Progress Report CRF grant: "Intra-dermal Imaging of Subjects with Cystinosis using Confocal Microscopy and Natural History study"

August 22th, 2022

A. Abstract

This study intends to gather intra-dermal images of the skin from patients with cystinosis as a method to estimate the quantity of cystine crystals in this tissue compartment. The tissue cystine crystal density most likely has an inverse correlation with disease outcome and may correlate with long-term efficacy of treatment and compliance with therapy. We are planning to use this method to monitor the therapeutic impact of the stem cell treatment during the upcoming stem cell transplant clinical trial for cystinosis at UCSD. With the support of the Cystinosis Research Foundation, we purchased a Caliber Vivascope® 3000 in November 2015. So far, we imaged 66 patients with cystinosis and 30 healthy controls. Using ImagePro imaging software, new algorithms were generated to recognize and quantify specifically the cystine crystals. The data revealed striking differences between patients and compliance correlation. Therefore, the aim of this study is to investigate intra-dermal images of the skin as an alternative method to monitoring the levels of cystine crystal accumulation in the skin and compare those relationships to the current method of blood cystine levels, to the patients' health status and to their genetic mutations. This study may potentially provide a method for an easier, noninvasive and inexpensive way for quantifying intradermal cystine crystals, and therefore response to available therapies. We are building a database of descriptive epidemiology and clinical outcome for the patients with cystinosis through the collected medical records. Altogether, this project aims at improving the follow up of patients affected with cystinosis, increase our understanding of the pathology, and building a Natural History for cystinosis. These data will also be critical for evaluating the outcome of the stem cell gene therapy clinical trial currently ongoing at UC San Diego.

B. Progress Report and Study Design

We have acquired intra-dermal images of cystinosis patients and healthy controls using a handheld confocal microscope, the Caliber ID Vivascope 3000. Currently we have a total of 119 subjects enrolled in the study, 77 cystinosis patients and 42 healthy controls (Table 1). We imaged the patients and controls about 2 cm behind the earlobe in the mastoid region about 200 microns deep corresponding to 78 images with a step size of 2.8 microns for each subject. Using ImagePro, we were able to automatically process all the images in an unbiased manner to select and measure the skin cystine accumulation. The ImagePro macro we previously developed {Bengali, 2021 #3473} allows for reproducible analysis of skin cystine crystals and we have optimized a set of macros that will be able to be run precisely and unbiasedly by anyone. The 2D and 3D images collected in 2017, 2018, 2019 and 2022 have been analyzed and showed significantly increased cystine crystal quantity in patients compared to healthy controls (Figure 1 and 2, respectively), continuing to show that our new crystal analysis platform is an effective new tool to specifically quantify the cystine crystals in the skin.

Table 1: Current and previous enrollment and tests obtained

Total Number of Subjects Enrolled 2017	Total Number of Subjects Enrolled 2018	Total Number of Subjects Enrolled 2019	Total Number of Subjects Enrolled 2022	Confocal Imaging 2017	Confocal Imaging 2018	Confocal Imaging 2019	Confocal Imaging 2022
37	69	100	18	36	56	63	18

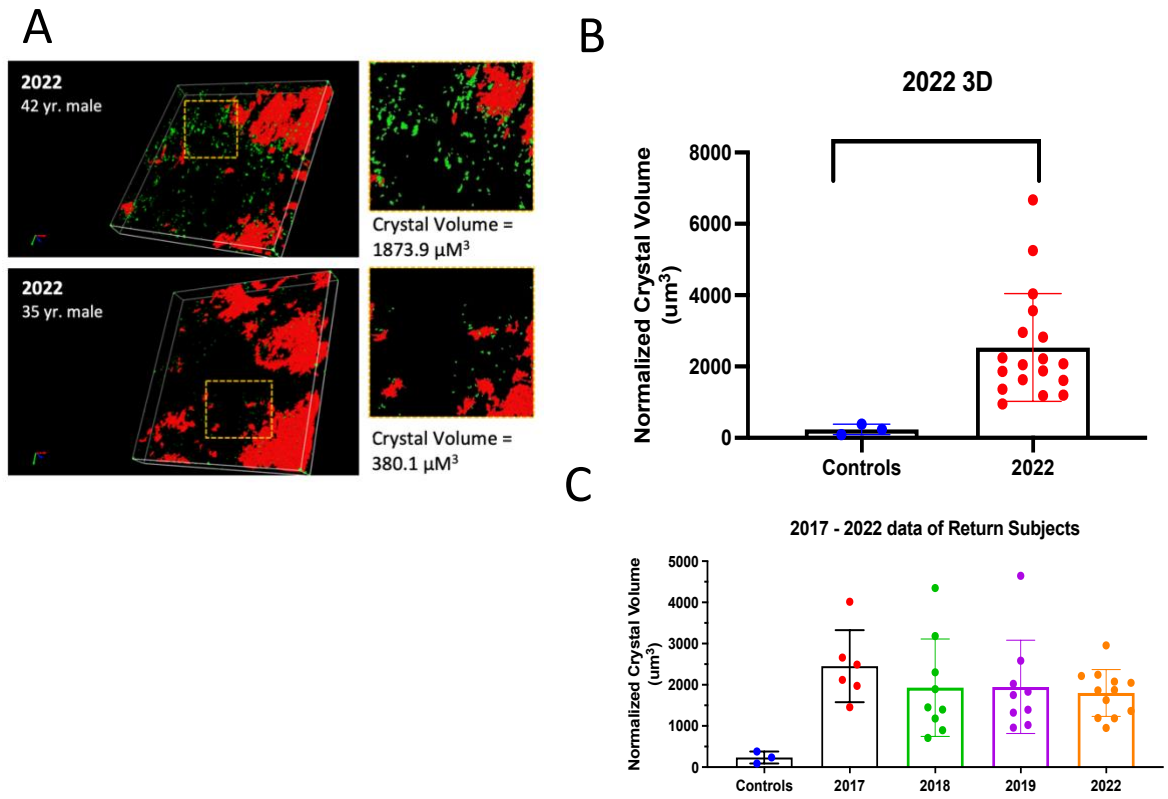

Figure 1: 3D imaging of the skin and quantification. (**A**) 3D-reconstruction of Z-stacks confocal images of a patient with cystinosis (upper panel) and healthy control (lower panel). (**B**) Quantification analysis of the 3D-confocal images of the skin of cystinosis patients and healthy controls acquired in 2022. Significantly more crystals are observed in cystinosis patients compared to healthy controls. (**C**) Longitudinal quantification analysis of the skin 3D-confocal images of returning cystinosis patients from 2017, 2018, 2019 and 2022, and the healthy controls from 2022. *p<0.05

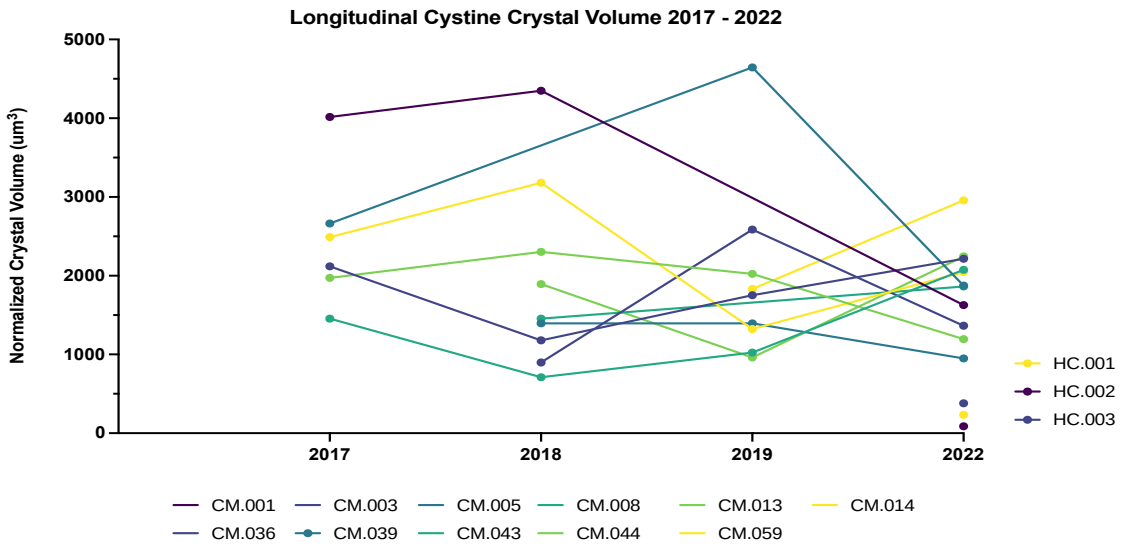

Figure 2: Longitudinal data of 3D skin confocal imaging of 11 returning patients at Day of Hope 2022 conference and 3 Healthy Controls. Quantitative non-invasive intradermal confocal imaging shows increased crystals within cystinosis patients.

Results from the 3D-confocal imaging in Figure 2 suggests, 55% of returning patients had an increase of cystine crystals when previous timepoint is compared to 2022 data. As the patients age, we expected to see an increase in cystine crystals due to crystal accumulation over time. However, 45% of returning patients exhibited a decrease in cystine crystals in 2022. This could be due to the area of imaging but we will also analyze these patients' medical histories to understand why there was a decrease in cystine crystals. We are currently working to obtain medical records from the patients' primary care and nephrology providers for the patients.

Conclusion: This observational study continues to gather intra-dermal images of male and female cystinosis patients at different ages (from 1 to 58-year-old). Our results suggest this unbiased automated imaging to quantify the cystine crystals could become an important tool for patients monitoring due to its noninvasive methodology that allows to visualize and quantify the cystine crystal deposits within the skin. Due to the noninvasive nature of this testing, we have been able to obtain cystine crystal quantification of children as young as 1 years old. Tracking the skin cystine crystals for the same patients across several years has allowed us to estimate the changes in cystine crystal counts over time. Continuing to observe these changes and collecting the most information possible on their health status, laboratory levels, white blood cell cystine levels, compliance to cysteamine and genetic mutations would allow us to understand why we are seeing an increase or decrease in cystine crystals. This will deepen our understanding of the different factors in cystinosis progression.

Moving forward, we plan to enroll new patients and continue imaging to increase our sample size and monitor currently enrolled patients. With this study we are continuing to demonstrate the usefulness of using the In-Vivo Skin Confocal Analysis and biomarkers to follow cystinosis patients and build the natural history of cystinosis.