103 EXPLORATORY SEGREGATION OF PATIENTS UPON THEIR LEVELS OF ANTI- MITOCHONDRIAL ANTIBODIES (AMAS) REVEALS ASSOCIATIONS BETWEEN AMAS AND DISEASE MANIFESTATIONS

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Background Mitochondria are intracellular organelles derived from the endosymbiosis between an α-proteobacterium and a primitive eukaryotic cell. Mitochondria thus display pro-inflammatory and antigenic properties, when released into the extracellular milieu.

Several cross-sectional studies reported increased levels of anti-mitochondrial antibodies (AMAs) in patients with systemic lupus erythematosus (SLE) and the antiphospholipid syndrome (APS). These autoantibodies also displayed correlations with the SLE disease activity index 2000 (SLEDAI-2K) and associations with various clinical manifestations (e.g. lupus nephritis, thromboses, carotid plaque). In the present study, we aim to detect AMAs against either whole organelles (AwMA), mitochondrial DNA (mtDNA) or RNA (mtRNA) through time in samples from patients included in the SLICC cohort.

Methods Clinically relevant variables (e.g., sociodemographic variables, disease-specific outcomes including death and arterial vascular events (AVE)) were documented and biosamples were harvested upon patient enrolment in the SLICC cohort, as well as at each follow-up visit. AMA levels were measured by in-house direct ELISAs whereas SLE autoantibodies were detected by clinical laboratories. Healthy individuals, defined as having no known illnesses and infectious symptoms at the time of the blood draw, were recruited. 90% confidence intervals were calculated for both limits of the 95% nonparametric two-sided reference intervals for values measured in healthy donors. AMA values were segregated into 3 categories: Normal values were determined as within the inner limits of the range while values outside this range were characterized as abnormal, either lower or higher than the reference interval. (figure 1). Marginal Cox models with AMAs in 3 categories were adjusted for covariables and are presented as [hazard ratio (95% CI)]. Interactions with sex were tested in models with the AMAs as continuous variables.

Results Sera from healthy individuals (n=126) or SLE patients included in the SLICC cohort, from their inclusion, up to 7 years of follow-up (n=1114 patients at baseline, 3577 samples in total). AwMA displayed lower correlations with antibodies to mitochondrial nucleic acids (versus AmtDNA: r_s=0.37, and vs AmtRNA: r_s=0.38), while antibodies to mitochondrial DNA or RNA shown higher correlations (r_s=0.59). During our preliminary analyses on the distribution of the variables, We made intriguing observations regarding patients with AMA levels that were either lower or higher than those of healthy individuals. This information led us to categorize SLE patients as described in the methods and in figure 1. For each of the three antibodies assessed, SLE patients displayed more abnormal AMA levels at baseline than controls. The percentage of patients with higher levels of AwMA and AmtRNA increased at subsequent follow-up visits, while a slight decrease was observed for AmtDNA (figure 2). SLE patients with higher levels of AwMA showed higher risks of death [2.12 (1.18-3.83)]. It is of interest that an inverse relationship was found between AmtRNA and AVEs, with a small subset of patients with low levels of AmtRNA (n = 4), this autoantibody was associated with increased risks of this manifestation [4.46 (1.71-11.66)]. Additionally, patients with higher levels of AmtDNA and AmtRNA displayed increased risks of lupus nephritis [respectively: 3.05(2.05-4.54), and 1.56(1.12-2.18)]. Interestingly, there was an interaction with sex for AmtRNA levels effect on AVEs [males: 0.32 (0.11-0.99). Females: 1.56 (1.11-2.19)], and AmtDNA association with nephritis was only significant in female patients [4.00 (2.51-6.36)] (table 1).

Conclusion These results show that AMAs display different associations with disease manifestations in various clusters of patients. These results prompt for further analyses by machine-learning in order to delineate clusters of clinical interests by adding AMAs in the routine serological assessment of SLE autoantibodies.

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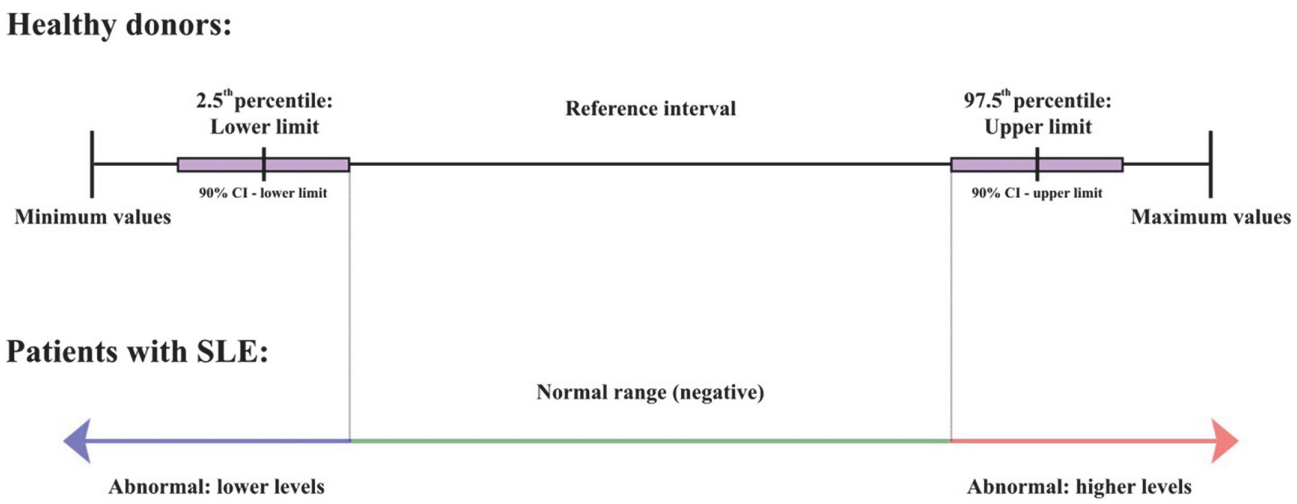
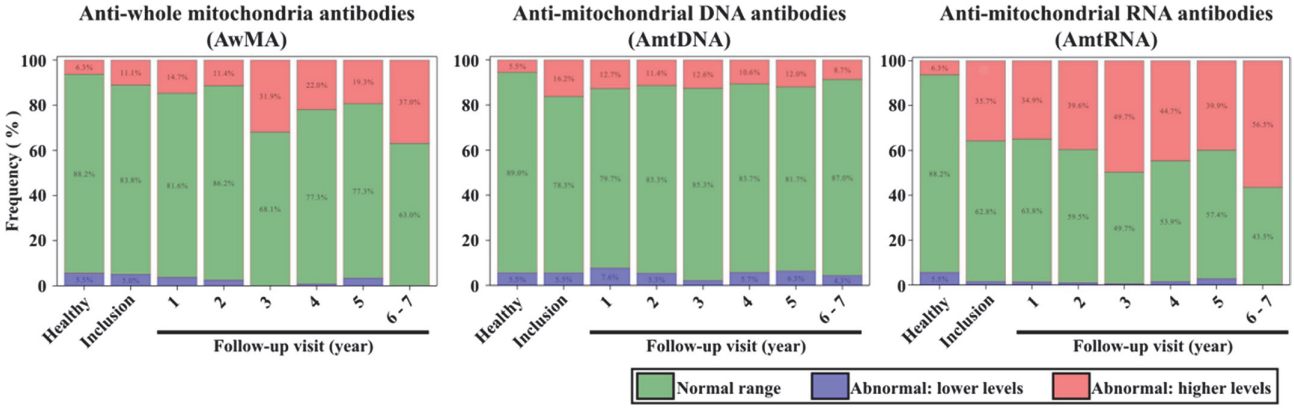
LAY ABSTRACT The mitochondrion is a part of the cell that controls various biological mechanisms (e.g., energy supply, whether the cell should live or die, control, or produce various cellular components). They are derived, through evolution, from a microbe. Mitochondria may sometimes be jettisoned out of their host cell and subsequently elicit immune reactions – including the production of antibodies. Previous studies



Abstract 103 Figure 1 Segregation of the patients in categories. For each of the AMA assessed, values were divided into 3 categories. 95% nonparametric two-sided reference intervals were calculated, using values measured in healthy donors, to define a reference interval. In patients with SLE, values were considered as normal if they were included within the inner limits of the reference interval. Values outside this interval were characterized as abnormal (i.e. higher or lower levels).



Abstract 103 Figure 2 Evolution of the positivity to the three anti-mitochondrial antibodies assessed. Autoantibodies (i.e., IgG) to whole organelles (AwMA), mtDNA (AmtDNA) or mtRNA (AmtRNA) were assessed in sera from healthy individuals (n=126) or patients included in the SLICC cohort (n=1114 patients at baseline, 3577 samples in total). Through time, the frequency of patients with higher levels of AwMA appears to increase, while a decrease of the positivity to AmtDNA was detected.

Abstract 103 Table 1 Associations between AMAs and clinical manifestations of the disease.

	AwMA	AmtDNA	AmtRNA
AMA as categorical variables			
Deaths	levels: 1.74 (0.66	Lower levels: 1.12 (0.51 - 2.46)	Lower levels: 1.42 (0.21-9.67)
	levels: 2.12 (1.18	Higher levels: 1.28 (0.77 - 2.14)	Higher levels: 1.39 (0.89-2.18)
Arteriovascular events (AVEs):	levels: 0.90 (0.41	Lower levels: 0.45 (0.17-1.20)	**Lower levels: 4.46 (1.71 - 11.66)**
	levels: 0.57 (0.20	Higher levels: 1.27 (0.62 - 2.58)	Higher levels: 1.24 (0.85 - 1.80)
Lupus nephritis	levels: 1.08 (0.55	Lower levels: 0.33 (0.041 - 2.59)	Lower levels: 2.65 (0.95 - 7.34)
	levels: 0.90 (0.49	**Higher levels: 3.05 (2.05 - 4.54)**	**Higher levels: 1.56 (1.12 - 2.18)**
AMA as continuous variables			
Arteriovascular events (AVEs):	0.65 (0.29 - 1.44)	0.96 (0.43 - 2.15)	**Females: 1.56 (1.11 - 2.19)**
			Males: 0.32 (0.11 - 0.99)
Lupus nephritis	0.83 (0.17 - 4.06)	**Females: 4.00 (2.51 - 6.36)**	**1.68 (1.34 - 2.11)**
		Males: 1.38 (0.51 - 3.76)	

Marginal Cox models, adjusted for covariables. AMA were either segregated in three categories (i.e., normal range, lower or higher levels, as presented in figure 1), or considered as continuous variables. For this approach, male and female patients are shown separately when an interaction with sex was observed. Data are hazard ratio (95% CI) with significant results in bold.

indicated that patients with autoimmune conditions such as systemic lupus erythematosus (SLE) and the antiphospholipid syndrome (APS) have antibodies against mitochondria in their blood stream. Presence of these antibodies was associated with increased disease activity and clinical manifestations of these diseases (e.g. kidney disease, arterial vessel disease). In this study, we studied blood samples harvested by an international group dedicated to the study of SLE [i.e., the SLE International Collaborating Clinics (SLICC) cohort] and observed that patients may be clustered into groups, upon their levels of antibodies and/or sex, allowing to have a better appreciation of their risks of death, vascular events, and kidney disease. These results might lead to improved diagnosis and/or prognosis in SLE and thus, in improved care and quality of life for the people living with lupus.

104 SLE AUTOANTIBODIES TO CASEIN KINASE II: POTENTIAL MEDIATORS OF IMMUNOPATHOLOGY

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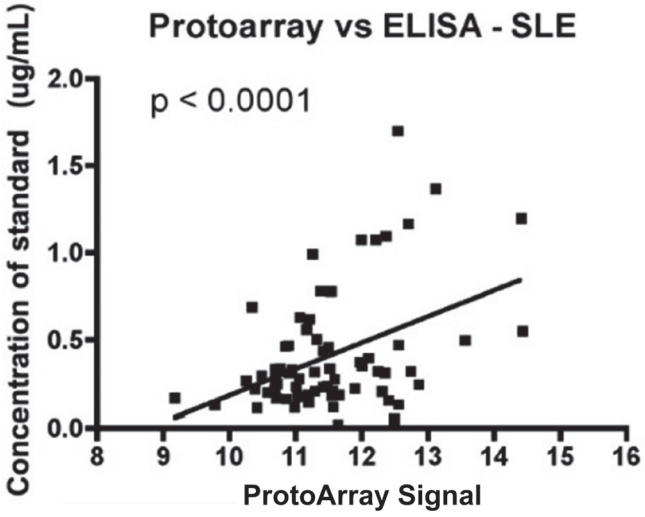
Background Casein kinase II (CSNK2) is a key regulator of cell activation, proliferation, and apoptosis. While primarily an intracellular molecule, it has recently been appreciated that CSNK2 is expressed on cell surfaces, where it functions as an ectoenzyme with broad regulatory activity. Its presence of cell surfaces raises the possibility that autoantibodies to CSNK2 might interfere with its function and mediate immunopathology. A role for CSNK2 autoantibodies was suggested 30 years ago in animal models. More recently, we have used protein array technology (Protoarray, Invitrogen Technologies) to investigate the full spectrum of SLE autoantibodies. A study of patient sera from three different cohorts (Philadelphia, Romania, and People's Republic of China) showed that autoantibodies to the alpha (catalytic) subunit of CSNK2 were among the top seventeen self specificities found in common among these diverse lupus sera. In the present study, we developed a formal ELISA to investigate autoantibodies to CSNK2 in two well studied collections of SLE sera.

Methods We developed an ELISA binding assay for CSNK2 autoantibodies. We inhibited the ELISA to show antigen specificity, and we performed Western blots to show that the lupus sera recognized recombinant antigen. We used the clinical information about both cohorts to look for clinical correlations with the presence of this autoantibody.

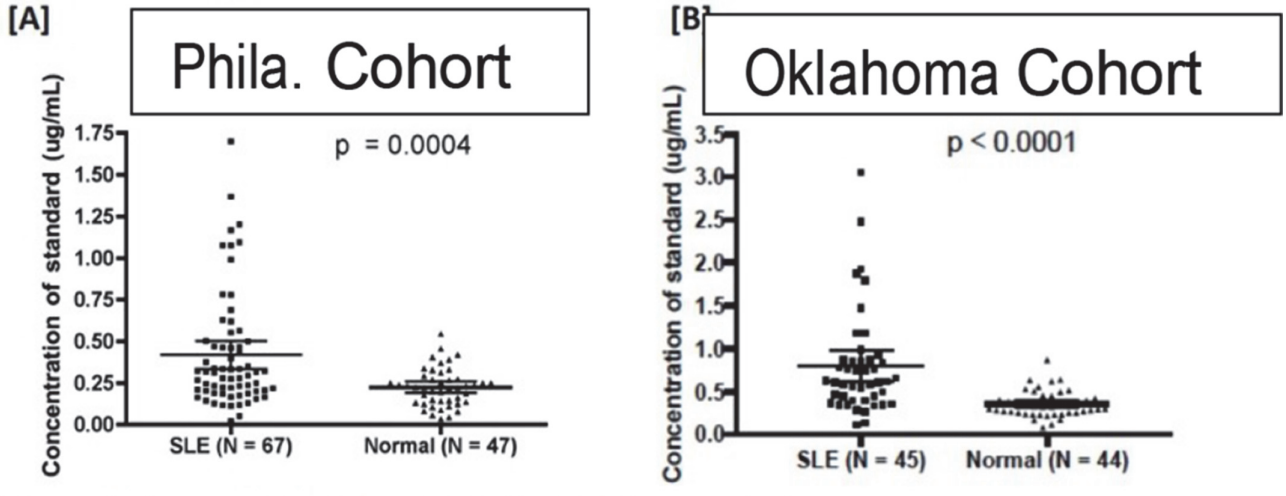
Results Using recombinant human alpha subunit of human CSNK2 (Lifespan Technologies), we developed an ELISA to test binding of lupus and control sera to CSNK2. We examined 114 SLE sera and age and sex matched controls from Philadelphia, and 99 paired lupus sera from Oklahoma City.

We showed by Western blot that lupus sera bound to the same molecular weight recombinant CSNK2 as rabbit antisera to this protein.

The presence of autoantibodies to CSNK2 correlated with overall SLEDAI and with new onset arthritis in the two groups. Other correlations were inconsistent.



Abstract 104 Figure 2 Protoarray correlates with ELISA



Abstract 104 Figure 1 Indirect ELISA for anti-CSNK2α2 in two cohorts. Panel A shows ELISA signal strength for α-CSNK2α2 in temple Lupus cohort and matched controls (N=114). Panel B shows ELISA signal strength for α-CSNK2α2 in Oklahoma Lupus cohort and matched controls (N=99).