Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 46. Risk Profiling in Lupus

7. **Start and End Date of Research Project:** 7/20/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Nancy J. Olsen, MD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 107,100

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Olsen, Nancy	Principal Investigator	5% Yr1; 3% Ext	$23,982.71
Berg, Arthur	Biostatistician	3.1% Yr1	$6,587.22
Liao, Duanping	Biostatistician	4.7% Yr1	$18,362.52
McAloose, Carl	Research Technician	26.5%Yr1; 12% Ext	$18,692.32
He, Fan	Research Asst	12.5% Yr1	$10,539.56
Colon-Rodriguez, Sol	Statistician	8% Yr1	$6,981.44
Carter, Jamie	Nurse/ LPN	25.4% Yr1	$21,953.89

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the

application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Diagnosis and Treatment of Preclinical Systemic Lupus Erythematosus	☒NIH ☐ Other federal (specify:____) ☐ Nonfederal source (specify:)	April, 2013	$3,813,512	$ not funded

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We will submit to NIH on March 1, 2014 a U34 Planning Grant, entitled "Hydroxychloroquine treatment for prevention of systemic lupus erythematosus"

12. Future of Research Project. What are the future plans for this research project?

The U34 proposal will provide support for planning and submitting a multicenter clinical trial proposal under the UM1 mechanism, which is a clinical trial implementation cooperative agreement. The trial will be a randomized, placebo-controlled, multicenter project to give hydroxychloroquine to individuals at high risk for development of systemic lupus erythematosus.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No___X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes__X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    We were able to create a database in Access that permits us to analyze multiple variables from our patients. We are able to add new patients to the database.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

We collaborated with University of Texas Southwestern Medical center, with whom we are writing the U34 application. The co-principal investigators on this proposal are from Hershey Medical Center and UT Southwestern.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project

work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The objectives of this research are to develop insights into profiling immune features in individuals who are at high risk for development of systemic lupus erythematosus (SLE). The long term goals are to develop a Lupus Risk Calculator and then to design an early intervention trial to ameliorate or abort this disease. The risk calculator will be modeled on similar quantitative scales that are useful for personalizing risks of cardiovascular events and that are being currently applied to the early identification and preclinical treatment of patients with Type I diabetes mellitus. Elements of the calculator tool will include demographic features and immunologic measures including autoantibodies, cytokines, lymphocyte subsets and levels of genes expressed in peripheral blood cells. The high risk population we propose to study is composed of individuals who have sought medical care for evaluation of antinuclear antibody (ANA) positivity and have some additional elements suggestive of lupus. These individuals are classified as having incomplete lupus (ILE). Since ANA is generally considered a requirement to establish a diagnosis of SLE, this population is already enriched for individuals who are at risk for developing this disease. The problem of ANA positivity is relatively common, and 12-15% of our requests for new patient consultations in rheumatology are for evaluation of the significance of ANA positivity, which assures sufficient subjects for study. Developing tools to better assess the significance of a positive ANA in terms of disease risk will permit early disease detection and effective triage of patients from primary care to specialty clinics. An advantage of identifying high risk patients in the early stages of lupus is that interventions to ameliorate, abort or prevent disease could be tested. A significant body of available data suggests that hydroxychloroquine is an excellent candidate therapeutic for an early intervention trial. The one year period of support requested will enable collection of sufficient data to establish feasibility of this approach and support an application for extramural funding to proceed with the long term objectives.

For this project, we propose the following two specific research aims:
1. To determine the prevalence of high risk markers for SLE in 50 subjects with ILE who will be studied at two different time points, separated by at least 4 months.
2. To determine the immunologic and gene expression correlates of hydroxychloroquine treatment in ILE patients.

The results of these studies will provide insights into the variables that will be useful to develop as biomarkers of lupus risk in early patients and will define a suitable population and approach for a subsequent intervention trial.

SLE is a multiorgan and chronic illness that afflicts primarily persons younger than 40 years of age. Consequences of SLE include life-threatening organ damage and lupus nephritis is a major cause of end stage renal disease. Other significant manifestations include seizures and cerebrovascular accidents, hematologic abnormalities including hemolytic anemia, thrombocytopenia and lymphopenia, polyserositis, blood clotting disorders, avascular necrosis and accelerated cardiovascular disease. Challenges to treatment include the relapsing and remitting course with unpredictable flares of disease activity as well as the heterogeneous nature and diversity of manifestations. Disease flares carry the risk of organ damage, which then accumulates over time. So even though treatments and survival rates have improved over the past two decades, many challenges remain.

One fundamental concept for improving outcomes of a chronic disease such as SLE is to diagnose and treat early in the course before irreversible damage has occurred, and this is an overarching goal of the planned trial. This principle has now permeated the approach to rheumatoid arthritis (RA), in which disease-modifying therapy is routinely started prior to the development of joint damage. The early diagnosis of RA has been facilitated in part by the development of serologic tests with high specificity (anti-citrullinated protein antibodies or ACPAs) and also by the well-documented benefits of relatively safe treatments such as with methotrexate. These approaches have led to improved outcomes for RA patients.

Achieving the long term objective to target early lupus disease stages will require development of identifiers, which may include biomarkers, that have prognostic importance. That is, it must be possible to identify a patient who is at high risk of developing SLE with sufficient reliability to justify intervention with a therapeutic agent. Based on previous studies, our own clinical experience and a review of the literature, the candidate agent for this intervention is hydroxychloroquine (HCQ). So the initial steps are to find markers of risk and to then assess the potential for HCQ to ameliorate that risk. During this study period we also investigated some of the immunologic effects of HCQ. Consideration has been given to various ways to validate this approach. One is to examine individuals who have some clinical features of lupus but who do not fully satisfy the criteria for this disease. If these individuals have significant positivity for antinuclear antibodies (ANAs), an element that is essentially required for SLE diagnosis, then this step serves as a type of filter to focus on individuals at higher lupus risk (Figure 1).

Figure 1 Significant ANA positivity filters the population to a higher risk subgroup which contains patients with early SLE. However, positive identification of the relatively few cases of incipient SLE within that group (circle) is inexact using currently available tools.

Several straightforward steps can be taken to increase the risk profile of the ANA-positive pool. The first is that, if ANA testing were prompted by a clinical finding or symptom, then the pre-test probability of an SLE diagnosis is significantly higher than if the result were obtained as part of a population survey. This idea follows from studies in rheumatoid arthritis showing that when rheumatoid factor and antibodies to citrullinated proteins (ACPAs), are ordered by a health care provider rather than surveyed in the general population, the pre-test probability of RA is increased by 17-fold. Futhermore, a recent study by van de Stadt and colleagues (December, 2013) clearly shows that a starting point of symptomatic arthralgias is very useful for predicting subsequent development of RA. These results indicate that when symptoms are present and medical care is sought the likelihood that an underlying disease is present is increased. For these reasons, our studies focus on ANA+ individuals who are referred to our clinics, rather than other options such the use of first-degree relatives (FDRs) of patients, an approach that has been utilized to detect preclinical cases of Type I diabetes mellitus (T1DM) and which is being pursued in some studies of SLE (www.sissle.org). There are distinct advantages to use of the patient-based approach for our proposed trial. One is that the individual subjects are already identified as patients rather than as unaffected healthy persons. This will facilitate design of an intervention with the proposed treatment, hydroxychloroquine (HCQ), in those who show risk features. Treatment of unaffected FDRs, even with a very safe drug like HCQ, would be more likely to pose ethical issues, since they are not technically patients seeking care.

Another advantage of the patient-based population is that the timeline to accumulation of disease features that clearly point in the direction of SLE is likely to be shorter in the patients than in unaffected first degree relatives; this concept is supported by our previous autoantibody analyses. A third is that these patients are readily available in our clinics; a significant number of new patient referrals for ANA evaluations (12-15% of those at Hershey Medical Center) provide a large recruitment pool.

Within the ANA+ referred patient population, other basic demographic and laboratory

features are likely to further define higher lupus risk. One is female gender, since 85% of SLE patients are female. Another is age, since increasing age, especially after menopause in females, is associated with decreased incidence of lupus. Racial disparities also exist. The presence of suggestive clinical features is another category that likely adds to SLE risk. This parallels what has been described in longitudinal studies of early synovitis in which joint complaints and symptoms such as morning stiffness and responses on visual analog measures can identify a group at risk for development of RA. For lupus, this is where the ILE model is especially useful. As first described by Greer and Panush in 1989, these patients exhibit some features of lupus but do not satisfy sufficient criteria for classification as SLE. An example of this would be an ANA positive person with a photosensitive malar rash and chest pain suggestive of pleurisy. A significant subset of ILE patients are likely to will progress to SLE.

Results

Patients: The 50 individuals recruited during this grant period were added to an existing set of previous samples, permitting analysis of over 100 individuals with either SLE or ILE. Individuals completed questionnaires about habits, such as use of tobacco products, pain profiles and assessments of abilities to do activities of daily living. Biological samples were collected including Paxgene tubes for later isolation of RNA, serum, whole blood for later isolation of DNA and in some cases peripheral blood mononuclear cells were separated and frozen in liquid nitrogen.

Autoantibody measurements: The serum samples were used to measure autoantibodies. All patients had measurement of ANA using an ELISA kit (Inova Diagnostics) and some also had measurement of autoantibodies to thyroglobulin and the complement component C1q; both of these were done with ELISA kits (Abcam).

Previous studies have suggested that antibodies to C1q are relatively specific for SLE, especially patients with nephritis. On the other hand antibodies to thyroglobulin are commonly associated with autoimmune thyroid disorders, which might be an underappreciated contribution to ANA positivity in patients referred to rheumatologists.

Results of these analyses (Figure 2) suggest that both ILE and SLE patients have similar positivity for anti-TG. On the other hand, elevated levels of antibodies to C1q are more commonly seen in patients with SLE ($P=0.045$). The three SLE patients in this sample with the highest anti-C1q levels all had a history of nephritis, though none had active renal disease at the time of sampling.

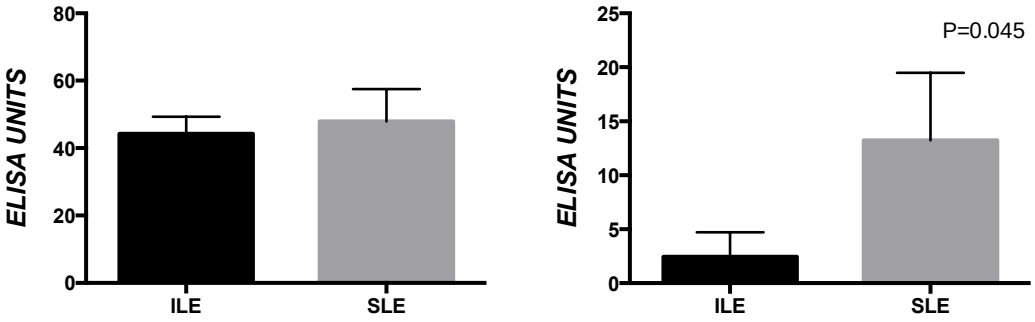


Figure 2 Measurement of antibodies to thyroglobulin (TG; left) and C1q (right) in patients with ILE (N=15) and SLE (N=16). Results are shown as mean values and SEM. P value calculated using a 2-tailed t-test.

Looked at another way, there is a generally inverse correlation between elevated antibodies to TG and C1q (Figure 3). No individual had elevated levels of both of these autoantibodies in a serum sample. These profiles suggest that if a patient with ILE was found to have elevated levels of anti-C1q, this would be a marker of elevated lupus risk. The anti-C1q positivity is not highly prevalent, however, so while this might be a specific marker it is not sufficiently sensitive to be used in a stand-alone screen. As a component of a risk profile though, this would be a cost-effective addition.

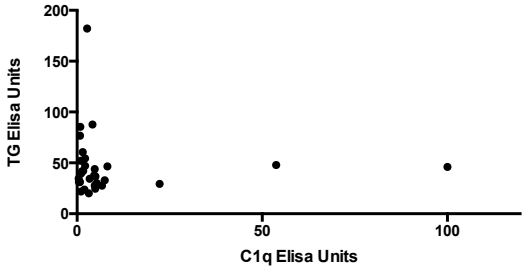


Figure 3 Comparison of antibodies to thyroglobulin (TG) and C1q in ILE and SLE patients. No individual patient had high levels of both autoantibodies.

Gene expression profiles: The use of PAXgene tubes permits collection of blood samples that can later be used for isolation of RNA and study of levels of expressed genes. We have in previous studies documented that these samples are stable when frozen at -20 degrees centigrade for at least several years. The samples collected in the present study were used to measure several genes of interest in the Type I interferon gene signature that has been associated with SLE. The objective of these measurements is to determine whether a set of markers that are measureable in blood would be useful components of a risk profile. For these measures, three genes were chosen: MX1, OAS1 and IFI27. Our previous studies had suggested that some ILE patients did express elevated levels of these genes and therefore this could provide insights into determining whether those were patients at risk for progression to SLE.

A comparison of these results in HC, ILE and SLE (Figure 4) shows general elevation of values in the patient groups. However, the specificities have some differences with OAS1 showing significant elevation in many ILE patients while IFI27 elevation is most marked in

SLE patients. Using the HC group, an upper limit of normal expression was defined as the HC mean value + 2 standard deviations above that mean. With this definition, thirteen ILE patients were found in whom all 3 specificities were elevated. This group if IFN-high ILE was compared to 13 ILE patients in whom none of these specificities were elevated over the normal range.

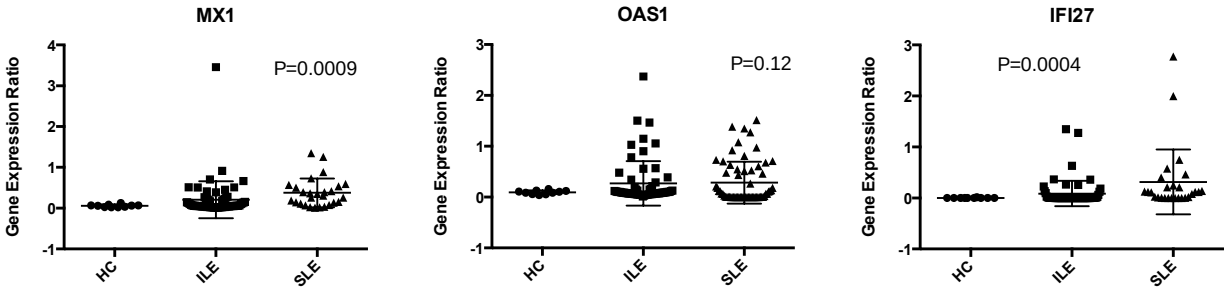


Figure 4 Gene expression levels for three specificities in the type I IFN signature measured in peripheral blood samples from healthy controls (HC), incomplete lupus patient (ILE) and systemic lupus patients (SLE). P values by one-way ANOVA.

Table 1: Comparison of ILE Subgroups defined as IFN High or IFN Low			
	IFN High	IFN low	P value
Criteria (number)	1.84	2.15	0.3
SLEDAI (score)	1.08	2.30	0.70
Age (years)	47	49	.64
Other positive labs*	10/13	1/13	0.0010
APL antibodies	0/13	2/13	---
CCP antibodies	0/13	1/13	---

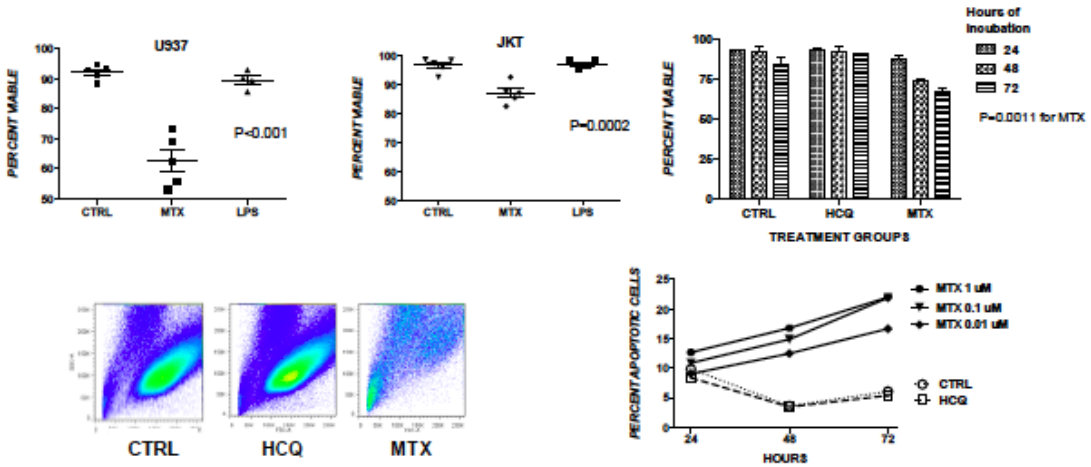
*antibodies to DNA, histone, SSA, SSB, Scl70, RNP, CENPB or low Complement

The comparison of IFN high and IFN low group showed no significant differences in terms of the number of SLE criteria satisfied, the SLE disease activity index (SLEDAI) or age. However, it was noted that a very high proportion of the IFN High group had other autoantibodies or low levels of serum complement (10/13) compared to the low IFN group (1/13) and this was a highly significant difference (P=0.0010, Fisher's Exact Test). Other autoantibodies, specifically to phospholipids or citrullinated proteins (CCP) were only present in the IFN low group. This result suggests that endogenous activities related to Type I interferon are associated with a broadened array of autoantibodies or immune activation (low complement levels). Since it has been shown that the number of autoantibodies increases in the years prior to diagnosis (Arbuckle et al, 2003), these high IFN ILE patients are potentially ones in which the progression to disease is a higher risk.

Longitudinal analyses: The original plan to carry out longitudinal analyses is still in progress and results of these studies are incomplete at this time. At least 10 patients have baseline samples prior to starting HCQ, some return visits have been completed and others are still being followed. The compliance with HCQ is very high, and this will be quantitated as part

of the planning grant application to justify feasibility of the treatment. One patient had interruption of dosing due to some confusing symptoms that brought her to the emergency department, but HCQ was subsequently restarted. One ILE patient in the IFN low group has had disease progression to the point where she is now unable to hold her job due to cognitive and other difficulties. Measurement of IFN levels after she started HCQ showed no significant changes.

<u>In vitro HCQ analyses:</u> To develop insights into what other gene or cytokine pathways might be targeted by HCQ in vivo, studies were carried out in vitro using the human monocyte/macrophage cell line U937. This target cell was chosen because many of the effects of HCQ are intracellular in lysosomal compartment and other studies have suggested that the actions of HCQ are largely mediated by monocytes in humans. Furthermore, in other studies, we have shown that methotrexate has effects on apoptosis and gene expression pathways. Unlike MTX, HCQ did not reduce cell viability or induce markers of apoptosis in U937 cells (Figure 5).



<u>Figure 5</u> Viability of U937 cells was reduced by MTX (top left) but not by HCQ (top right) and cell features measured by flow cytometry were not changed with HCQ (bottom left). Apoptotic effects measured by flow cytometry were mediated by MTX but not HCQ (bottom right).

Examination of gene expression effects showed that while MTX induced JUN and FOS in U937 cells, HCQ had no effect (Figure 6). But two other genes in the JUN pathway, JNK1 and JNK2 appeared to be modestly increased by HCQ (bottom panels; Figure 6). These results suggest possible biomarkers of HCQ effect that could be sought in treated patients.

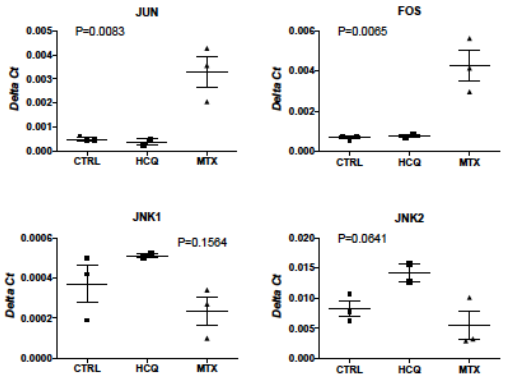


Figure 6: Gene expression effects mediated by HCQ or MTX in U937 cells. While MTX induced JUN and FOS expression (top panels), HCQ had no effect on these levels, but may induce expression of the map kinases JNK1 and JNK 2 (bottom panels).

Summary and future directions:

Patients with ILE may provide insights into early stages of SLE. Other studies suggest that treatment of ILE patients with HCQ may delay or prevent SLE onset. The studies supported by the CURE funds have permitted us to expand significantly our ILE database and provided us with important data to suggest what markers will be potentially useful in the transition to lupus. However, the recent review of our NIH proposal suggested that clinical rather than immunologic endpoints will be of greatest interest. A model for this might be recent studies showing that accumulation of symptoms in patients with nonspecific joint pains can lead to a prediction algorithm for onset of rheumatoid arthritis. While our recently reviewed application to study this further was not funded, we received encouragement from the NIH staff to resubmit this as a planning grant proposal for a placebo-controlled HCQ trial in patients with ILE. Preparation of this proposal is in progress with collaboration of three other rheumatology sites in major US academic medical centers.

The primary objective of the trial will be to determine the efficacy of HCQ in slowing accumulation of lupus features in ILE patients over 24 months. The clinical (N=11) and immunologic (N=6) criteria of the new (2013) SLICC classification system will be used to quantitate this response. The two groups, HCQ and Placebo, will be compared using a 2-tailed t-test. The enrolled group of 200 patients, 100 in each treatment group, is assumed to have a dropout rate of 10%, leaving 90 in each treatment group. To estimate power of HCQ to affect a significant difference in clinical features, data were examined in three clinical studies in which patients with either cutaneous lupus or ANA+ immune thrombocytopenia (which might be considered a form of ILE) were treated with HCQ. The CLASI score for skin activity was observed in one of these studies to be decreased from 10.1 to 4.5 after 16 weeks on HCQ. Assuming a standard deviation of 5, groups of 90 patients each have a greater than 98% power to detect a significant difference. Two other studies suggest that the HCQ response rate is 55-60%. If the placebo response is assumed to be 20%, then the power to detect a significant treatment effect with group sizes of 90 is again greater than 98%. Another requirement, though, is how much progression in ILE is expected during 24 months. If no significant accumulation of criteria takes place, then effects of HCQ on this outcome will not be valid. Several studies, including our own, suggest that progression of ILE to SLE occurs in 15-17% of patients over 3-5 years. In

our own small study, if the initial group were restricted to individuals less than age 45 years, then progression to SLE happened in 43%. The proposed design does not require satisfying SLE criteria, just accumulation of additional criteria, which therefore might conservatively be estimated to be at least 50%. The time period is shorter (24 months) but this is counterbalanced in part by the use of a more sensitive set of criteria. Based on these features, we make the following assumptions: 45 of the 90 placebo patients will progress and (0.6x45)=27 of the active patients will progress. These values predict a greater than 98% power to detect a significant difference.

Secondary clinical objectives, including two indices of disease activity, BILAG and SLEDAI will be similarly analyzed. The planned study will incorporate a significant mechanistic component, with analysis of candidate biomarkers for both ILE progression and HCQ action. The outcome of the planning grant will be a UM1 proposal designed to carry out the multicenter clinical trial.

Presentations/abstracts:

Dr. Olsen is scheduled to present findings related to this ILE project at two international meetings in 2014:

1. "Pre-Lupus and Early Lupus Cohorts: What have we learned?", 9th European Lupus Meeting, 24 April 2014, Athens, Greece.
2. "Lupus: Is prevention a goal?", Lupus 2014, 19 September 2014, Quebec City, Quebec, Canada. The abstract for this meeting will be published in Arthritis Care & Research.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and

19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Organ damage in high-risk patients with systemic and incomplete lupus syndromes	Nancy J. Olsen Maha Yousif Azza Mutwally Melinda Cory Nada Elmagboul David R. Karp	Rheumatology International 33:2585-2590	November 2012	☐Submitted ☐Accepted ☒Published
2.Methotrexate induces production of IL-1 and Il-6 in the monocytic cell line U937	Nancy J Olsen Charles F Spurlock Thomas M Aune	Arthritis Care and Research	September 2013	☐Submitted ☐Accepted ☒Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes__X_____ No_____

If yes, please describe your plans:

We are continuing to analyze data on our 110 enrolled patients, which includes the 50 individuals enrolled with CURE funds and expect to be able to generate additional publishable information for submission.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No_____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH
Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Nancy J. Olsen	POSITION TITLE Professor of Medicine, Chief of Rheumatology, Hallowell Chair in Rheumatology
eRA COMMONS USER NAME (credential, e.g., agency login)	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Brown University, Providence RI	Sc.B.	06/73	Biology
University of Chicago, Chicago IL	M.S.	06/77	Immunology
University of Chicago, Chicago IL	M.D.	06/77	Medicine

A. Personal Statement
The investigator has been working for three decades in the field of rheumatologic and autoimmune disease. This experience includes studies ranging from basic laboratory investigation in animal models to translational and clinical studies in patients with autoimmune disorders.

B. Positions and Honors
Positions and Employment
1977-1980 Intern and Resident, Internal Medicine, Medical College of Virginia
1980-1983 Fellow, Rheumatology, University of Texas Southwestern Medical School, Dallas
1983-1984 Instructor, Rheumatology, University of Texas Southwestern Medical School, Dallas
1985-1992 Assistant Professor, Medicine, Vanderbilt University, Nashville TN
1985-2004 Staff, Rheumatology, Nashville Veteran's Administration Medical Center
1986-2004 Courtesy Staff, Nashville Metro General Hospital
1996-2004 Adjunct Faculty, Meharry Medical College, Nashville TN
1992-2000 Associate Professor, Medicine, Vanderbilt University, Nashville TN
1999-2004 Associate Professor, Microbiology and Immunology, Vanderbilt University, Nashville TN
2000-2004 Professor, Medicine, Vanderbilt University, Nashville TN
2004-2010 Professor and McGee Foundation Chair in Arthritis Research, UT Southwestern, Dallas TX
2007-2010 Director, Rheumatology Training Program, UT Southwestern, Dallas TX
2010- Adjunct Professor, University of Texas Southwestern Medical Center, Dallas TX
2010- Professor, Chief of Rheumatology, Penn State Hershey Medical Center, Hershey PA
2012- Director, Rheumatology Training Program, Penn State MS Hershey Medical Center

Other Experience and Professional Memberships
1981- Fellow, American College of Rheumatology
1986- Member, American Association of Immunologists
1995 Elected member, Southern Society for Clinical Investigation
2000-2006 Member, Examining Board Rheumatology, ABIM
2007- Associate Editor, Arthritis Research & Therapy
2002-7 Member, Chair (2005-7), Allergy,Immunology, Transplantation Research Committee, NIH-NIAID
2004-2005 NIH/NIAMS DSMB, GIFT Trial (Gabapentin in Fibromyalgia)
2008, 2009 NIH/NIAID Review Panels: Statistical Data Center; Loan Repayment Program, R03 awards
2008-10,12-13 Arthritis Foundation, national grant review panel
2008-2011 Arthritis Advisory Committee, FDA

2011- ACTS study section, CSR, NIH
2012 NIH/NIAMS P60 grant review panel, November 2012
2013 NIH/NIAMS RFA-AI-12-260 Autoimmunity Centers of Excellence, Panel Chair, Nov 2013

Honors and Awards

1977 American Medical Women's Association Citation
1977 Mary Roberts Scott Memorial Prize, University of Chicago
1982-1985 Arthritis Foundation Postdoctoral Fellow
1984 Senior Rheumatology Scholar Award
2001 Mary Etta Larsen Hope Award, Lupus Foundation of America, Nashville Chapter
2003-2006 Clinical Scholar Educator Award, American College of Rheumatology
2008 Metro Ogryzlo Lecturer, University of Manitoba
2012 Invited lecturer, Dept. Pathology and Microbiology, University of Nebraska Medical Center
2013 Invited lecturer, Rheumatology Grand Rounds, Univ. Pittsburgh Medical Center
2014 Invited speaker: The European Lupus Meeting, Athens Greece, April 23-26

C. Selected Peer-reviewed Publications (Selected from a total of 123 peer-reviewed publications)

1. Li QZ, Zhou J, Lian Y, Zhang B, Branch VK, Carr-Johnson F, Karp DR, Mohan C, Wakeland EK, **Olsen NJ**: Interferon signature gene expression is correlated with autoantibody profiles in patients with incomplete lupus syndromes. Clin Exp Immunol 159:281-291, 2010. PMC2819494
2. Li QZ Karp DR, Quan J, Branch VK, Zhou J, Lian Y, Chong BF, Wakeland EK, **Olsen NJ**: Risk factors for ANA positivity in healthy persons. Arthritis Res Ther 13(2):R38, 2011. PMC3132017
3. **Olsen NJ**, Li QZ, Quan J, Wang L, Mutwally A, Karp DR: Autoantibody profiling to follow evolution of lupus syndromes. Arthritis Res Ther 14(4):R174, 2012 PMC3580568
4. **Olsen NJ**, Karp DR: Autoantibodies and SLE – the threshold for disease. Nature Rev Rheumatol December 2013 doi: 10.1038/nrrheum.c013.184.
5. Quan J, Lakhanpal A, Reddy MM, Zaman S, Li QZ, German DC, **Olsen NJ**, Kodadek T, Karp DR: Discovery of biomarkers for systemic lupus erythematosus using a library of synthetic autoantigen surrogates. J Immunol Methods 2013 Nov 20 doi: 10.1016/j.jim.2013.11.004.
6. Wandstrat AE, Carr-Johnson F, Branch V, Gray H, Fairhurst AM, Reimold A, Karp D, Wakeland EK, **Olsen NJ**: Autoantibody profiling to identify individuals at risk for systemic lupus erythematosus. J Autoimmun 27:153-60, 2006.
7. Li QZ, Zhour J, Wandstrat AE, Carr-Johnson F, Branch V, Karp DR, Mohan C, Wakeland EK, **Olsen NJ:** Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. Clin Exp Immunol 147:60-70, 2007.
8. Bellatin MF, Han M, Fallena M, Fan L, Donglan X, **Olsen N**, Branch V, Karp D, Stastny P: Production of autoantibodies against citrullinated antigens/peptides by human B cells. J Immunol 188: 3542- 3550, 2012
9. Dooley MA, Jayne D, Ginzler EM, Isenberg D, **Olsen NJ**, Wofsy D, Eitner F, Appel GB, Contreras G, Lisk L, Solomons N; ALMS Group: Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. N Engl J Med 365:1886-95, 2011.
10. Chong BF, Tseng LC, Lee T, Vasquez R, Li QZ, Zhang S, Karp DR, **Olsen NJ**, Mohan C: IgG and IgM autoantibody differences in discoid and systemic lupus patients. J Invest Dermatol doi:10.1038/jid.2012.207, 2012. PMCID:PMC3465644
11. Chong BF, Song J, **Olsen NJ**: Determining risk factors for developing systemic lupus erythematosus in patients with discoid lupus erythematosus. Br J Dermatol 166:29-35, 2012. .
12. **Olsen NJ**, Schleich MA, Karp DR: Multifaceted effects of hydroxychloroquine in human disease. Seminars Arthritis Rheum doi: 10.1016/j.semarthrit.2013.01.001 [Epub ahead of print].
13. **Olsen NJ,** Yousif M, Mutwally A, Cory M, Elmagboul N, Karp DR: Organ damage in high-risk patients with systemic and incomplete lupus syndromes. Rheumatol Int. 33:585-2590, 2013.
14. Becker AM, Dao KH, Han BK, Kornu R, Lakhanpal S, Mobley AB, Li QZ, Lian Y, Wu T, Reimold AM, **Olsen NJ**, Karp DR, Chowdhury FZ, Farrar JD, Satterthwaite AB, Mohan C, Lipsky PE, Wakeland EK, Davis LS: SLE peripheral blood B cell, T cell and myeloid cell transcriptomes display unique profiles and each subset contributes to the interferon signature. PLoS One 8:e67003, 2013.

1BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Liao, Duanping	Professor of Epidemiology Vice Chair for Research Chief, Division of Epidemiology
eRA COMMONS USER NAME (credential, e.g., agency login) DPLIAO	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Tong Ji Medical University, China	MD	07/83	Medicine
Tong Ji Medical University, China	MS	07/86	Pediatric Science
University of North Carolina, Chapel Hill, NC	MPH	12/90	Epidemiology
University of North Carolina, Chapel Hill, NC	PhD	05/94	Epidemiology

A. Personal Statement

The objectives this project are to develop a Lupus Risk Calculator that uses preclinical Systemic Lupus Erythematosus (SLE) information and demographic factors to identify individuals at higher risk of progression to clinical SLE and to test treatment strategies to slow the progression of autoimmune responses in individuals with high risk of progression towards clinical SLE. My 25 years of collaborative research expertise involves the application of epidemiological approaches to investigate the progression and etiology of chronic disease, such as cardiovascular disease, metabolic disorders, and sleep-related problems. The four research projects briefly described below are most illustrative of my expertise necessary to successfully collaborate on the proposed project. I successfully applied for funding from NIH to initiate a population-based study of cardiac autonomic modulation. This study became the largest population based study in the US (*A Population based Study of Heart Rate Variability – the ARIC Study)*. I took responsibility for all aspects of this project, which was implemented by adding cardiac autonomic modulation components to the ongoing ARIC study. I have been fortunate to be among the first investigators to study cardiac autonomic control in the development of cardiovascular disease. I was intimately involved in the EPA-sponsored *Baltimore Elderly Epidemiology-Exposure*, in which I am fortunate to be one of the first investigators to hypothesize and report the associations between air pollution and cardiac electrophysiological parameters as one of the mechanisms responsible for the link between air pollution and cardiac disease risk. I am the leading investigator to develop, validate, and apply the GIS-based daily kriging approaches for the *Environmental Epidemiology of Arrhythmogenesis in Women Health Initiatives* (EEAWHI) study. In recent years, I have successfully developed collaborative studies in sleep related health studies. In these sleep-related studies, I have been able to develop the framework and the link between sleep-related disorders and cardiac and metabolic risks (*Sleep and Cardio-Metabolic Risk Factors: a Prospective Study of Adolescents*). I have contributed over 100 peer-reviewed articles in the fields of CVD epidemiology, air pollution epidemiology, and sleep disorders. My work has been widely cited, and I have been able to provide technical, methodological, and intellectual support to others in these fields. In summary, I am an experienced epidemiologist and I have a demonstrated record of successful and productive research projects in chronic disease epidemiology. My expertise and experience have prepared me to make important contribution to the proposed project by providing support in study design, data management, and data analysis, and on the interpretation of statistical analysis results to the research team.

B. Positions and Honors

Positions

1986-1988 Pediatrics Resident, Department of Pediatrics, Shanghai Medical University, China
1988-1989 Attending Pediatrician, Department of Pediatrics, Shanghai Medical University, China
1990-1993 Research Assistant, Department of Psychiatry, Univ. of North Carolina, Chapel Hill, NC
1991-1994 Research Assistant, Department of Epidemiology, Univ. of North Carolina, Chapel Hill, NC
1994-1995 Post Doctoral Fellow, Department of Epidemiology, Univ. of North Carolina, Chapel Hill, NC
1995-1998 Research Assistant Professor, Dept. of Epidemiology, Univ. of North Carolina, Chapel Hill, NC
1998-2001 Assist. Professor, Health Evaluation Sciences, Penn State College of Medicine, Hershey, PA

2001-2006 Assoc. Professor, Health Evaluation Sciences, Penn State College of Medicine, Hershey, PA
2006-Present Professor, Public Health Sciences, Penn State College of Medicine, Hershey, PA

Other Experience and Professional Memberships

1999-Present Steering Committee, Pennsylvania State University Institute of Environment
2001-2003 EPA's STAR Research Grants Review Panel
1998-Present NIH/NIEHS Review Panel – Special Emphasis Panels to review environmental pollution and health effects applications

Honors

1989-1990 International Clinical Epidemiology Network Fellowship, Univ. of North Carolina, Chapel Hill, NC
1993-1994 NIH Pre-Doctoral Traineeship in Cardiovascular Epidemiology, Nat'l Research Service Award
1994-1995 NIH Post-Doctoral Traineeship in Cardiovascular Epidemiology, Nat'l Research Service Award

C. Selected Peer-reviewed Publications (from 90 peer-reviewed publications)

1. Evans DL, Leserman J, Perkins DO, Stern RA, Murphy C, Tamul K, **Liao D**, van der Horst CM, Hall CD, Folds JD, Golden RN, Petitto JM: Stress associated reductions of cytotoxic T lymphocytes and natural killer cells in asymptomatic human immunodeficiency virus infection. *Am J Psychiatry* 152:543-550, 1995.
2. **Liao D**, Cai J, Brancati F, Crow R, Barnes RW, Tyroler HA, Heiss G. Association of vagal tone with serum insulin, glucose and diabetes mellitus -- The ARIC Study. *Diabetes Res Clin Pract* 30:211-221, 1995.
3. **Liao D**, Cai Jianwen, Barnes RW, Tyroler HA, Rantaharju P, Holme I, Heiss G. Cardiac autonomic function and the development of hypertension -- The ARIC Study. *Am J Hypertens* 9:1147-1156, 1996.
4. **Liao D**, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, Tyroler HA. The presence and severity of cerebral white matter lesions and hypertension, its treatment and control -- The ARIC Study. *Stroke* 27:2262-2270, 1996.
5. **Liao D**, Cai J, Rosamond W, Barnes R, Hutchinson R, Whitsel E, Rautaharju P, Heiss G. Cardiac autonomic function and incident CHD: a population based case-cohort study -- The ARIC Study. *Am J Epidemiol* 145:696-706, 1997.
6. Salomaa VV, **Liao D**, Kark JD, Wu KK, Stinson VL, Folsom A, Davis CE. The association of three components of fibrinolysis with fasting insulin and triglycerides. *Nutr Metab Cardiovasc Dis* 8:277-286, 1998.
7. Nelson JJ, **Liao D**, Sharrett AR, Folsom AR, Chambless LE, Shahar E, Szklo M, Eckfeldt J, Heiss G. Serum albumin as a predictor of incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol* 151:468-477, 2000.
8. Knuiman M, Chambless LE, **Liao D**, Wu K, Folsom A. Association of hemostatic variables with MRI-detected cerebral abnormalities. *Neuroepidemiology* 2001; 20: 96-104.
9. **Liao D**, Carnethon MR, Evans GW, Cascio WE, Heiss G: Lower Heart Rate Variability Is Associated with the Development of Coronary Heart Disease in Individuals with Diabetes - The ARIC Study. *Diabetes*. 2002; 51: 3524-3531.
10. **Liao D**, Heiss G, Chinchilli VM, Duan Y, Folsom AR, Lin H, Veikko Salomaa: Association of criteria pollutants with plasma hemostatic/inflammatory markers - A Population-Based Study. *J Expo Anal Environ Epidemiol*. 2005; 15: 319-328.
11. **Liao D**, Li X, Rodriguez-Colon SM, Liu J, Vgontzas AN, Calhoun S, Bixler EO: Sleep Disordered Breathing and Cardiac Autonomic Modulation in Children. Sleep Med. 2010; 11: 484-488. PMCID: PMC2857753
12. Rodríguez-Colón SM; Li X; Shaffer ML; He F; Bixler EO; Vgontzas AN; Cai J; **Liao D**: Insulin resistance and circadian rhythm of cardiac autonomic modulation. Cardiovascular Diabetology 2010; 9:85. PMCID in progress.
13. **Liao D**, Shaffer ML, Rodriguez-Colon SM, He F, Li X, Wolbrette DL, Yanosky J, Cascio WE: Acute Adverse Effects of Fine Particulate Air Pollution on Ventricular Repolarization. Environmental Health Perspective. 2010;118(7):1010–1015; PMCID: PMC2920900
14. He F, Shaffer ML, Rodriguez-Colon S, Bixler EO, Vgontzas AN, Williams RW, Wu R, Cascio WE, **Liao D.** Acute Effects of Fine Particulate Air Pollution on ST Segment Height - A Longitudinal Study. *Environmental Health* 2010, 9:68. PMCID: PMC2989950
15. Xian Li, Michele L. Shaffer, Sol M. Rodríguez-Colón, Fan He, Edward O. Bixler, Alexandros N. Vgontzas, Deborah L. Wolbrette, Chuntao Wu, Richard W. Ball, **D. Liao**: Systemic inflammation and circadian rhythm of cardiac autonomic modulation. Autonomic Neuroscience: Basic and Clinical. 2011. 162: 72–76.