PROJECT SUMMARY

Evaluation of the cutaneous microbiome in psoriasis

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I. PROJECT ID NUMBER, PUBLICATION MORATORIUM INFORMATION, PROJECT DESCRIPTION:

This manuscript is part of a pilot effort on the part of NIH staff and the Nature publishing group to provide a more convenient archive for "marker papers" to be published. These "marker papers" are designed to provide the users of community resource data sets with information regarding the status and scope of individual community resource projects. For further information see editorial in September 2010 edition of Nature Genetics (*Nature Genetics*, **42**, 729 (2010)), and the Nature Precedings HMP summary page.

Project ID: 46309
Project title: 16S rRNA from human cutaneous microbiome in Psoriasis
Moratorium length: 6 months

Psoriasis, a highly prevalent disease of humans of unknown cause, is a chronic inflammatory disorder primarily involving skin, with distinctive clinical characteristics. With the newly developed tools that facilitate microbiome research, it now is possible to assess whether the cutaneous microbiome plays a role in the pathogenesis of this disorder. Preliminary data from our studies suggest that the cutaneous microbiome in psoriasis is complex and possibly different from normal. To deal with this complexity, we propose to examine the cutaneous microbiome in relation to psoriasis with explorations at several taxonomic and informatic levels. Our overall objective is to examine how changes in the normal cutaneous microbiome contributes to the pathogenesis of psoriasis. Since causality is complex and often difficult to prove, our overall hypothesis is that there are alterations in the cutaneous microbiome in areas of skin affected by psoriasis in comparison with the range observed in clinically unaffected areas, or in healthy persons. We also hypothesize that the characteristics of the microbiome may affect clinical responses to the immunomodulatory agents used to treat psoriasis. An alternative hypothesis is that effective treatment of psoriasis with systemic immunomodulatory agents will not substantially affect the disordered microbial ecosystem. Such observations would provide evidence for the roles of the microbiota in this disorder. Since an important consideration in microbiome research is the optimal level (e.g. phylum, genus, species, strain, gene) at which to examine a scientific question, and we are not yet certain what are the optimal levels for psoriasis, this also will be examined. Our studies of psoriasis should allow development of both approaches and tools that will have general utility for microbiome research. To test our

hypothesis, we propose the following specific aims: 1. To understand the cutaneous microbiome species composition overlaying psoriatic lesions; 2. To investigate differences in metagenome content for psoriatic lesions compared to normal skin; 3. To identify differences in the transcriptional profiles of the microbiome and the host between normal skin and psoriatic lesions using high-throughput sequencing; and 4. To estimate the effects of systemic immunomodulatory therapy for psoriasis on microbiome composition. In total, these studies should help us understand the role of the microbiome in psoriasis pathogenesis.

The research subjects were categorized by a number of clinical factors including age, gender, race, family history of psoriasis, recent history of antibiotic use, and other recent treatments for psoriasis. Disease severity was assessed by duration of symptoms, percentage of body surface involved, and psoriasis area and severity (PASI) score. Multiple samples from each subject were further characterized by body site, and disease involvement of skin at the site. Samples were labeled with identifiers which include information about type of PCR primers used for amplification, and date of pyrosequencing run.

We now have collected specimens from 68 patients with psoriasis and 107 age-, gender-, and ethnicity-matched controls and established them in a physical repository. Vials with purified DNA from those samples have been barcoded for simplicity in the handling and study of them. We have established a single database that contains all the demographic and clinical information collected from the psoriasis patients and the demographic information from the control subjects.

In accordance with the guidelines of the Human Microbiome Project (HMP), there is a one year publication moratorium on data from this study starting from the time of data submission to dbGaP and to SRA at NCBI. The first dataset submission of UH2 phase data to SRA and to dbGaP was in May 2010.

DATA IDs

II. DATA QUALITY:

For the 16S samples with 454, the average read lengths were approx. 400 bp and the quality score was 30. For the whole genome shotgun metagenomic samples sequenced by 454, the average read lengths were 235 bases and the average quality score was 31.8. For the pilot internally transcribed spacer (ITS) studies for assessing fungal diversity, the assays produced ~20,000 good reads/sample, in which <4% of total reads were <200nt in length, and <1% of the total reads had an average QV<25.

III. DATA ANALYSIS AND PUBLICATION PLANS:

We have analyzed two different regions (V1-V3 and V3-V5) of 16S rRNA in 231 specimens provided to JCVI. These include 105 specimens sent in November, 2009, which involved 35 samples from 35 controls and 70 samples (paired lesion and non-lesion) from 35 matched psoriasis patients. In addition, we have analyzed 126 specimens sent in March 2010 that included

42 specimens from 42 controls and 84 samples from 42 psoriasis patients (same pairing and matching). We plan to compare the results of the two variable regions to determine which one is more informative.

Preliminary analysis confirmed that the sequence reads provide data useful for studies at the phylum and genus level. We will explore the taxonomic distribution and abundance of the 16S sequences across sample phenotype classes using several methods including UniFrac clustering, taxonomic abundance tables created with the Ribosomal Database Project (RDP) classifier, and OTU-based methods that cluster sequences based only on percentage identity.

We have conducted preliminary studies into the use of ITS regions as markers for examination of fungal diversity. JCVI developed and tested specimens from 12 patient and 6 control samples, by 454-XLR.

We also performed qPCRs for three of the most common bacterial genera (Propionibacterium, Staphylococcus, and Streptococcus), as well as for Eubacteria in total, and GAPDH as a measure of human DNA present the 231 specimens. Review of the HMP data concerning healthy persons (available through controlled access at the HMP DACC site)shows that the above mentioned genera are the 3-best represented genera in healthy skin. We constructed ratios of various genera to examine whether broad trends in microbial population structure might be present.

We hope to submit for publication the initial results of the 16S rRNA tag sequencing related to bacterial taxa in psoriatic lesions and clinically uninvolved skin from patients in comparison to healthy controls to examine for broad trends in population structure. We hope to submit this within the next 12 months before the termination of the embargo period.

IV. DATA RELEASE PLAN:

The J. Craig Venter Institute (JCVI), which will produce the 16S, whole genome shotgun and ITS sequence data for this project, and NYU will make all resources developed through this UH2contract available to the biomedical research community in keeping with NIAID (http://www3.niaid.nih.gov/research/resources/mscs/data.htm) and NHGRI (http://www.genome.gov/10506376) data release policies. Further, we will make every effort to coordinate data release with the Data Analysis Coordination Center (DACC) for the Human Microbiome Project. Consistent with these guidelines, the sharing of research data will include rapid dissemination of research data to the scientific community in order to maximize the public benefit of the data produced. Software tools resulting from this program will be freely released under an open source license with no restrictions and made available to all researchers in both the private and public sector with minimal restriction. When developing project plans, project staff will disclose NIAMS/NHGRI data release and reagent sharing (microorganism/nucleic acids) policies with collaborators. As a part of freely sharing the data with the research community, neither the JCVI nor NYU will file patent applications on the sequence data to be generated as a part of this project, and the sequence data will be deposited in accordance with the NIAMS/NHGRI data release policies.

V. CONTACT PERSONS:

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