Individuals meeting at least 2 of the 3 criteria were classified as having SS (SS +) whereas individuals meeting none or 1 of the criteria were classified as not having SS (SS -).

Demographics and other systemic comorbidities

Information on gender, age (20-39, 40-59, ≥ 60 years), highest level of education (high school, college), employment (employed, unemployed), country of residence (Argentina, China, Denmark, Japan, India, United Kingdom, United States), and self-reported ethnicity (Caucasian,

Hispanic, African American, Asian, Native American) were collected from the self-administered questionnaire. The presence of other systemic diseases (yes, no) was collected and confirmed by treating physicians who were contacted in writing when participants answered positively about specific systemic conditions. These conditions included RA, SLE, scleroderma, other connective tissue disease, Graves disease, Hashimotos thyroiditis, interstitial nephritis, primary biliary cirrhosis, autoimmune hepatitis, renal tubular acidosis, glomerulonephritis, lymphoma, hypothyroid, hyperthyroid, other thyroid, other liver, other renal, myxedema, and autoimmune or interstitial lung disease.

Statistical Analysis

Baseline characteristics of our SS sample were described using frequencies and percentages. HRQoL scores for the 8 SF-12v2 sub-scales and 2 component summary scores (PCS and MCS) were summarized by the mean, standard deviation, median, and interquartile range.

To determine individual predictors of HRQoL and depression, linear regression models were utilized to assess for marginal associations between these outcome measures with SS classification and other chosen variables. Multivariate linear regression models were also fitted to explore the effects of potential confounding variables, including demographic and socioeconomic variables, on these associations and their interactions. Candidate variables were limited to those that were marginally associated with these outcomes at the 10% significance levels. Variables were retained in the final model if they were found to be associated with the outcome at a significance level of at most 5% or if their removal resulted in a ≥10% change in estimated coefficients for the remaining covariates. A likelihood ratio test of interaction was performed to determine possible variation in scores due to interaction between country of residence and SS classification. Age and gender were adjusted in all models, regardless of statistical significance level. Diagnostic analyses were conducted to assess linearity, normality

of residuals, multicollinearity, and outliers. All analyses were conducted using Stata, version 12.0.

Results

Sample Characteristics

The majority of patients were female (91%), between the ages of 40 and 59 (50%), attended college (60%), and were currently unemployed (53%) (Table 1). Most of the SICCA participants were recruited from the US (37%), followed by Denmark (17%), Argentina (13%), and Japan (10%). Less than 10% of participants resided in China (9%), UK (9%), and India (5%). Fifty-four percent of SICCA participants were Caucasian, 29% reported to be of Asian/Pacific Islander ancestry, and 11% reported to be Hispanic. Less than 10% reported African American or Native American ancestry. Approximately one-third of participants were taking anti-cholinergic medications, and a quarter had other systemic diseases in addition to SS. Forty-five percent of SICCA participants fulfilled the ACR classification criteria for SS, including 21% who fulfilled the criteria with 2 positive objective tests and 24% with 3 positive objective tests. Fifty-six percent had 1 or none of the objective tests considered in the ACR criteria.

PHQ-9 and MCS/PCS SF12-v2 HRQoL Values

Forty percent of SICCA individuals did not report any depressive symptoms based on the PHQ-9 score (Table 2). Twenty-eight percent of individuals with SS had mild depression, followed by 17% with moderate depression, and 14% with significant depression. The mean PCS and MCS was 42.59 ± 11.03 and 46.01 ± 10.64, respectively (Table 3). The range of SF-12v2 sub-domain scores ranged from 41.47 ± 10.91 to 46.04 ± 11.43, with lowest value in the general health sub-domain and the highest value in vitality sub-domain.

Individual Predictors of Physical HRQoL, Mental HRQoL, and Depression

Univariate linear regression analyses were performed to identify individual predictors of HRQoL and depression. No significant difference in mean PCS was seen between fulfilling 1 SS criterion versus no criteria ($\beta = 0.12$, $p = 0.82$), so these subcategories were combined together as not having SS. Because fulfilling 2 and 3 SS criteria were both associated with a higher mean PCS score ($\beta = 1.75$, $p < 0.0001$ and $\beta = 4.89$, $p < 0.0001$, respectively) when compared to not having SS, these subcategories were combined together as having SS. Thus, a comparison between having SS and not having SS was examined, and we found that having SS was associated with higher mean PCS compared to not having SS (Table 4). Other individual predictors of higher mean PCS included younger age (between the ages of 20-40) and current employment. Living in Argentina, China, and Japan were associated with higher PCS compared to living in the US. Also, not taking anti-cholinergic medication and absence of other systemic diseases were associated with higher mean PCS.

No significant difference in mean MCS was seen between fulfilling 1 SS criterion versus no criteria ($\beta = -0.60$, $p = 0.25$), so they were combined as not having SS. Fulfilling 2 and 3 criteria were also associated with a similar mean MCS score ($\beta = 1.99$, $p < 0.0001$ and $\beta = 2.29$, $p < 0.0001$, respectively) when compared to not having SS, so they were combined together as having SS. Thus, we found that having SS was associated with higher mean MCS compared to not having SS (Table 5). Other predictors of higher mean MCS included older age (older than 60 years) and lack of anti-cholinergic medication use. Also, living in China and Denmark were associated with higher mean MCS compared to living in the US.

Similarly, no significant difference in mean PHQ-9 score was seen between fulfilling 1 SS criterion versus no criteria ($\beta = 0.19$, $p = 0.53$), so these subcategories were combined as not having SS. Fulfilling 2 and 3 criteria were associated with a similar mean PHQ-9 depression score ($\beta = -1.80$, $p < 0.0001$ and $\beta = -2.42$, $p < 0.0001$, respectively) when compared to not having SS, so they were combined together as having SS. Thus, we found that having SS was

associated with lower PHQ-9 depression scores compared to not having SS (Table 7). Other predictors of lower depression levels included older age (> 60 years) and employment. Living in China, Denmark, and Japan were predictors of lower depression scores compared to living in the US. Also, not taking anti-cholinergic medication and absence of other systemic disease were associated with lower PHQ-9 scores.

Adjusted Predictors of Physical HRQoL, Mental HRQoL, and Depression

The final multivariate regression model (Table 6) for PCS revealed that having SS was associated with a higher mean PCS compared to not having SS. Other predictors of higher mean PCS included being older than 60 years of age and employment. There was evidence of interaction between country of residence and SS classification when examining associations with mean PCS. Having SS and living in US, Denmark, and UK were associated with higher mean PCS values compared to not having SS. The interactions between SS classification and the other countries were not statistically significant (Table 9). Also, absence of systemic diseases and lack of anti-cholinergic medication use were predictors of higher mean PCS. The final model was adjusted for all other covariates including gender and age.

Our multivariate model for MCS (Table 6) revealed that having SS was associated with higher mean MCS score compared to not having SS. Being over 60 years of age and lack of anti-cholinergic medication use were associated with higher mean MCS compared to individuals without these characteristics. Living in Denmark and China were predictors of higher mean MCS compared to living in the US whereas individuals living in Argentina, Japan, and UK were predictors of lower mean MCS. There was no evidence of interaction detected between country of residence and SS status (Table 9) when examining associations with MCS. The final model was adjusted for all other covariates including gender and age.

In our final multivariate regression model for PHQ-9 score (Table 8), we found that having SS associated with lower mean PHQ-9 depression scores compared not having SS.

Other predictors of lower depression were being older than 60 years of age, employment, and not taking anti-cholinergic medication. Living in China, Denmark, and Japan compared to living in the US were also associated with lower levels of depression. No interaction was detected between country of residence and SS classification when examining associations with PHQ-9 score (Table 9). The final model was adjusted for all other covariates including gender and age. Having other systemic diseases was not associated with mean PHQ-9 score and not retained in the final model.

Discussion

This study is unique in that it explored the association between SS and HRQoL and depression using the SF-12v2 health survey and PHQ-9, respectively, in a large cohort of well-characterized adults participating in the SICCA registry. Our study demonstrated that having SS was associated with higher mean SF-12v2 PCS/MCS values and lower PHQ-9 depression scores compared to not having SS. The higher PCS scores revealed that having SS was associated with fewer physical limitations, disabilities, or decrements in well-being; a higher level of energy; and a better self-rating of health compared to not having SS. The higher mean MCS scores indicated that having SS was associated with better positive affect; lack of psychological distress and limitations in social or role activities due to emotional problems; and better self-rated health than not having SS. Similarly, lower PHQ-9 scores revealed that having SS was associated with less depressive symptoms compared to not having SS.

We were surprised by these findings since previous studies demonstrated that reduced HRQoL and higher depression levels were frequent conditions in SS (1-15, 29, 30). Studies that compared individuals with SS to individuals without autoimmune characteristics (but with symptoms of dry eyes and dry mouth) found that impaired HRQoL and psychological status were comparable in these two groups (5, 6). In contrast, we found in our analysis that having SS was associated with higher HRQoL and fewer depressive symptoms than not having SS.

This difference is likely attributed to the unique characteristics of our non-SS control group, which consisted of symptomatic SICCA participants who fulfilled 1 component or none of the SS criteria. We combined these 2 subcategories to form our control non-SS group since they shared comparable HRQoL and depression values. In fact, the exclusion of individuals fulfilling only 1 criteria in our control group did not reveal significant changes in mean HRQoL and depression scores when compared to individuals with SS, confirming that individuals who did not have a SS diagnosis were similar regardless of having one positive objective test. Because our control non-SS group is not representative of healthy individuals but rather comprised of individuals with suspected SS, they may have represented individuals with another underlying autoimmune rheumatic disease that has not been diagnosed. Because many autoimmune rheumatic diseases having overlapping disease profiles, individuals often cannot be categorized into an established clinical entity like SS or SLE (31). According to Jones *et al.*, approximately 40% of patients in general practice do not have a diagnostic label, leaving most patients psychologically and emotionally vulnerable (32). Thus, having a disease identity, that is, a definitive diagnosis of SS, may encourage individuals to get a better understanding of their disease and have better coping strategies in place, including effective ways to manage their symptoms. The lower HRQoL and higher depression levels seen in our control non-SS group may be largely driven by symptoms of dry eyes and dry mouth, which may not have been diagnosed and/or managed appropriately. Alternatively, this groups may also have symptoms of dry eyes and mouth related to depression.

We found site-specific PCS values among individuals with SS. The differences in PCS scores likely reflect the availability of treatment for SS within each country. There are several European trials that have used biological agents, such as rituximab, and hydroxychloroquine to treat systemic manifestations of SS, which have demonstrated good results (19, 33-36). Specifically, Gottenberg *et al*. found that median ESSDAI decreased from 11 (range 2-31) to 7.5 (range 0=26), $p < 0.0001$ in their French cohort (36). These trials most likely encouraged the

introduction of these treatments into routine care for SS in these countries, which could explain the higher PCS scores associated with most European countries. Individuals with SS living in the US were also associated with higher mean PCS compared to individuals without SS. There are some clinical trials using rituximab to treat SS in the US (34) that have also demonstrated some modest clinical benefits.

In our regression analyses, several demographic and socioeconomic factors were significantly associated with HRQoL and depression outcomes. We found that increasing age was strongly associated with higher HRQoL and fewer depressive symptoms. It is well established in the literature that aging is associated with an intrinsic reduction in anxiety and depression. A meta-analysis by Jorm *et al.* examined the occurrence of anxiety, depression, and general distress across the adult life span and found that there was an initial rise across age groups, which was then followed by a significant drop in anxiety and depression (37). As expected, individuals with other systemic diseases, including those individuals with depression who were currently taking anti-cholinergic medications, were associated with reduced HRQoL values and higher depression levels. This is not surprising since physical disability and depression is commonly attributed to chronic disorders like fibromyalgia (38), rheumatoid arthritis (39), osteoarthritis (40), and other chronic musculoskeletal disorders (41, 42). However, many of these demographic and socioeconomic associations are difficult to capture accurately since they reflect other psychological factors such as self-esteem and social/family support, which are difficult to measure but commonly associated with HRQoL and depression.

A unique strength of this analysis was the utilization of the well-characterized SICCA registry, which is an international database consisting of over 3500 registrants. Over 100 phenotypic variables and biospecimens were collected from registry participants and standardized across 9 international sites, allowing for consistency of the variables and large sample size. Trained SICCA team clinicians also performed uniform, protocol-driven clinical evaluations, which assessed for objective measures of oral, ocular, and rheumatological

involvement. Also, the inclusion criteria targeted individuals with signs and/or symptoms suggestive of SS, allowing the use of individuals who did not fulfill the ACR classification criteria as our control group.

Our study had limitations. Disease activity indices have been recently developed by the EULAR SS task force: 1) EULAR SS disease activity index (ESSDAI) to assess systemic activity (20) and 2) the EULAR SS patient-reported index (ESSPRI) to assess patients' symptoms (43). These indices were developed to evaluate clinical trial outcomes of new treatment modalities in SS and to also suggest thresholds to be used as entry criteria and response criteria (44). SICCA preceded the establishment and validation of these indices; thus, they were not included as outcome measures in SICCA and therefore not available for this analysis. Our study was based on registry data, and only data collected at the time of the baseline questionnaire could be used for this study. Like any cross-sectional study design, this work is limited in its ability to establish causal relationships. Secondly, because these survey questions were based on patient recollection, we had to rely on self-report data, which is an inherent weakness in our analyses. We also could not exclude the possibility of recruitment bias in which patients who were more physically fit and had higher energy/motivation participated or were selected to participate in the registry, resulting in an underestimation of the true burden of SS on HRQoL. And lastly, our control group was not representative of the general population but rather consisted of symptomatic individuals, which may have skewed our results.

In our analysis, we found that individuals with SS had better overall HRQoL and lower depression levels compared to individuals without SS. This observation may reflect unique characteristics of this cohort, or alternatively, suggest that having a disease identity is important among coping strategies and disease management. We found that individuals with SS living in countries more aware of SS were associated with better physical HRQoL, highlighting the need for management of symptoms associated with SS. These findings also emphasize the importance of assessing patient symptoms and objective measures to arrive to a diagnosis or

provide skillful patient management to address patient concerns. Our results have the potential to influence policy makers to allocate health care sources and funding to optimize the multi-disciplinary care needed for individuals with SS or symptoms suggestive of SS.

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Table 1: Baseline clinical characteristics of individuals enrolled in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

Characteristics	Number[a]	Frequency[b] (%)
Gender		
Female	3185	91
Male	309	9
Age (years)		
20-39	602	17
40-59	1732	50
≥60	1158	33
Self-Reported Ethnicity		
African American	106	3
Asian/Pacific Islander	1001	29
Caucasian	1894	54
Hispanic	372	11
Native American	118	3
Site		
Argentina	441	13
China	333	9
Denmark	610	17
India	161	5
Japan	368	10
United Kingdom	312	9
United States	1289	37
Highest Education		
High School	1379	40
College	2059	60
Employment		
Employed	1624	47
Unemployed	1868	53
Anticholinergic medications		
Yes	1055	32
No	2281	68
Other systemic disease		
Yes	965	27
No	2549	73
Satisfaction of ACR[c] SS Classification Criteria[d]		
0 criteria	628	18
1 criteria	1308	37
2 criteria	745	21
3 criteria	833	24

[a] Column count for each variable may not add up to total number of participants (n=3514) in the analysis due to missing data
[b] May not total 100% due to rounding
[c] American College of Rheumatology
[d] Satisfaction of at least 2 criteria satisfies the American College of Rheumatology classification criteria for SS

Table 2: Summary statistics for the 9-item Patient Health Questionnaire (PHQ-9) among individuals enrolled in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

PHQ-9 Depression Severity	Frequency[a]	Percent[b]
No Depression (0-4)	1338	40
Mild Depression (5-9)	939	28
Moderate Depression (10-14)	579	17
Moderately Severe Depression (15-19)	299	9
Severe Depression (20-27)	176	5

[a] Column count for each variable may not add up to total number of participants (n=3514) in the analysis due to missing data
[b] May not total 100% due to rounding

Table 3: Summary statistics for the SF-12v2 Health Survey among individuals enrolled in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

	Mean	SD[a]	Median	IQR[b]
Total Physical Component Summary (PCS) Score	42.59	11.03	43.73	34.38-51.73
Total Mental Component Summary (MCS) Score	46.01	10.64	46.73	38.75-54.50
SF-12v2 subscales:				
Physical Functioning	44.48	11.32	41.32	33.45-54.25
Role limitations due to Physical Problems	42.61	10.66	40.54	32.07-49.00
Bodily Pain	42.59	11.81	39.69	30.67-48.71
General Health	41.47	10.91	47.75	33.84-57.69
Vitality	46.04	11.43	49.07	39.23-58.90
Social Functioning	44.87	10.91	39.11	30.22-48.01
Role limitations due to Emotional Problems	43.72	11.58	45.89	35.49-56.28
Mental Health	45.95	10.61	47.00	41.26-52.74

[a] Standard deviation
[b] Interquartile range

Table 4: Individual predictors of Physical Component Score (PCS) among individuals participating in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

	Total (n)[a]	Mean Scores	Unadjusted β[b]	95% Confidence Interval	P-value
Gender					
Female	3159	42.49	reference		
Male	305	43.63	1.14	-0.16, 2.43	0.09
Age (years)					
20-40	596	45.06	reference		
40-60	1717	41.67	-3.38	-4.41, -2.36	<0.0001
>60	1149	42.69	-2.37	-3.45, -1.28	<0.0001
Site					
Argentina	428	43.22	1.97	0.79, 3.15	0.001
China	327	47.04	5.79	4.49, 7.10	<0.0001
Denmark	598	40.46	-0.79	-1.84, 0.25	0.14
India	161	40.06	-1.19	-2.96, 0.57	0.19
Japan	363	47.36	6.10	4.85, 7.36	<0.0001
United Kingdom	311	42.37	1.11	-0.22, 2.45	0.10
United States	1276	41.25	reference		
Highest Education					
High School	1361	42,43	reference		
College	2047	42.79	0.35	-0.41, 1.11	0.36
Employment					
Employed	1610	45.64	5.70	4.99, 6.41	<0.0001
Unemployed	1853	39.94	reference		
Anticholinergic medications					
Yes	1048	37.91	reference		
No	2406	44.63	6.72	5.96, 7.49	<0.0001
Other systemic disease					
Yes	952	40.03	reference		
No	2512	43.56	3.53	2.72, 4.35	<0.0001
ACR[c] SS Classification					
SS -	1813	41.02	reference		
SS +	1557	44.47	3.44	2.71, 4.18	<0.0001

[a] Column count for each variable may not add up to total number of participants (n=3514) in the analysis due to missing data

[b] Regression coefficients (β) for each variable estimate the difference in the mean PCS between the indicated level and the chosen reference level

[c] American College of Rheumatology

Table 5: Individual predictors of Mental Component Score (MCS) among individuals participating in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

	Total (n)[a]	Mean Score	Unadjusted β[b]	95% Confidence Interval	P-value
Gender					
Female	3159	46.00	reference		
Male	305	46.14	0.13	-1.12, 1.38	0.83
Age (years)					
20-40	596	45.26	reference		
40-60	1717	45.16	-0.10	-1.08, 0.89	0.84
>60	1149	47.69	2.43	1.38, 3.47	<0.0001
Site					
Argentina	428	42.96	-2.40	-3.54, -1.26	<0.0001
China	327	51.76	6.40	5.14, 7.67	<0.0001
Denmark	598	47.38	2.02	1.01, 3.03	<0.0001
India	161	46.86	1.50	-0.20, 3.21	0.09
Japan	363	45.21	-0.15	-1.37, 1.06	0.81
United Kingdom	311	44.75	-0.61	-1.90, 0.68	0.35
United States	1276	45.36	reference		
Highest Education					
High School	1361	46.10	reference		
College	2047	46.07	-0.02	-0.75, 0.70	0.95
Employment					
Employed	1610	46.30	0.53	-0.18, 1.24	0.14
Unemployed	1853	45.77	reference		
Anticholinergic medications					
Yes	1048	43.29	reference	3.16, 4.68	<0.0001
No	2406	47.21	3.92		
Other systemic disease					
Yes	952	45.58	reference		
No	2512	46.18	0.60	-0.19, 1.39	0.14
ACR[c] SS classification					
SS -	1813	45.02	Reference		
SS +	1557	47.20	2.17	1.46, 2.89	<0.0001

[a] Column count for each variable may not add up to total number of participants (n=3514) in the analysis due to missing data
[b] Regression coefficients (β) for each variable estimate the difference in the mean MCS between the indicated level and the chosen reference level
[c] American College of Rheumatology

Table 6: Predictors of Physical Component Score (PCS) and Mental Component Score (MCS) among individuals participating in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

	Physical Component Score (PCS)		Mental Component Score (MCS)	
	β[a] (95% CI)[b]	P-value	β[a] (95% CI)[b]	P-value
Gender				
Male	0.80 (-0.41, 2.01)	0.20	0.26 (-0.97, 1.50)	0.68
Female	reference		reference	
Age				
20-40	reference		reference	
40-60	-1.99 (-2.94, -1.04)	<0.0001	0.52 (-0.45, 1.49)	0.29
>60	1.34 (0.29, 2.39)	0.01	3.41 (2.37, 4.44)	<0.0001
Site				
Argentina	1.70 (0.61, 2.80)	0.002	-2.43 (-3.55, -1.30)	<0.0001
China	3.15 (1.82, 4.47)	<0.0001	5.20 (3.85, 6.55)	<0.0001
Denmark	-0.54 (-1.53, 0.45)	0.29	1.89 (0.88, 2.90)	0.002
India	-2.30 (-4.12, -0.47)	0.01	0.28 (-1.58, 2.13)	0.77
Japan	4.13 (2.93, 5.32)	<0.0001	-1.60 (-2.82, -0.38)	0.01
United Kingdom	-0.56 (-1.84, 0.71)	0.39	-1.87 (-3.18, -0.57)	0.005
United States	reference		reference	
Employment				
Yes	5.68 (4.95, 6.42)	<0.0001		
No	reference			
Anticholinergic medications				
Yes	reference		reference	
No	4.97 (4.18, 5.76)	<0.0001	3.38 (2.58, 4.19)	<0.0001
Other systemic disease				
Yes	reference			
No	2.75 (1.98, 3.51)	<0.0001		
ACR[c] SS classification				
SS -	Reference		Reference	
SS +	1.96 (1.25, 2.67)	<0.0001	1.31 (0.59, 2.04)	<0.0001

[a] Regression coefficients (β) for each variable estimate the difference in the mean PCS or MCS between the indicated level and the chosen reference level, adjusting for the remaining variables in the table.
[b] 95% Confidence Interval
[c] American College of Rheumatology

Table 7: Individual predictors of 9-item Patient Health Questionnaire (PHQ-9) among individuals participating in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

	Total (n)[a]	Mean Score	Unadjusted β[b]	95% Confidence Interval	P-value
Gender					
Female	3053	7.50	reference		
Male	278	6.96	-0.54	-1.29, 0.21	0.16
Age (years)					
20-40	571	7.23	Reference		
40-60	1629	8.23	1.00	0.42, 1.58	0.001
>60	1129	6.43	-0.80	-1.42, -0.19	0.01
Site					
Argentina	433	9.20	0.81	0.16, 1.45	0.01
China	333	2.98	-5.41	-6.12, -4.69	<0.0001
Denmark	603	6.90	-1.49	-2.06, -0.92	<0.0001
India	0				
Japan	311	8.26	-1.96	-2.65, -1.28	<0.0001
United Kingdom	368	6.43	-0.13	-0.86, 0.60	0.73
United States	1283	8.39	reference		
Highest Education					
High School	1265	7.42	reference		
College	2043	7.45	0.03	-0.40, 0.46	0.89
Employment					
Employed	1565	6.80	-1.23	-1.65, -0.82	<0.0001
Unemployed	1765	8.04	reference		
Anticholinergic medications					
Yes	1053	9.96	reference		
No	2268	6.29	-3.67	-4.10, -3.23	<0.0001
Other systemic disease					
Yes	899	7.96	reference		
No	2432	7.27	-0.69	-1.16, -0.22	0.004
ACR[c] SS classification					
SS -	1176	8.47	reference		
SS +	1488	6.27	-2.19	-2.61, -1.78	<0.0001

[a] Column count for each variable may not add up to total number of participants (n=3514) in the analysis due to missing data
[b] Regression coefficients (β) for each variable estimate the difference in the mean PHQ-9 score between the indicated level and the chosen reference level
[c] American College of Rheumatology

Table 8: Predictors of 9-item Patient Health Questionnaire (PHQ-9) among individuals participating in the Sjögren's International Collaborative Clinical Alliance (SICCA) Registry (n = 3514)

	PHQ-9 Score		
	Adjusted β[a]	**95% Confidence Interval**	**P-value**
Gender			
Male	-0.63	-1.33, 0.07	0.08
Female	reference		
Age (years)			
20-40	reference		
40-60	0.30	-0.24, 0.84	0.28
>60	-2.35	-2.95, -1.75	<0.0001
Site[b]			
Argentina	0.84	0.23, 1.46	0.007
China	-4.22	-4.95, -3.48	<0.0001
Denmark	-1.62	-2.17, -1.07	<0.0001
Japan	-0.84	-1.50, -0.17	0.01
United Kingdom	0.81	0.10, 1.53	0.03
United States	reference		
Employment			
Employed	-1.83	-2.25, -1.41	<0.0001
Unemployed	reference		
Anticholinergic medications			
Yes	reference		
No	-2.66	-3.10, -2.21	<0.0001
ACR[c] classification criteria			
SS -	reference		
SS +	-1.30	-1.70, -0.90	<0.0001

[a] Regression coefficients (β) for each variable estimate the difference in the mean PHQ-9 score between the indicated level and the chosen reference level, adjusting for the remaining variables in the table.
[b] PHQ-9 scores were not available for the India site
[c] American College of Rheumatology

Table 9: Interaction between country of residence and SS status with Physical Component Summary (PCS), Mental Component Summary (MCS), and 9-item Patient Health Questionnaire (PHQ-9) depression scores

	Physical Component Summary (PCS) Score		Mental Component Summary (MCS) Score	PHQ-9 Depression Score
Test for Interaction	P = 0.05		P = 0.10	P = 0.88
	β[a] (95% CI)[b]	**p-value**	**β[a] (95% CI)[b]**	**β[a] (95% CI)[b]**
Argentina	0.64 (-1.30, 2.57)	p = 0.52	n/a	n/a
China	-0.03 (-2.87, 2.81)	p = 0.98	n/a	n/a
Denmark	4.16 (2.36, 5.95)	p < 0.0001	n/a	n/a
India	0.24 (-3.27 3.75)	p = 0.89	n/a	n/a
Japan	2.01 (-0.06, 4.08)	p = 0.06	n/a	n/a
United Kingdom	3.51 (1.27, 5.75)	p = 0.002	n/a	n/a
United States	1.65 (0.54, 2.77)	p = 0.04	n/a	n/a

[a] Regression coefficients (β) for each variable estimate the change in PCS/MCS/PHQ-9 scores among individuals with SS (compared to individuals without SS) in each country indicated, adjusting for other socioeconomic and demographic variables.
[b] 95% Confidence Interval

Utilization of Dental Care Services Among Women Participating in the Sjögren's Syndrome International Collaborative Clinical Alliance (SICCA) Registry

Introduction

Dental care utilization is essential among individuals with SS due to the debilitating effects of salivary hypofunction. Saliva plays multiple roles in the oral cavity including the buffering of acidity, mediating taste sensation, lubricating mucosal surfaces, and performing antimicrobial duties. Thus, chronic salivary hypofunction predisposes individuals with SS to oral damage, soft tissue infection, and dental caries, which can have a major impact on basic daily functions (1, 2). In general, individuals with SS report poorer oral health and greater dental needs than individuals without SS. In Denmark, Christensen *et al*. found that those with SS had more teeth extracted, more trouble with their teeth in their lifetime, and higher dental expenses compared to control groups (3). Similarly, in the US, individuals with SS reported having higher out-of-pocket dental expenses, more frequent dental visits, and more dental treatments compared to controls (4).

The majority of oral complications and high dental costs can be prevented with routine dental care and preventative measures such as daily application of topical fluoride. However, no studies have comprehensively characterized the utilization of dental care among individuals with SS to explore the association between SS and utilization of dental care while controlling for demographic and socioeconomic factors. By doing so, the burden of SS on an individual and societal level can be evaluated with the goals of improving access to dental care in overall health maintenance and policy planning.

Previous studies describing oral health in SS have focused on specific population groups and/or featured only a few oral health outcomes, resulting in limited generalizability and comprehensiveness. In this analysis, we took advantage of the well-characterized Sjögren's

International Clinical Collaborative Alliance (SICCA) dataset, which is the first international data registry that uses a single questionnaire and standardized methodology to collect data on a wide array of outcome measures, including oral health and dental care experiences among individuals. This resource provides the unprecedented opportunity to 1) evaluate selected oral health parameters and dental care utilization patterns in an international group of women with SS or signs suggestive of SS and 2) explore predisposing, enabling, and need factors that may explain utilization (or lack of utilization) of dental care services, particularly preventative dental care, in this population.

Materials and Methods

Study sample

Data was obtained from the SICCA registry. The cohort (5) and eligibility criteria are described in more detail in the Introduction. This analysis was based on women enrolled in the SICCA registry and for whom data was available for analysis as of September 30, 2013 after all data collected as part of the registry had undergone comprehensive clean-up. The SS classification used in this study was developed by the SICCA investigative team and endorsed by the American College of Rheumatology (ACR) (6), which requires fulfillment of two of the following requirements: 1) positive serum anti-SSA (Ro) and/or anti-SSB (La) or [positive RF and ANA titer ≥ 1:320]; 2) ocular staining score ≥ 3 using lissamine green or fluorescein to diagnose keratoconjuncitivitis sicca (7); or 3) presence of focal lymphocytic sialadenitis with a focus score ≥1 focus/4mm^2 in their labial salivary gland biopsy (8).

Men (n=309) were excluded to limit the number of confounding variables in this study. Women who were edentulous, or nearly edentulous (with < 5 teeth), (n=127) were also excluded because one of our main outcomes pertained to use of preventative dental care measured by whether or not one had received a dental cleaning in the previous year. Although baseline characteristics are presented for women from the India site (n=100), they were not

included in subsequent analyses since this subgroup was too small to contribute significantly to the analyses.

Variables and Measurements

All participants underwent a protocol-driven oral examination for evaluation of their dentition, oral mucosa, and salivary flow. Participants were also asked to answer questions regarding their oral symptoms and utilization of dental care services. All SICCA questionnaires and data collection forms and protocols are available for review at http://sicca.ucsf.edu.

Utilization of dental care services, defined by binary or categorical indicators of self-reported use of such services in the past 12 months, were the outcome variables of this analysis. These services included: 1) Having regular dental care, that is, a dentist or dental clinic that they visit on a regular basis to get their teeth examined or cared for (yes, no); 2) Length of time since the they were last treated or examined by a dentist (< 12 months, 1-3 years, > 3 years or never); and 3) Dental procedures in the past 12 months including a dental cleaning (yes, no) and dental restorations/crown (yes, no). In subsequent analyses, we focused on dental cleaning as the primary outcome variable since we believed that this variable was a more accurate reflection of regular dental care.

Analyses to determine predictors of preventative dental care were based on the Andersen Behavioral Model of Health Service (Figure 1), a behavioral model proposed by Andersen and his colleagues to conceptualize utilization of health services (9, 10). The model, also known as the *Predisposing-Enabling-Need (PEN)* model, assumes that an individual's use of health services is a result of characteristics of the population at risk. Relevant population characteristics include predisposition to use services, factors that enable or impeded use, and need for care. Predisposing factors are variables associated with the individuals receiving care, specifically, characteristics that existed prior to onset of disease. Age (20-39, 40-59, > 60), highest education (high school graduate, university/college), race/ethnicity (Asian/Pacific

Islander, Caucasian, and Other), and country of residence (Argentina, China, Denmark, Japan, UK, and US consisting of sites from UPenn, JHU, and UCSF) were selected as predisposing factors in this analysis. Enabling factors are those that are related to healthcare access and include the logistical aspects of obtaining care like the availability of personal and community resources. Enabling variables included employment (part or full time employed, unemployed) and referral by a dentist (yes, no). Need variables are the immediate cause of health service use perceived by either the patient or health care provider. Need variables selected for this analysis were SS status (yes, no), perception of oral health (excellent-good, fair-poor), number of teeth (6-16 teeth, > 16 teeth), number of dental caries (none, 1-5, > 6), perception of dry mouth (yes, no), dry mouth duration (years), difficulty swallowing (yes, no), ability to eat a cracker without fluid (yes, no), frequency of brushing (< 2x/day or never, ≥ 2x/day), and frequency of flossing (< 1x/day or never, ≥1x/day)

Statistical Analyses

Baseline characteristics of individuals participating in the SICCA registry were described using frequencies and percentages. Contingency tables were constructed to compare baseline characteristics among women with and without SS and to assess statistically significant differences in distribution across and within sub-groups. To determine individual predictors of preventative dental care, logistic regression models were performed to determine marginal associations between individual PEN variables and dental cleaning in the past year (yes, no). An odds ratio (OR) was obtained to measure the magnitude and direction of the association between each predictor variable and the likelihood to receive an annual professional dental cleaning. To address confounding by other variables, multivariate logistic regression models were constructed to refine these relationships by controlling for other covariates. The initial multivariate logistic regression model included PEN predictor variables found to be individually associated with the outcome at the 0.10 significance level. Predictors were retained in the final

model if they were found to be associated with the outcome at a significance level of at most 0.05 or if removal of these variables resulted in a ≥ 10% change in ORs among the other covariates in the model. Also, predictors were removed if they were interpreted as an intermediate, or mediator, variable. An overall test of interaction was performed to determine possible variation in ORs due to interaction between country of residence and SS classification. I assessed model goodness of fit by using the Hosmer-Lemeshow statistical test. The statistical software Stata, version 12.0 was used for all statistical analyses in this study.

Results

Sample Characteristics

There were 2974 dentate women (with at least 5 teeth) who participated in the SICCA registry as of September 2013 (Table 10). Forty-seven percent (n=1388) of SICCA participants fulfilled the ACR criteria for SS whereas 53% (n=1586) did not. The highest proportion of women with SS (35%) and non-SS (39%) were recruited by US sites. The majority of women with and without SS were in their fourth through fifth decades of life and reported to be Caucasian. Most women with SS (51%) were employed in a part or full-time occupational activity whereas the majority of non-SS women (57%) were unemployed. Less than 10% of women in both subgroups were referred to the registry by their dentist.

Oral Health Parameters

The majority of women with SS reported a fair to poor perception of their oral health (63%), had no active dental caries at the time of the SICCA oral exam (59%), had difficulty swallowing (56%), reported the inability to eat a cracker without fluid (60%), brushed at least twice a day (85%), but did not floss daily (63%) (Table 11). The majority of women without SS also reported a fair to poor perception of their oral health (53%), had no active dental caries at the time of the SICCA oral exam (73%), brushed at least twice a day (88%), but did not floss

daily (55%). Half of the women without SS reported the inability to eat a cracker without fluid and difficulty swallowing.

Utilization of dental care

Surprisingly, only 42% of women with SS compared to 58% women without SS ($p <$ .0001) reported that they had a dentist or dental clinic they visited on a regular basis (Table 12). Similarly, only 44% of women with SS compared to 56% of women without SS ($p <$ .0001) reported having seen their dentist in the last year, and fewer women with SS reported having a dental cleaning in the past 12 months (40% versus 60% among those without SS, $p <$ .0001).

Predictors of dental care

Univariate analyses were performed to identify individual predictors of dental care utilization (Table 13). Of note, we found that women with SS were less likely to receive a dental cleaning in the past year. In contrast, the following characteristics were associated with higher utilization of dental care: being over the age of 40, having a college education, living in the US, referral from a dentist to the SICCA registry, having excellent perception of their oral health, having greater than 16 teeth, having less than 5 dental caries, having dry mouth, difficulty swallowing, inability to eat a cracker without fluid, brushing twice a day or more, and flossing once a day or more.

To identify predictors of dental care use while controlling for other confounding influence and mediation by other covariates in our PEN model, multivariate analyses were performed (Table 14). We found that the adjusted odds of receiving a dental cleaning in the past 12 months among women with SS were 30% less than corresponding odds for women without SS. Similarly, when compared to women living in the US, we found that women residing in Argentina, China, Japan, and UK were 70-90% less likely in terms of odds to receive an annual dental cleaning. Comparison of annual preventative care use between women living in Denmark

and those living in the US did not yield a statistically significant difference. Although some interaction was detected between country of residence and SS classification, the presence of the interaction resulted in very little change in the adjusted odds ratios for the other variables included in Table 14. Women who had an excellent or good perception of their oral health (OR=1.77; 95% CI=1.38-2.28; $p < 0.0001$), more than 16 teeth (OR=1.97; 95% CI=1.33-2.93; $p = 0.001$), and reported flossing 1x/day or more (OR=1.82, 95% CI=1.42-2.34; $p < 0.0001$) were also more likely to receive an annual dental cleaning compared to women without these characteristics. We also found that specific socio-economic characteristics represented independent effects of annual preventative dental care use. Women who were referred by their dentist to the SICCA registry (OR=51.93; 95% CI=34.78-77.55; $p < 0.0001$), women aged 60 years and older (OR=1.60; 95% CI=1.13-2.26; $p = 0.01$), and those who had a college/university education (OR=1.41; 95% CI=1.10-1.82; $p < 0.0001$) were more likely to see a dentist for professional cleaning compared to women without these characteristics. It was unclear whether dental need variables served as confounding or intermediary effects (mediation) on SS classification, but exclusion of most of these need variables in our multivariate model did not change other covariates significantly and/or were not associated with having an annual dental cleaning. Thus, number of dental caries, perception of dry mouth, difficulty swallowing, ability to eat a cracker without fluid, and frequency of brushing were not retained in the final model. The model had adequate fit at an acceptable level according to the Hosmer-Lemeshow statistical test (p=0.26).

Discussion

This is the first analysis that provides a comprehensive description of oral health among a large international cohort of women with SS or with signs and symptoms suggestive of SS. As expected, we found that women with SS who had enrolled in SICCA had poor oral health perceptions and symptoms. In particular, we found that the majority of women with SS reported

difficulty swallowing and were unable to eat a cracker without fluid. Previous studies have also demonstrated high dental needs among individuals with SS. For instance, the majority of individuals with SS living in Denmark felt that the condition of their teeth was poor, reported higher need for fillings, and were dissatisfied with their own teeth (3). Soto-Rojas *et al.* also found that 86% of patients with SS living in Mexico had cervical or atypical caries, and 74% were co-infected with candidiasis at their first clinical visit (11).

Despite increased dental needs among individuals with SS, only 44% of our sample of women with SS visited a dentist in the past year. Interestingly, one study actually found a high annual prevalence of dental visits among individuals with SS. Lu *et al.* found higher frequencies of annual visits up to 8 years prior to their baseline visit date among their cohort of individuals with SS living in Taiwan compared with their controls (12). These visits included treatment of caries, pulpitis, gingivitis, stomatitis, and periodontitis. Because a dental visit can include various types of care, including restorative and/or other emergency dental treatment that are not performed routinely, a measure of visit versus non-visit may not be an accurate indicator of regular utilization of dental care services. Thus, in this analysis, we used preventative dental care (dental cleaning versus non-dental cleaning) in the past year as a more accurate measure of utilization of regular dental care.

We applied the behavioral model developed by Anderson and his colleagues to determine individual characteristics that are more likely to cause women to seek dental care. This model has been shown to effectively explain dental care utilization in other populations (13, 14, 15) but has not yet been applied to a population with SS or with early signs suggestive of SS. A study by Gilbert *et al*. used Andersen's model of health behavior to predict dental utilization of older adults (65 years old and older) living in Florida and found that perceived importance of oral health, college education, high dentate status, and high income were significantly associated with utilization of regular dental visits (16). Similarly, Evashwick *et al*. found in their analysis of 883 older adults living in Washington that dental need, measured by an

index of self-perceived dental problems, was the strongest determinant of dental care use (14). Like these previous studies, our model revealed that older age, having a college education, referral to the registry by a dentist, good perception of one's oral health, and good oral hygiene habits were predictors of regular utilization of dental care among our sample of SICCA women.

Surprisingly, when we fitted a multivariate logistic regression model to explain predictors of regular dental care while controlling for other relevant covariates, we found that having SS was associated with low utilization of dental care. This is consistent with the high dental needs reported by individuals with SS in our study. This observation is likely explained by the reality that women with SS are unaware of the benefits of routine dental care or the burden of their systemic disease interferes with regular use of dental care. Our findings are in accord with a study by McGlynn *et al.* that found that people living with chronic diseases receive only 56% of recommended preventative health care services in the US (17). Thus, not only should the benefits of routine dental care be emphasized among this high-risk group, but we must also educate physicians about the importance of referring their patients with SS to dentists for routine dental exams and appropriate prevention, such as fluoride treatment. Additionally, policy makers should be encouraged to broaden coverage for those living with SS to make access to dental care easier.

We also found that country of residence was strongly associated with utilization of dental care. We hypothesized that utilization of dental care would be higher in countries that offered universal health coverage compared to the US, where insurance coverage can be quite fragmented (18). Interestingly, we found that women living in other countries were less likely to receive a dental cleaning compared to the US, with the exception of Denmark, for which we found no statistical significant difference with the US with respect to utilization of preventative dental care. This was unexpected since a comparative analysis of how health insurance design affects access to care in eleven countries, including the UK, found that US adults were more likely to incur high medical expenses, to spend more time on insurance paperwork and disputes,

or to have payments denied (19). Although this analysis argued for comprehensive health reforms in the US, our analysis suggests that health reforms may be needed in other countries as well, particularly for individuals living with SS. In addition, the cultural and social beliefs must be taken into account when making cross-country comparisons. This may explain why the US, a country without universal health care, demonstrated higher utilization of preventative dental care compared to other countries with universal health care due to increased awareness and social acceptability of routine dental care in the US. Additionally, these results may also be a reflection that US women who enrolled in SICCA had overall higher socioeconomic status than women living in other countries.

There were some limitations to this study. Due to the confines of a cross-sectional study based on data collected from a questionnaire, some important variables were not included in the analysis, including private or public dental insurance coverage (or both) and other health beliefs. Employment status and country of residence were chosen as proxies for these variables; however, they may not have adequately represented the characteristics we were interested in. In addition, there may have been some recall errors. For instance, participants may not have recalled the exact number of dental visits or type of visit they made in the previous year. And finally, we restricted our analyses to women participating in SICCA in order to limit the number of confounding effects in our study; however, this likely caused a loss of statistical power and inhibited the generalization of our results. Rather, our results reflect behaviors of a select group of cases and controls, specifically women who have access to an academic clinical setting or have more severe symptoms (consistent with or suggestive) of SS.

This analysis focused on the oral health status of individuals with SS and their utilization of dental care services. Although individuals with SS have an increased susceptibility to dental caries and other oral disease due to impaired salivary function, they are underusing dental care services. Since dental restorative treatment is costly and requires maintenance, increasing access to dental care will likely decrease the burden of disease and overall dental costs.

Regular dental visits allow dental health professionals to provide preventive services, early diagnosis, and treatment. Increasing awareness of the benefits of routine dental care and access to dental care among individuals living with SS should be made a priority.

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Figure 1. Behavioral model of health care services utilization adapted to explain utilization of dental care services in SICCA Registry participants.

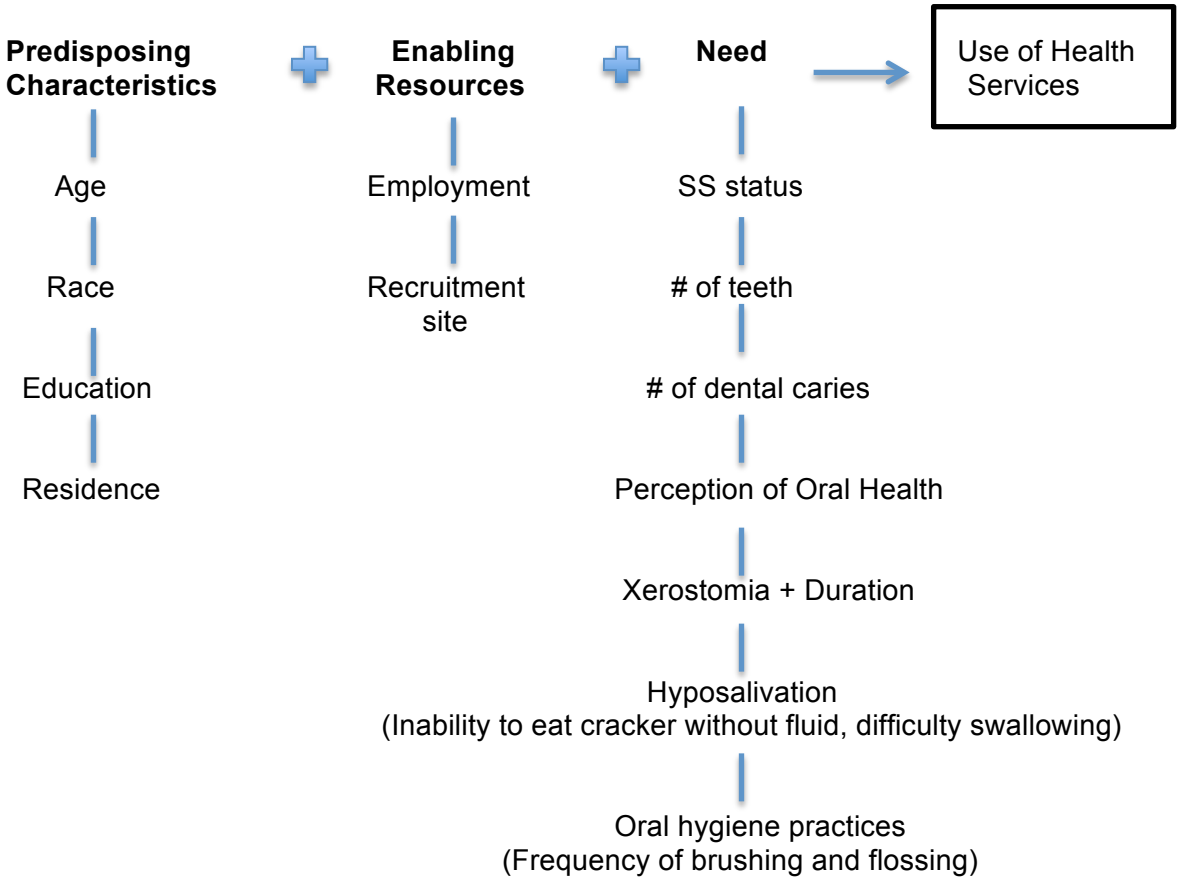

Table 10: Demographic and socio-economic characteristics of 2974 women participating in the Sjögren's International Collaborative Clinical Alliance (SICCA), stratified by Sjögren's syndrome (SS) according to the American College of Rheumatology Classification Criteria for SS

	SS n[a] (%)[b]	Non-SS n[a] (%)[b]	*p*-value
# of Participants	1388	1586	
Age (in years)			0.001
20-39	284 (20)	242 (15)	
40-59	695 (50)	816 (52)	
60+	409 (29)	526 (33)	
Race / Ethnicity[c]			<0.0001
Asian / Pacific Islander	549 (40)	269 (17)	
Caucasian	608 (44)	1032 (65)	
Other	230 (17)	283 (18)	
Country of Residence			<0.0001
Argentina	138 (10)	226 (14)	
China	234 (17)	57 (4)	
Denmark	151 (11)	378 (24)	
India	68 (5)	32 (2)	
Japan	179 (13)	136 (9)	
United Kingdom	131 (9)	134 (8)	
United States	487 (35)	623 (39)	
Highest Education			0.06
High school	548 (40)	576 (37)	
College/education	818 (60)	995 (63)	
Employment			<0.0001
Currently employed[d]	712 (51)	687 (43)	
Unemployed	675 (49)	898 (57)	
Recruitment			0.003
Dentist	78 (6)	134 (8)	
Non-dentist[e]	1309 (94)	1446 (92)	

[a] Column count for each variable may not add up to total number of women with SS (n=1388) and without SS (n=1586) in the analysis due to missing data
[b] % = frequency of individuals by column. May not total 100% due to rounding
[c] Self-reported on baseline questionnaire
[d] Currently employed full or part time
[e] Non-dentist: rheumatologist, ophthalmologist, other doctor, website/internet, advertisement, support groups, friends, other

Table 11: Oral health parameters of 2974 women participating in the Sjögren's International Collaborative Clinical Alliance (SICCA), stratified by Sjögren's syndrome (SS) status according to the American College of Rheumatology Classification Criteria for SS

	SS n[a] (%)[b]	Non-SS n[a] (%)[b]	*p*-value
# of Participants	1388	1586	
Perception of oral health			<0.0001
Excellent – good	517 (37)	747 (47)	
Fair – poor	870 (63)	839 (53)	
Number of teeth			0.87
6-16 teeth	109 (8)	122 (8)	
>16 teeth	1279 (92)	1464 (92)	
Number of dental caries			<0.0001
None	815 (59)	1151 (73)	
1-5 caries	421 (30)	363 (23)	
>6 caries	152 (11)	72 (5)	
Does your mouth feel dry?			0.70
Yes	1250 (90)	1435 (90)	
No	138 (10)	151 (10)	
Mean dry mouth duration (years)	6.15[c]	5.64[d]	0.09
Difficulty swallowing			0.001
Yes	778 (56)	788 (50)	
No	610 (44)	798 (50)	
Eating a cracker without fluid			<0.0001
Yes	560 (40)	794 (50)	
No	828 (60)	788 (50)	
Frequency of brushing			0.03
<2x/day, never	202 (15)	189 (12)	
≥2x/day	1186 (85)	1396 (88)	
Frequency of flossing			<0.0001
<1x/day, never	875 (63)	868 (55)	
≥1x/day	513 (37)	718 (45)	

[a] Column count for each variable may not add up to total number of women with SS (n=1388) and without SS (n=1586) in the analysis due to missing data
[b] % = frequency of individuals by column. May not total 100% due to rounding.
[c] SD=2.17, Median=0.75, Range=0.16-2.14
[d] SD= 7.39, Median=3.46, Range=0.58-15.17

Table 12: Utilization of dental care services among 2974 women participating in the Sjögren's International Collaborative Clinical Alliance (SICCA), stratified by Sjögren's Syndrome (SS) according to the American College of Rheumatology Classification Criteria for S

Dental Services	SS n[a] (%)[b]	Non-SS n[a] (%)[b]	Total	*p*-value
# of Participants	1388	1586		
Regular dental care[c]				<0.0001
Yes	942 (42)	1318 (58)	2260	
No	446 (62)	268 (38)	714	
Last seen by a dentist for any procedure:				<0.0001
< 12 months	1046 (44)	1330 (56)	2376	
1-3 years	196 (55)	159 (45)	355	
>3 years or never	78 (55)	65 (45)	143	
Dental procedures in the past 12 months:				
Cleaning by a dentist or hygienist:				<0.0001
Yes	763 (40)	1140 (60)	1903	
No	625 (58)	446 (42)	1071	
Tooth restored or crown made:				0.80
Yes	718 (46)	828 (54)	1546	
No	670 (47)	758 (53)	1428	

[a] n = number of individuals within each row variable.
[b] %= frequency of individuals within each row variable. May not total 100% due to rounding
[c] Access to a dentist or dental clinic that they visit on a regular basis to get your teeth examined, cleaned, or cared for

Table 13: Individual predictors of preventative dental care use among 2874 women participating in the Sjögren's International Collaborative Clinical Alliance (SICCA), excluding 100 women from India.

	Received dental cleaning n[a](%)[b]	Total	Unadjusted Odds Ratio (OR)	95% CI	*p*-value
# of Participants	**1902**	**2874**			
Age					
20-39	280 (55)	506	ref		
40-59	932 (65)	1442	1.48	1.20-1.81	0.001
60+	689 (75)	924	2.47	1.88-2.98	<0.0001
Employment					
Currently employed[c]	900 (66)	1370	0.96	0.82-1.12	0.59
Currently unemployed	1001 (66)	1502	ref		
Highest Education					
High school	578 (55)	1050	ref		
College/education	1316 (73)	1808	2.18	1.86-2.56	<0.0001
Country of residence					
Argentina	145 (40)	364	0.12	0.10-0.16	<0.0001
China	40 (14)	291	0.03	0.02-0.04	<0.0001
Denmark	459 (88)	529	1.23	0.91-1.65	0.18
Japan	163 (52)	315	0.20	0.15-0.26	<0.0001
United Kingdom	160 (60)	265	0.29	0.21-0.38	<0.0001
United States	935 (84)	1110	ref	ref	
Referral source:					
Dentist	1866 (79)	2376	47.0	34.0-66.8	<0.0001
Non-Dentist[d]	36 (7)	498	ref		
Sjögren's Syndrome status					
SS	762 (58)	1320	0.50	0.42-0.58	<0.0001
Non-SS	1140 (73)	1554	ref		
Perception of oral health					
Excellent to good	963 (81)	1191	3.34	2.81-3.98	<0.0001
Fair to poor	939 (56)	1682	ref		
Number of teeth					
6-16 teeth	103 (46)	223	ref		
>16 teeth	1799 (68)	2651	2.46	1.87-3.24	<0.0001
Number of dental caries					
None	1355 (71)	1901	2.57	1.94-3.42	<0.0001
1-5 caries	440 (58)	755	1.40	1.07-1.96	0.02

>5 caries	107 (49)	218	ref		
Does your mouth feel dry?					
Yes	1741 (67)	2597	1.47	1.14-1.89	0.003
No	161 (58)	277	ref		
Difficulty swallowing					
Yes	1059 (70)	1510	1.45	1.24-1.69	<0.0001
No	843 (62)	1364	ref		
Eat a cracker without fluid					
Yes	849 (64)	1324	ref		0.03
No	1050 (68)	1546	1.18	1.01-1.38	
Frequency of brushing					
≥2x/day	1738 (67)	2572	1.76	1.39-2.25	<0.0001
<2x/day, never	163 (54)	301	ref		
Frequency of flossing					
≥1x/day	996 (84)	1190	4.41	3.68-5.28	<0.0001
<1x/day, never	906 (524)	1684	ref		

[a] n=number of individuals who received a dental cleaning in the past 12 months within each row variable. Column count for each variable may not add up to total number of women who received a cleaning (n=1902) due to missing data.
[b] %= frequency of individuals within each row variable.

Table 14: Multivariate logistic regression model for predictors of preventative dental care use among 2874 women participating in the Sjögren's International Collaborative Clinical Alliance (SICCA), excluding 100 women from India.

	Received dental cleaning[a] n[b](%)[c]	Total	Adjusted Odds Ratio (OR)	95% CI	*p*-value
# of Participants	**1902**	**2874**			
Age					
20-39	280 (55)	506	Ref		
40-59	932 (65)	1442	1.08	0.80-1.47	0.59
60+	689 (75)	924	1.60	1.13-2.26	0.01
Highest Education					
High school	278 (53)	521	ref		
College/university	1316 (73)	1808	1.41	1.10-1.82	<0.0001
Country of residence					
Argentina	145 (40)	364	0.11	0.08-0.16	<0.0001
China	40 (14)	291	0.05	0.03-0.08	<0.0001
Denmark	459 (88)	529	1.19	0.79-1.79	0.40
Japan	163 (52)	315	0.32	0.21-0.48	<0.0001
United Kingdom	160 (60)	265	0.22	0.15-0.32	<0.0001
United States	935 (84)	1110	ref		
Referral source:					
Dentist	1866 (79)	2376	51.93	34.78-77.55	<0.0001
Non-Dentist[d]	36 (7)	498	ref		
Sjögren's Syndrome status					
SS	762 (58)	1320	0.71	0.56-0.90	<0.0001
Non-SS	1140 (73)	1554	ref		
Perception of oral health					
Excellent to good	963 (81)	1191	1.77	1.38-2.28	<0.0001
Fair to poor	939 (56)	1682	ref		
Number of teeth					
6-16 teeth	103 (46)	223	ref		
>16 teeth	1799 (68)	2651	1.97	1.33-2.93	0.001
Frequency of flossing					
≥1x/day	996 (84)	1190	1.82	1.42-2.34	<0.0001
<1x/day, never	906 (54)	1684	ref		

[a] Hosmer-Lemeshow goodness of fit, p=0.26
[b] n=number of individuals who received a dental cleaning in the past 12 months within row variable. Column count for each variable may not add up to total number of women who received a dental cleaning (n=1902) due to missing data.
[c] %= frequency of individuals within row variable.

Germinal Center Structures and Lymphoma Development Among Individuals in the Sjögren's Syndrome International Collaborative Clinical Alliance (SICCA) Registry

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration of exocrine glands, mainly affecting the lacrimal and salivary glands and causing the clinical symptoms of dry eyes and dry mouth. Individuals with SS typically develop a serologic profile of autoantibodies directed against ribonucleoproteins Ro/SSA and La/SSB, antinuclear antibodies (ANA), and rheumatoid factor (RF) (1). This state of B-cell hyperactivity places individuals with SS at an increased risk for development of non-Hodgkin's lymphoma, particularly of the marginal zone type (formerly lymphoma of mucosa-associated lymphoid tissue (MALT) subtype), for which individuals with SS have an estimated 9-44 fold increased risk compared to healthy controls (2-6). In comparison to other rheumatic diseases, individuals with SS have the greatest risk for development of lymphoma (7), highlighting the need for better understanding of biologic mechanisms responsible for this serious complication.

Some important genetic events have been implicated in the development of lymphoma in SS. Germline and somatic variants in the *tumor necrosis factor, alpha-induced protein 3 (TNFAIP3)* gene (8) and *B-cell activating factor (BAFF)* gene (9) have been identified within labial salivary glands (LSG) and/or genomic DNA of individuals with SS who developed lymphoma. Because this transformation may take years to develop, it is challenging to measure this outcome among individuals with SS. Thus, substantial work has sought to identify early risk factors for lymphomagenesis in this population. Clinical and laboratory features have been identified as predictors of lymphoma development, which include recurrent or chronic enlargement of the parotid and/or submandibular glands (4, 6, 10), lymphadenopathy (1, 4, 10), cryglobulinemia (4, 11), splenomegaly (1, 4, 6), low levels of serum complement factors C3 and C4 (1, 4-6, 11), lymphocytopenia (1, 4-6), leukopenia (1, 6), hypergammaglobulinemia (1, 6,

11), skin vasculitis or palpable purpura (4-6, 11), peripheral neuropathy (4), glomerulonephritis (4), anemia (1, 6), and chronic leg ulcers (10). Also, monoclonal immunoglobulin heavy chain gene rearrangements in lymphoplasmacytic infiltrates of labial salivary glands in SS has shown to be a useful marker for predicting the progression to lymphoma in SS (12, 13).

More recent work provides evidence that ectopic GC-like structures in labial salivary glands (LSGs) are precursors to lymphoma among individuals with SS. Germinal centers (GC) are aggregates of proliferating B-cells within peripheral lymphoid tissues, normally found in the spleen, lymph nodes, Peyer's patches, and tonsils. These structures form in response to an exogenous antigen through T-cell dependent antibody response and reach its maximum size within 2 weeks after initial immunological response (14). Ectopic GC structures have been described as important drivers of immune dysregulation in autoimmune disorders, like rheumatoid arthritis (15), psoriatic arthritis (16), and myasthenia gravis (17). Because ectopic GC-like structures have been associated with some B-cell lymphomas like Burkitt's lymphoma (18), there is speculation that these structures may also be associated with B-cell lymphoma development in autoimmunity. Ectopic GC-like structures within LSG have been described in approximately 25% of individuals with SS in the literature (19-21). Theander *et al.* showed that 6/7 Swedish individuals (86%) with SS who later developed lymphoma had GC-like structures within their LSG biopsies at the time of SS diagnosis (22). Similarly, Bombardieri *et al.* found that 6/8 (75%) of British individuals with SS who later developed lymphoma also had these structures within their LSGs (23). Further, GC-like structures in SS have been associated with a more severe serologic measures (elevated titers, serum anti-Ro/SSA and anti-La/SSB, and IgG levels ≥ 15.3 g/L) (24) and gene expression profiles (CCL11, CXCL13, CXCR5) (19, 25), which may be etiologically relevant to the development of lymphoma in SS. Thus, GC-like formation is an attractive target for investigation in SS given its location within target tissue and its development many years before transformation to lymphoma, facilitating earlier detection and intervention for high-risk patients.

GC-like formation has been characterized in a large collection of LSG biopsies among participants in the Sjögren's International Collaborative Clinical Alliance (SICCA) registry. This registry features a comprehensive collection of clinical data, biopspecimens (LSG biopsies frozen and paraffin embedded, saliva, tears, plasma, and serum), and full genome-wide genetic profiles (>2.5 million genotypes) from over 3,500 participants enrolled from 9 recruitment sites in 7 countries within the past decade. This resource represents an unprecedented opportunity to study the pathogenesis of SS and related complications. The primary objective of this study was to determine the relationship between clinical, serological, and genetic risk factors of lymphoma with GC-like formation in LSGs of individuals with SS.

Material and Methods

Study Sample

Data was collected from participants enrolled in the SICCA registry. The cohort (26) and eligibility criteria are described in more detail in the Introduction. This present analysis was based on all individuals with SS enrolled in the SICCA registry and for whom phenotype data and fixed LSGs were available for H&E histopathological review as of March 2012. Only participants who demonstrated focal lymphocytic sialadentitis (FLS) or sclerosing FLS (S/FLS) on histopathological review were included in this study since GC-like formation is preceded by the presence of these histological findings. The study featured individuals with SS of homogenous European or East Asian ancestry based on genetic ancestry analysis since they represented the two largest ancestry groups in SICCA. SS classification was determined using the American College of Rheumatology (ACR) classification criteria for SS, which was developed by the SICCA investigative team and endorsed by the ACR in 2011 (27). A diagnosis of SS requires fulfillment of at least two of the following requirements: 1) positive serum anti-SSA (Ro) and/or anti-SSB (La) or [positive RF and ANA titer ≥ 1:320]; 2) ocular staining score ≥3 using lissamine green or fluorescein to diagnose keratoconjuncitivitis sicca

(28); or 3) presence of FLS with a focus score ≥1 focus/4mm^2 in labial salivary gland biopsy specimen (29).

Variables and Measurements

Data Collection

At the time of SICCA entry, all participants completed a standardized baseline questionnaire that featured questions relating to demographics, socioeconomic background, and general health. All answers were self-reported. At each site, trained SICCA team clinicians (including oral medicine specialists, ophthalmologists, and rheumatologists) performed focused protocol-driven clinical evaluations, which assessed for oral, ocular, and rheumatological features. All serological test results were obtained from the same Quest laboratory. SICCA questionnaires, data collection forms, and protocols are available for review at http://sicca.ucsf.edu.

Labial Salivary Gland Biopsy

Labial salivary gland (LSG) biopsies were obtained on the vast majority of SICCA participants at the time of the oral examination. Five to eight minor salivary glands were collected from the lower labial mucosa. Half of the collected specimens were fixed in formalin and then embedded in paraffin while the other half were preserved as frozen glands. A histopathological diagnosis was assigned to each fixed specimen independently by two histopathologists at UCSF with any disagreements resolved by conference with a third pathologist. Diagnoses included: non-specific chronic inflammation, sclerosing chronic sialadenitis, granulomatous inflammation, focal lymphocytic sialadentitis (FLS), sclerosing FLS (S/FLS), lymphoma, or within normal limits if no abnormality was detected. Specimens that exhibited FLS were examined for other histopathological characteristics, which included: number of lymphocytic foci, area of salivary gland tissue in the specimen, focus score (in

foci/4mm^2), GC-like formation, epimyoepithelial islands, and parenchymal fatty replacement. GC-like formation was identified by light microscopy in H&E stained sections based on the presence of at least one defined spherical or ovoid aggregate of mononuclear cells showing an organized zonation of centroblasts and centrocytes occurring within a dense surrounding aggregate of lymphocytes containing a small proportion of plasma cells (29). The presence of GC-like structures was recorded as positive (+) or negative (-).

SICCA Genetic Data

Our literature review identified candidate genes that previously demonstrated genetic association with lymphoma development in SS. We focused on genetic variants within 2 candidate genes, *TNFAIP3* (rs13192841, rs2230926, rs6922466) and *BAFF* (rs9514827). The *TNFAIP3* locus at 6q23 encodes the A20 protein, which plays an important role in regulating inflammatory responses through the NF-κb pathway. The *TNFAIP3* variants were chosen due to previous association with multiple autoimmune diseases as well as the transformation to lymphoma in the setting of autoimmunity (30-33). Specifically, rs2230926 has demonstrated an increased risk of lymphoma transformation among individuals with SS (8). Similarly, *BAFF* regulates B-cell activity and has also been implicated in the pathogenesis of autoimmunity and lymphoma (34-40). Specifically, the rs9514827 variant has been associated with SLE and SS as well as lymphoma development in SS (9, 37, 40).

Genotype data was generated from blood DNA of SICCA participants as part of the SICCA Genome-wide Association Study (GWAS) performed in collaboration with the Center for Inherited Disease Research (CIDR) with the Illumina HumanOmni 2.5-4v1_H Quad marker set (41). Quality control (QC) measures included filters based on single nucleotide polymorphism (SNP) and missing samples (≥2%), unexpected relatedness, non-Mendelian inheritance, and chromosomal regions of anomaly (>10 Mb). A total of ~1,500,000 SNPs passed all QC filters.

Other Clinical and Demographic Characteristics

The clinical predictors of lymphoma consisted of palpable lymphadenopathy (cervical, axillary, or inguinal), skin vasculitis (petechial, purpura, nodules, or ulcers), and splenomegaly. The serologic markers included in this analysis were anti-SSA/Ro, anti-SSB/La, RF, and ANA. A past history of lymphoma was also collected and confirmed by their treating physicians. These characteristics were recorded as being present (yes) or absent (no). Levels of complement C3, complement C4, and immunoglobulin G (IgG) were recorded as continuous measures. Other demographic variables included gender (male, female), age, and history or current use of tobacco products (yes, no). Symptoms of dry eyes (yes, no), dry mouth (yes, no), and salivary flow rate were also recorded.

Statistical Analyses

Baseline characteristics of individuals participating in the SICCA registry were described using frequencies and percentages for categorical variables and means and ranges for continuous measures. To examine the association of GC-like formation with serologic and clinical risk factors of lymphomagenesis in SS, we used chi-square statistics and Fisher's exact test. A non-parametric Mann-Whitney U test was used to assess statistically significant differences between continuous measurements.

Genetic association testing was performed using PLINK, a publicly available open-source whole genome association analysis toolset (42). In particular, we performed logistic regression with an allelic model for SNPs (0, 1, or 2 copies of the minor allele), adjusting for potential covariates. To account for background genetic ancestry, we utilized principal components analysis (PCA) using the EIGENSTRAT program (43) to identify ancestry outliers and to adjust for intra-European and intra-Asian substructure. Analyses were stratified by ethnicity to control for genetic variability due to ethnic differences. Results for the two ancestry subgroups were formally combined using meta-analytic techniques if heterogeneity $p > 0.10$. To

counteract problems of multiple comparisons, we applied the Bonferroni correction so that a $p < 0.01$ was considered to be statistically significant.

Results

European and Asian individuals with SS demonstrated GC-like formation on LSG sections

Population structure revealed clustering of 5 distinct subgroups based on genetic ancestry analysis (Figure 3), for which homogenous subgroups of European and East Asian (excluding India) individuals were identified. Three hundred five individuals of European and 329 individuals of Asian ancestry met the ACR classification criteria for SS and had LSG sections with FLS or F/SLS available for histopathologic review (Tables 15 and 16). Twenty percent (n=62) of European SS patients and 11% (n=35) of Asian SS patients had GC-like structures in their LSGs sections at their baseline visit.

Association of GC-like formation with other well-established predictors of lymphoma in SS

The association of GC-like formation with other markers of lymphoma development in SS was evaluated within European and Asian subgroups. Among the European subgroup, participants with SS and GC-like formation were significantly more likely to be RF positive ($p = 0.004$), have a higher focus score ($p = 0.02$), have lower unstimulated whole saliva ($p = 0.007$), hypergammaglobulinemic ($p = 0.01$), have low C4 complement levels ($p = 0.01$), and have bilateral parotid enlargement ($p < 0.001$) compared to SS participants without GC-like formation. Within the Asian subgroup, there was a suggestive association of bilateral parotid enlargement with GC-like formation ($p = 0.07$) among individuals with SS.

GC-like formation is associated with genetic variants in BAFF *and* TNFAIP3

Three SNPs, rs2230926, rs6922466, and rs13192841, were examined in the *TNFAIP3* gene region (Table 17). The rs2230926 is a coding polymorphism, resulting in the substitution of

phenylalanine by cysteine at residue 127 (T > G, F127C) and is located near a deubiquitinating motif responsible for the de-ubiquitylating activity of A20 protein (Figure 4). We did not find a significant association between the rs2230926 variant with GC-like formation in the European subgroup, the Asian subgroup, or their meta-analysis. The other two variants, rs13192841 and rs6922466, are noncoding polymorphisms. The rs13192841 (G > A) variant is located upstream of the *TNFAIP3* region whereas the rs6922466 (A > G) variant is located downstream of the *TNFAIP3* region. We found the rs6922466 variant to be significantly associated with GC-like formation in the Asian subgroup only (OR=2.08; 95% CI: 1.18-3.65; p=0.01). No significant association was found between rs13192841 and GC-like formation in our analysis.

The rs9514827 variant is located in the regulator region of *BAFF* (Figure 5). *BAFF* is a ligand for receptors that activates the NF-κb pathway and is essential for the formation and maintenance of B cells. We identified the rs9514827 variant of the *BAFF* gene to be associated with GC-like formation in a meta-analysis of the European and Asian subgroups (OR:0.67, p=0.02) (Table 17).

Minor allele frequencies of variants seen in GC-formation and lymphoma development in SS

We identified 22 SICCA participants with lymphoma, comprising of 20 individuals with a history of lymphoma and 2 with lymphoma identified during SICCA LSG histopathological review (n=2) (Table 18). Fifteen individuals with lymphoma were classified as having SS. Five individuals with SS had GC-like structures at the time of their LSG biopsy (33%), 9 did not have these structures (60%), and 1 did not undergo a LSG biopsy (7%).

We compared allele frequencies of the 4 genetic variants in lymphoma cases (n=4) and GC+ cases (n=62) within our European subgroup (Table 19). We did not include data for individuals of Asian ethnicity due to the lack of cases (n = 1). We found comparable minor allele frequencies (MAFs) between individuals with GC-like structures versus lymphoma for rs6922466 (0.24 versus 0.25) and rs9514827 (0.22 versus 0.25). We also found similar MAFs

between individuals without GC-like structures versus no lymphoma for rs6922466 (0.21 versus 0.21) and rs9514827 (0.35 versus 0.32).

Discussion

The most serious complication associated with SS is the development of marginal zone B-cell (MALT) lymphoma, particularly MALT. Since it may take years for lymphoma to develop in SS, investigators have identified early risk factors of malignancy in SS as a measure of disease severity. Recent evidence suggests that the presence of GC-like structures in LSGs may predict the development of lymphoma in SS. Given the greater prevalence of GC-like formation compared to lymphoma development in SS, GC-like formation provides greater power to identify genetic variants that will likely influence the risk of lymphoma development in SS. In our analysis, we found that GC-like formation in SS was associated with serologic markers, clinical manifestations, and genetic risk variants for lymphoma. In addition, we found significant ethnicity differences in clinical presentation and genetic susceptibility to GC-like formation in SS.

Among our SS cohort, 20% (n=62) of the European subgroup and 11% (n=35) of the Asian subgroup exhibited GC-like structures in their LSG sections at the time of their baseline visit. GC-like formation has been well documented in SS and typically detected in approximately 25-30% of individuals with SS (19-21). A recent meta-analysis of GC-like formation in SS found that the mean prevalence of GC structures was 25.1 ± 5.0% (range of 18.33 to 33.3%) using H&E criteria (20). Because the majority of studies have been conducted in SS cohorts of European ancestry, the prevalence of GC-formation observed in our European subgroup complements these prior studies. However, the prevalence of GC-like structures seen in our Asian subgroup was much lower than expected. Also, there was a surprising lack of serologic markers and clinical manifestations associated with GC-like formation in the Asian subgroup compared to the European SS subgroup. Taken together, our findings suggest that there are significant ethnicity differences in the disease profile of SS and the progression of SS

severity. In fact, Zhao *et al*. highlighted this difference in their study when they found that their Han Chinese patients with SS showed particular clinical manifestations, systemic involvement, and immunological alterations compared to other previous studies performed in other ancestry groups (44).

We identified 1 variant, rs6922466 (A>G) of the *TNFAIP3* gene, which is a noncoding polymorphism downstream of *TNFAIP3* gene, to be significantly associated with GC-like formation in the Asian subgroup. We found that Asian individuals with the minor allele (G) were twice as likely (in terms of odds) to form GC-like structures compared to those with the major allele (A). We were unable to detect a statistically significant association between the rs6922466G variant and GC-like structures in our European subgroup (OR = 0.75, p = 0.28). The rs6922466 variant has been found to be associated with multiple autoimmune rheumatic diseases in multiple studies (30-33) and has recently gained interest for its role in lymphoma transformation in (SS and non-SS) autoimmune settings (45-49). Nocturne *et al*. was the first to investigate the rs6922466G variant for association with lymphomagenesis in blood and tumor DNA of individuals with SS, but they were unable to detect a significant association (OR: 0.81, p = 0.56) in their French cohort (8). In our study, we found this genetic risk locus to be associated with GC-like formation among individuals of Asian ethnicity only, revealing that differences in ethnicity can be associated with different susceptibility loci. In fact, recent GWAS performed in European and Han Chinese SS cohorts showed very little overlapping *TNFAIP3* loci associated with SS development (50, 51) (Table 20). This highlights a powerful genetic component to SS and its complications.

A second genetic variant in *BAFF*, rs9514827 (A>G), was found to be suggestively associated with GC-like formation in our meta-analysis of the Asian and European subgroups. We found that the rs9514827G variant was associated with a 0.67 increase in the odds of developing GC-like structures compared to the major allele (A) in both subgroups. Increased serum and tissue *BAFF* levels have been observed in patients with autoimmune rheumatic

diseases such as SLE, SS, and RA (34-38). Among patients with SS specifically, strongly expressed *BAFF* protein has been identified within infiltrating inflammatory cells of the labial salivary glands (38). Nezos *et al.* were the first to describe a genetic association between *BAFF* germline variants and lymphoma development among individuals with SS (8). However, they did not detect a significant association between rs9514827 and high-risk of developing lymphoma (as defined by the presence of lymphoma or adverse predictors of lymphoma including salivary gland enlargement, purpura and low complement C4 levels) versus SS individuals at low-risk for lymphoma (OR: 0.97, 95% CI; 0.61-1.41, p=0.73). The lack of association seen in their study is likely due to the heterogeneity of their high-risk group. Nezos' study included 52 individuals with a history of lymphoma and 30 individuals with clinical and serological predictors of lymphoma development, which may or may not be genetically relevant to lymphomagenesis. In our analysis, we did not detect significant genetic heterogeneity in rs9514827 between the European and Asian subgroups, so we were able to perform a meta-analysis consisting of 97 individuals with GC structures. In doing so, we detected a suggestive association between the rs9514827 and GC-like formation in both groups. Here, we show that there can be similar genetic susceptibility to GC-formation in SS across ethnic groups, particularly, of the rs9514827 variant. Thus, while a broad range of genetic variation exists across ethnicity groups, there is also considerable similarity in genetic mechanisms across groups.

In general, we were surprised to find such genetic variability between European and Asian subgroups. This may be due to differences in genetic mechanisms among specific ancestry groups such as absence/presence of target variants, allelic heterogeneity, and differences in linkage disequilibrium. Also, we were surprised by the lack of significant associations detected between GC-like formation and genetic variants among European and Asian individuals in our study. This lack of association could likely be attributed to low power of our study. For instance, as reported previously by Nocturne *et al.* (8), to detect an OR of 3.36

with a *p* of 0.01 between lymphoma development in SS and rs2230926, our study only had 43% to detect such an association among our European subgroup and 4% power among our Asian subgroup.

The formation of GC-like structures as a precursor to lymphoma has been questioned in the literature. While there are several studies in the literature that demonstrate GC structures as a predictor of lymphoma development in SS (19, 22, 23), Johnsen *et al*. recently showed a lack of association between GC-like structures and lymphoma development in their Norwegian SS cohort (52). We found that 33% (n=5) of SICCA individuals who developed lymphoma or had a history of lymphoma also demonstrated GC-like structures in their LSG. Due to lack of available lymphoma cases, we did not have sufficient power to determine the magnitude of association between lymphoma development and genetic variants directly. However, similar allele frequencies seen in our lymphoma and GC cases suggest that similar genetic changes may be associated with both processes and share similar biological mechanisms. However, the relationship between individuals with a history of lymphoma development and GC-like formation in SS is unclear since lymphoma development may have preceded SS development as an unrelated event. Surprisingly, there were no allelic changes in the rs2230926 variant among SS individuals with lymphoma in our study. Nocturne *et al.* previously showed an association between rs2230926G and lymphoma in a French SS cohort (8); however, the absence of the rs2230926 variant in our study is likely a reflection of the scarcity of lymphoma cases (n=4) in the SICCA registry.

There were several strengths to this study. Given the greater prevalence of GC-like formation compared to lymphoma among SS patients, the use of GC-like formation in this study allowed for more power to detect genetic variants. This retrospective study also takes advantage of a unique resource for studying SS based on the size of the SICCA registry, including the extensive phenotypic data available and biospecimen collection from 9 international sites. This study also benefited from the availability of a genome-wide set of

genetic markers that have been recently generated for all SICCA participants to identify homogenous genetic ancestry groups for analysis. And finally, with the application of the new ACR classification criteria, our cohort consisted of a homogenous group of SS individuals who have demonstrated objective oral, ocular, and systemic characteristics specific to SS.

There were many areas in this study that can be improved upon, most notably, the classification of GC-like formation based on H&E staining. Surprisingly, our estimated prevalence of GC formation was lower than reported by other studies. Although GC formation can be identified through H&E staining, it is only sufficient for the identification of moderate to large-sized structures, allowing for the possibility for missing smaller GC structures. Studies using IHC staining of CD21 follicular dendritic cells reported an average GC+ prevalence of 34%, which is higher than the mean prevalence of GC+ formation of 25.1% identified from H&E staining (20, 23, 53). IHC has been performed for only some of the SICCA specimens since the SICCA budget did not support wide use of IHC. Therefore, since not all GC structures were formally identified, misclassification might have decreased the actual prevalence of GC formation, and resulted in lower power for our analyses. Further directions will include the extension of this work to include a more definitive identification of GC formation using anti-CD21 IHC staining (54, 55) on all available SICCA LSGs. Also, a broader genetic analysis of the full SICCA GWA data as well as the analysis of more genetic variants related to SS-related lymphomagenesis, GC-like formation in SS (and other non-SS setting), and lymphoma development more generally will be performed. These studies will be followed by analyses using bioinformatics tools to assess the biological function of the most strongly associated genetic variants and to also prioritize genes/variants for further investigation.

In conclusion, we found that ethnic differences in genetic susceptibility contribute to SS and its complications, such as the formation of ectopic GC-like structures in LSGs. We also provide some evidence to show that similar allele frequencies are seen in both GC-like formation and lymphoma development in SS, suggesting similar biological mechanisms.

Therefore, these ectopic structures show promising potential as an objective histopathological marker of more severe disease, specifically lymphoma development, in SS and may represent an important opportunity for identifying those individuals at greatest risk for this disease complication. Early recognition can allow for more rigorous follow-up for the detection and early intervention of lymphoma development among populations with SS. However, further investigation is needed to fully elucidate the role of GC-like formation in lymphoma development in these populations.

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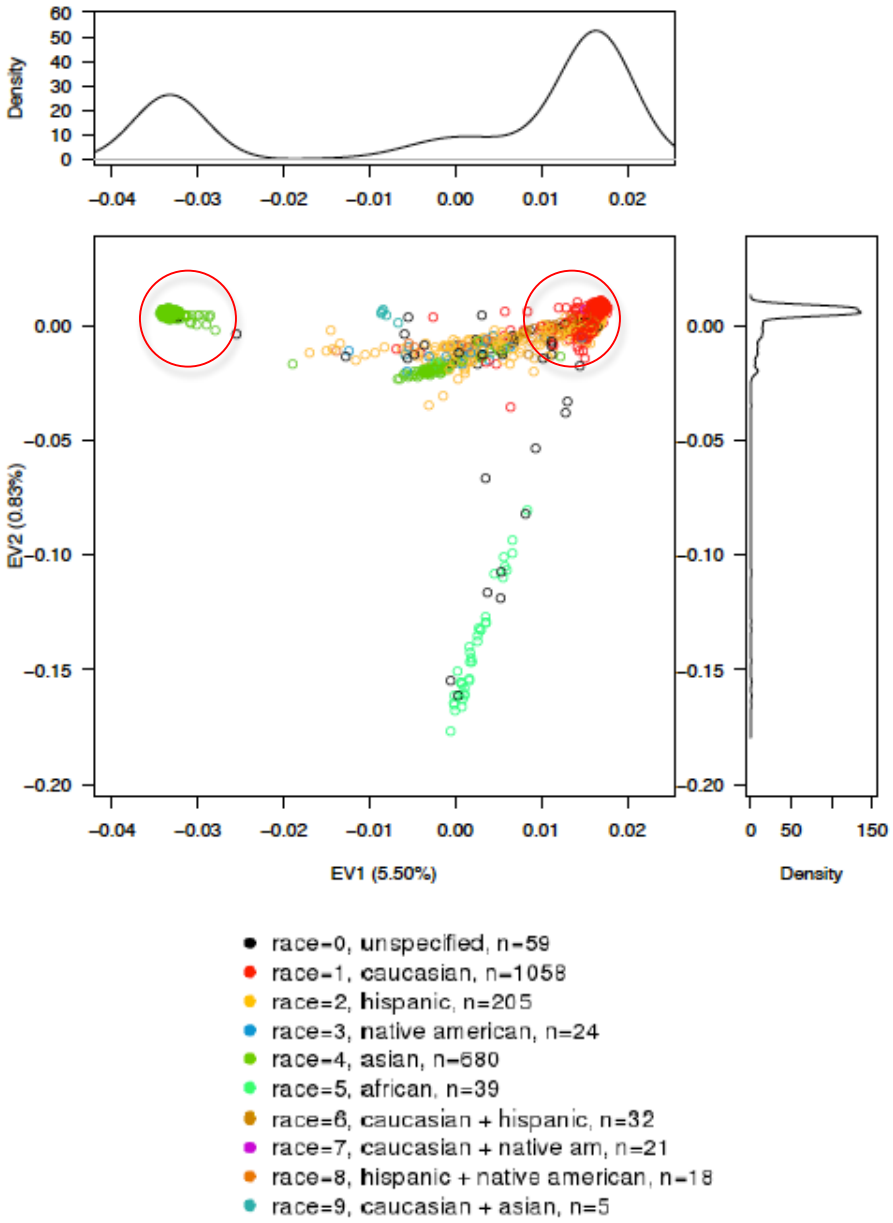

Figure 2: Principal component analysis of 2,141 unrelated study subjects.
Clustering of distinct ethnic groups are seen and used to define homogenous European and Asian subgroups (circled). Axis labels indicate the percentage of variance explains by each eigenvector. Analysis performed in EIGENSTRAT (41).

Table 15: Characteristics of individuals with Sjögren's Syndrome (SS) of European ancestry that meet the American College of Rheumatology classification criteria for SS

	Germinal Centers with Focal Lymphocytic Sialadenitis[a]	No Germinal Centers with Focal Lymphocytic Sialadenitis[a]	Total	p-value[b]
Participants (%):	**62 (20.3)**	**243 (79.7)**	**305**	
Gender:				
Women (%)	57 (91.9)	222 (91.4)	279 (91.5)	0.66
Age, mean years [SD]	55.9 [14.7]	56.2 [12.0]	56.2 [12.5]	0.73
Smoking, ever (%)	25 (40.3)	111 (45.7)	136 (44.6)	0.24
Symptoms of:				
Dry mouth (%):	60 (96.8)	232 (95.5)	292 (95.7)	0.65
Dry eyes (%):	56 (90.3)	229 (94.2)	285 (93.4)	0.27
Positive serum:				
Anti-SSA (Ro) (%)	44 (71.0)	167 (68.7)	211 (69.2)	0.73
Anti-SSB (LA) (%)	36 (58.1)	112 (46.1)	148 (48.5)	0.11
RF (%)	51 (82.3)	144 (59.3)	195 (63.9)	**0.004**
ANA ≥1:320 (%)	56 (90.3)	196 (80.7)	252 (82.6)	0.11
C3, mean [SD]	117.8 [29.7]	117.9 [27.5]	117.6 [27.8]	0.95
C4, mean [SD]	19.3 [7.86]	22.7 [8.46]	21.9 [8.40]	**0.01**
Gammaglobulin levels (mg/dL), median [range]	1555 [588-4648]	1319 [215-5548]	1354 [215-5548]	**0.01**
Focus score, median [range]	4.3 [1-11.3]	2.5 [0.1-12]	2.9 [0.1-12]	**0.02**
Unstimulated whole saliva, (ml/min); median [range]	0.08 [0-2.5]	0.25 [0-2.9]	0.22 [0-2.9]	**0.007**
Parotid enlargement:				**<0.0001**
Unilateral (%)	4 (6.45)	15 (6.17)	19 (6.23)	
Bilateral (%)	13 (21.0)	28 (11.5)	41 (13.4)	
Lymphadenopathy* (%)	9 (14.5)	25 (10.3)	34 (11.1)	0.34
Skin vasculitis° (%)	3 (4.84)	8 (3.29)	11 (3.61)	0.56
Splenomegaly (%)	0	0	0	0

*Lymphadenopathy = cervical, axillary, inguinal lymphadenopathy;
°Skin vasculitis = petechia, purpura, ulcer, nodules
[a] ( ) = percentage; [ ] = standard deviation
[b] The bold numbers denote statistically significant associations ($p < 0.05$)

Table 16: Characteristics of individuals with Sjögren's Syndrome (SS) of Asian ancestry that meet the American College Rheumatology classification criteria for SS

	Germinal Center with Focal Lymphocytic Sialadenitis[a]	No Germinal Centers with Focal Lymphocytic Sialadenitis[a]	Total	p-value[b]
Participants (%):	**35 (10.6)**	**294 (89.4)**	**329**	
Gender:				
Women (%)	35 (100)	287 (97.6)	322 (97.9)	0.36
Age, mean years [SD]	49.2 [12.4]	49.6 [14.0]	49.6 [13.8]	0.60
Smoking, ever (%)	7 (20.0)	39 (13.3)	46 (14.0)	0.28
Symptoms of:				
Dry mouth (%):	29 (82.9)	255 (86.7)	284 (86.3)	0.53
Dry eyes (%):	27 (77.1)	227 (77.2)	254 (77.2)	0.99
Positive serum:				
Anti-SSA (Ro) (%)	27 (77.1)	252 (85.7)	279 (84.8)	0.18
Anti-SSB (LA) (%)	19 (54.3)	154 (52.4)	173 (52.6)	0.92
RF (%)	26 (76.5)	195 (66.3)	221 (67.4)	0.23
ANA ≥1:320 (%)	31 (88.6)	266 (90.8)	297 (90.6)	0.67
C3, mean [SD]	111.7 [24.8]	107.5 [25.3]	107.9 [25.3]	0.34
C4, mean [SD]	24.22 [7.51]	23.3 [9.80]	23.4 [9.57]	0.59
Gammaglobulin levels (mg/dL), median [range]	1793 [752-3180]	1810 [549-5940]	1807.5 [549-5940]	0.85
Focus score, median [range]	3.6 [1-9.8]	2.55 [0.2-12]	2.6 [0.2-12]	0.67
Unstimulated whole saliva, (ml/min), median [range]	0.27 [0-2.1]	0.27 [0-6.2]	0.27 [0-6.2]	0.51
Parotid enlargement:				0.07
Unilateral (%)	1 (2.86)	11 (3.74)	12 (3.65)	
Bilateral (%)	10 (28.6)	43 (14.6)	53 (16.1)	
Lymphadenopathy* (%)	1 (2.86)	13 (4.42)	14 (4.26)	0.67
Skin vasculitis° (%)	0	9 (3.14)	9 (2.80)	0.29
Splenomegaly (%)	1 (2.86)	7 (2.38)	8 (2.43)	0.83

*Lymphadenopathy = cervical, axillary, inguinal lymphadenopathy;
°Skin vasculitis = petechia, purpura, ulcer, nodules
[a] ( ) = percentage; [ ] = standard deviation
[b] The bold numbers denote statistically significant associations ($p < 0.05$)

Table 17: Association of *TNFAIP3* and *BAFF* genetic variants with GC-like formation among Sjögren's Syndrome individuals of European and Asian Ancestry.

Gene	Chr	SNP	Minor Allele[a]	Euro MAF[b]	European OR (p-value[c])	Asian MAF	Asian OR (p-value[c])	Test for hetero p-value	Meta-analysis OR (p-value[c])
TNFAIP3	6	rs13192841	A	0.26	1.07 (0.80)	0.13	0.7923 (0.58)	0.54	0.99 (0.95)
TNFAIP3	6	rs2230926	C	0.04	1.11 (0.85)	0.06	1.333 (0.53)	0.79	1.23 (0.55)
TNFAIP3	6	rs6922466	G	0.24	0.75 (0.28)	0.20	**2.075 (0.01)**	**0.009**	n/a
BAFF	13	rs9514827	G	0.33	0.64 (0.059)	0.43	0.6952 (0.16)	0.828	0.67 (0.02)

[a] Reference allele; [b] Minor allele frequency; [c] The bold numbers denote statistically significant associations ($p < 0.01$)

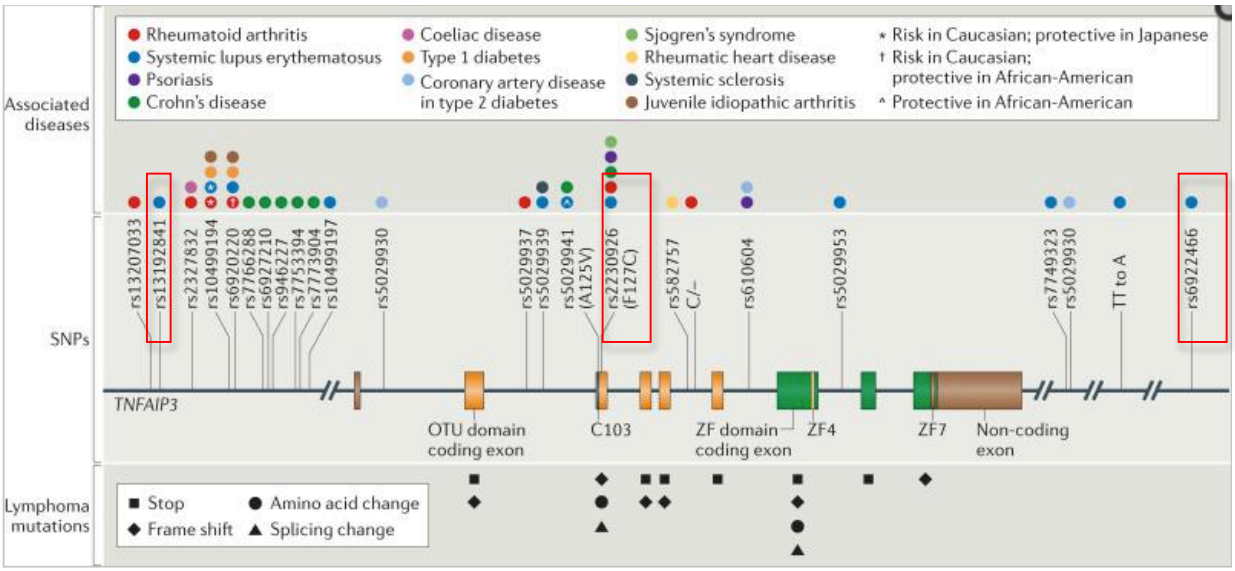

Figure 3: Polymorphisms or mutations in *TNFAIP3* and human diseases. **Figure and figure legend taken from Ma *et al*.**
The figure shows a schematic of the *TNFAIP3* gene locus, which encodes A20. Exons encoding the amino-terminal domain are shown in orange and exons encoding the carboxy-terminal zinc fingers (ZFs) of A20 are shown in green. The C103, ZF4 and ZF7 motifs are highlighted. Non-coding exons, including AT-rich sequences at the end of exon 9, are shown in brown. Human germline single-nucleotide polymorphisms (SNPs) in the TNFAIP3 locus that are associated with autoimmune and autoinflammatory diseases are indicated, as are somatic mutations that have been identified in the coding exons of *TNFAIP3* in human B cell lymphomas. Highlighted variants (rs2230926, rs6922466, rs13192841) were analyzed in this present study. No significant association was seen between rs2230926 and rs13192841 with GC-like formation in European or Asian subgroups (or their meta-analysis). We found that rs6922466 was significantly associated with GC-like formation (OR: 2.075, p = 0.009) in the Asian subgroup only.

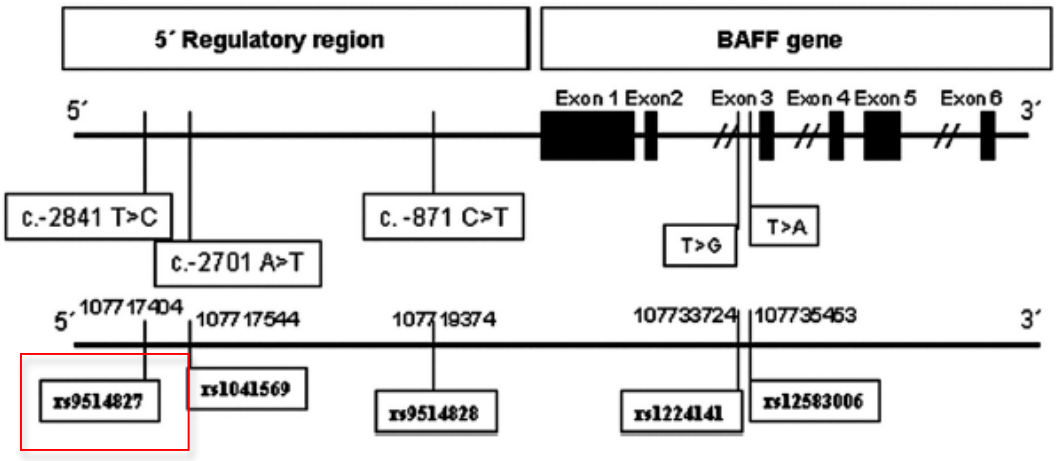

Figure 4: *BAFF* gene and 5' regulatory region. **Figure taken from Nezos *et al*.**
The figure shows a schematic of the *BAFF* gene locus and the 5' regulatory region. Selected polymorphisms are associated with SS or lymphoma susceptibility. The highlighted variant (rs9514827) was analyzed in this present study. We found that rs9514827 was suggestively associated with GC-like formation (OR: 0.67, p = 0.02) in the meta-analysis of the European and Asian subgroups.

Table 18: SICCA participants with Sjögren's Syndrome and a history of lymphoma or a diagnosis of lymphoma based on labial salivary gland biopsy (n = 20)

Location	Diagnosis	Year of Diagnosis	SS Status[a]	GC-like structures[b]
Parotid gland	Marginal Zone lymphoma	2010	SS +	GC +
Minor salivary gland	Diffuse large B-cell lymphoma	2000	SS -	GC -
Bone marrow	Follicular lymphoma	2006	Missing	Missing
Stomach	MALT[c] lymphoma	2010	SS +	GC +
Thoracic	Non-Hodgkin's lymphoma	2009	SS +	GC -
Unknown	Non-Hodgkin's lymphoma	1987	SS -	Missing
Minor salivary gland	MALT lymphoma	2007	SS +	GC -
Lacrimal gland	MALT lymphoma	2008	SS -	GC -
Axillary and inguinal lymph nodes	Follicular cell lymphoma	2004	SS +	GC -
Lacrimal gland	MALT lymphoma	2008	SS -	GC -
Unknown	Non-Hodgkin's lymphoma	1987	SS -	Missing
Unknown	Non-Hodgkin's lymphoma	2010	SS +	GC +
Retroperitoneal	Non-Hodgkin's lymphoma	2009	SS -	Missing
Bone Marrow	Large B-cell lymphoma	1999	SS +	Missing
Palate	Marginal zone lymphoma	2012	SS +	GC -
Cutaneous	MALT lymphoma	2010	SS +	GC -
Conjunctiva	Marginal zone lymphoma	2008	SS +	GC -
Minor Salivary gland	MALT lymphoma	2009	SS +	GC +
Unknown	Non-Hodgkin's lymphoma	2005	SS +	GC -
Unknown	Non-Hodgkin's lymphoma	1995	SS +	GC -
Unknown	Non-Hodgkin's lymphoma	2008	SS +	GC -
Unknown	Non-Hodgkin's lymphoma	2007	SS +	GC +

[a] According to the American College of Rheumatology classification criteria for Sjögren's Syndrome (25);
[b] Germinal center-like formation identified through H&E staining
[c] Mucosa-associated lymphoid tissue

Table 19: Allele frequencies of *TNFAIP3* and *BAFF* genetic variants of individuals with Sjögren's Syndrome of European Ancestry with lymphoma and germinal center formation (n = 305)

Gene	Chr	SNP	Minor Allele	Lymphoma+ MAF[a] (n = 4)	Lymphoma- MAF[a] (n = 301)	Germinal Center + MAF[a] (n = 62)	Germinal Center - MAF[a] (n = 243)
TNFAIP3[b]	6	rs13192841	A	0	0.21	0.24	0.20
TNFAIP3[b]	6	rs2230926	C	0	0.04	0.04	0.03
TNFAIP3[b]	6	rs6922466	G	0.25	0.21	0.24	0.21
BAFF[c]	13	rs9514827	G	0.25	0.32	0.22	0.35

[a] Minor allele frequency; [a] *Tumor Necrosis Factor, alpha-induced protein (TNFAIP3)*; [b] *B-cell activating Factor (BAFF)*

Table 20: *TNFAIP3* single nucleotide polymorphisms associated with Sjögren's Syndrome (SS) and SS-related lymphomagenesis

SNP	Alleles[a]	Cohort	OR[b], p-value (SS+ vs non-SS)	OR[b], p-value ([SS+, lymphoma+] vs [SS+, lymphoma-])
rs13192841	A/G	French[c]	0.94 (p=0.52)	1.12 (p=0.72)
rs2230926	G/T	French[c]	1.26 (p=0.24)	3.26 (p=0.011)
	G/T	Chinese[d]	1.08 (p=0.67)	-
rs6922466	G/A	French[c]	0.99 (p=0.91)	0.84 (p=0.6)
rs5029939	G/C	Chinese[d]	1.0 (p=0.99)	-
	G/C	Chinese[e]	1.67 ($p=7.75 \times 10^{-9}$)	-
rs6933404	T/C	European[f]	1.29 ($p=6.53 \times 10^{-8}$)	-
rs35926684	GA/G	European[f]	1.29 ($p=7.21 \times 10^{-8}$)	-

[a] Minor/major alleles; minor as reference allele [b] OR = odds ratio; [c] Nocturne *et al* (8); [d] Sun *et al* (546; [e] Li *et al* (51); [f] Lessard *et al* (50)

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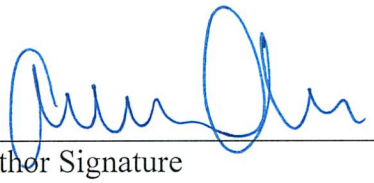

Author Signature

June 10, 2015

Date