Genetics in Sjögren’s syndrome-related lymphomagenesis

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ABSTRACT
Primary Sjögren’s Syndrome (pSS) is a common chronic autoimmune disease affecting 0.5-1% of the population characterized by the lymphocytic infiltration of exocrine glands. Patients suffering from pSS have a 13-fold higher risk of developing non-Hodgkin’s lymphoma (NHL) compared to the general population. Although the pathogenetic events leading from benign autoimmunity to malignancy remain unknown, several mutations have been suggested as possible drivers of this process. The aim of the current research proposal is to investigate the putative role of genetic factors in the pathogenesis of SS-related lymphoproliferation. Blood samples from the Biobank in the Department of Experimental Physiology of the Medical School of the University of Athens - including pSS patients with or without NHL - from rheumatoid arthritis individuals and healthy controls would be analyzed for polymorphisms of methylation enzyme MTHFR, B-cell activating factor (BAFF) as well as a mutation of its receptor BAFF-R His159Tyr, and polymorphisms of the DNA repairing enzyme TREX-1 and the NF-κB pathway inhibitor TNFAIP3/A20 with real time polymeric chain reaction (PCR). The mutation of the LILRA3 immunoglobulin, previously associated with both autoimmunity and lymphoma, will be also evaluated by a PCR-based assay. The results would be compared across the different sub-populations in order to determine whether any particular genetic factors and/or mutations can act as prognostic markers for pSS-related lymphoproliferation and propose new treatment approaches (i.e. targeted therapies) that may benefit these patients.

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Keywords: Sjögren’s syndrome, B-cell activating factor, Methylation alterations, Genetic factors.
BACKGROUND/OBJECTIVE
Primary Sjögren’s syndrome (pSS) is a common chronic autoimmune disease affecting 0.5-1% of the population. It is characterized by lymphocytic infiltration of mainly salivary and lacrimal exocrine glands resulting in oral and ocular dryness, although virtually any organ system can be affected. A cardinal feature of pSS is B-cell hyperactivity, manifested by the presence of hypergammaglobulinemia and various autoantibodies, with the B-cell activating factor (BAFF), a survival factor for B-cells, being a central contributor.1 Depending on the underlying pathophysiological mechanisms, pSS-related systemic manifestations can be largely classified into those related to the presence of peri-epithelial infiltrates in exocrine and parenchymal organs and to those resulting from immunocomplex deposits as a result of B-cell hyperactivity (purpura, peripheral neuropathy, and glomerulonephritis).2 The latter contributes to a disease subset affecting approximately 10% of patients associated with increased risk for B-cell lymphoma development and high mortality rates (high-risk phenotype). Amongst all autoimmune diseases, pSS has the highest risk for development of non-Hodgkin’s lymphoma (NHL). Unfortunately, no preventative strategy is available for the high-risk pSS subset to date. The underlying pathogenetic events leading from benign autoimmunity to malignant transformation remain elusive, although genetic contributors such as mutations of the p53 gene, t(14:18) translocation, as well as variants of the B-cell activating factor (BAFF) gene, a recently described His159Tyr mutation of its receptor (BAFF-R), and a mutation of the tumor necrosis factor alpha-induced protein 3 (TNFAIP3) gene - an NF-κB pathway regulator - have been also associated with SS MAL lymphomagenesis.3,5 Furthermore, epigenetic alterations mainly involving methylation pathways and DNA-repair mechanisms have been recently proposed as important pathogenetic contributors for both autoimmune disorders and cancer development. However, data on SS lymphomagenesis are scarce.10-16

AIM OF THE STUDY
To investigate the putative role of genetic factors in the pathogenesis of SS-related lymphoproliferation.

STUDY PARTICIPANTS
In our Biobank in the Department of Experimental Pathology of the Medical School of the University of Athens, we have collected 270 pSS patients with or without NHL: 150 rheumatoid arthritis (RA) patients, served as disease controls, and 180 healthy controls (HC). Sjögren’s Syndrome patients and RA patients fulfill the revised international classification criteria for SS17 (Vitali, Bombardieri et al. 2002) and the American College of Rheumatology classification criteria for RA18 (Arnett, Edworthy et al. 1988) respectively. The SS lymphoma group consists of both patients with MALT and patients with non-MALT lymphoma fulfilling the WHO classification criteria. Exclusion criteria for all groups are age younger than 18 years old and the presence of other systemic autoimmune diseases. All groups will be age- and gender-matched, of Caucasian origin. Peripheral blood and serum samples are collected and stored in the Autoimmune Biobank Registry of the Physiology Department of the University of Athens. Informed consent for participation in the study was obtained from all subjects and the study has been approved by the Ethics Committee of University of Athens and Laiko General Hospital.

STUDY PROTOCOL
We aim to collect more patients and controls including patients from other Rheumatology clinics and laboratories in the area of Athens, Greece. Then we will extract genomic DNA from the peripheral blood samples as well as the minor salivary gland tissues of all study participants. Polymorphisms of the B-cell activating factor (BAFF) and a mutation of its receptor BAFF-R His159Tyr will be evaluated by PCR-RFLP assays. The mutation of the LILRA3 immunoglobulin, previously associated with both autoimmunity and lymphoma,17,21 will be also evaluated by a PCR-based assay. Polymorphisms of the DNA-repairing enzyme TREX-1 and the NF-κB pathway inhibitor TNFAIP3/A20 will be also evaluated by commercial available TAOXMAN SNP Genotyping Real-Time PCR assays. Furthermore, functional implications will be further explored by quantitative determination (Real-Time PCR) of relevant genes (mRNA transcripts) and/or by western blot analysis of both target gene proteins and other downstream proteins from the level of peripheral blood cells and available salivary gland tissues. Given that polymorphisms of the methylating enzyme MTHFR have been associated with lymphomagenesis, C677T (Ala222Val) and A1298C (Glu429Ala) gene variants will be assessed.22,28 Additionally, putative epigenetic factors implicated in SS lymphomagenesis will be investigated through the global DNA methylation analysis. Alteration in global DNA methylation status will be investigated in DNA samples derived from both minor salivary gland tissues and peripheral blood. The methylation of Cpg islands of the promoter of the long interspersed nuclear element 1 (LINE-1) will be evaluated by pyrosequencing. LINE-1 elements have been used as an indicator of global DNA methylation status, because of their abundant presence in the genomes of many eukaryotic organisms. Statistical analysis will be performed with SNPStats and Shesis softwares for SNPs prevalence, as well as with SPSS and Graph Pad Prism software in order to reveal accurate biomarkers for the prediction of lymphoma development in the setting of SS.
SIGNIFICANCE
Although the link between SS and lymphoma development had first been appreciated more than four decades ago, the underlying pathogenetic events mediating the transition of benign autoimmunity to a malignant disorder remains largely unresolved. Our findings could help us to understand the disease heterogeneity, define the prognostic value of novel biomarkers for pSS-related lymphoproliferation and propose new treatment approaches (i.e. targeted therapies) that may benefit these patients.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES