FD/MAS is a debilitating rare disease caused by a postzygotic mutation of the gene GNAS that leads to mosaic G αs activation and inappropriate production of intracellular cAMP. FD/MAS affects bones, skin, and several hormone-producing (endocrine) tissues. Patients develop areas of abnormal fibrous tissue in their bones. Replacement of bone with fibrous tissue may lead to fractures, uneven growth, pain, and deformity. FD/MAS presents in different ways, and diagnosis and treatment can be difficult.

2018 Team FD Awardees: Spring 2020 Update

The Fibrous Dysplasia Foundation (FDF) and the University of Pennsylvania Orphan Disease Center announced the four winners of the 2018 Million Dollar Bike Ride research grants in December 2018, and the awardees began their studies in early 2019. These important grants fund innovative research to investigate fibrous dysplasia/McCune-Albright syndrome (FD/MAS). Each investigator recently provided a review of their progress to date, and their summaries have been edited and condensed for clarity below.

Research Summaries and Progress

Dr. Charles Hoffman, Boston College
Identification and Characterization of Novel Cell-Permeable, Small Molecule Adenylyl Cyclase Inhibitors for Future Development as Drugs to Treat FD/MAS

FD/MAS is caused by mutations in the *GNAS* gene that cause it to overstimulate a group of proteins called adenylyl cyclases (ACs) that make the signaling molecule cyclic AMP (cAMP). Rather than trying to inhibit GNAS itself as a way of reducing these elevated cAMP levels, we have sought to find compounds that inhibit the ACs found in the types of cells responsible for FD/MAS. We engineered yeast cells to express specific mammalian ACs of interest, and also used cultured human cells, including an osteoblast cell line. With funding from the 2018 MDBR award we confirmed the ability of two groups of molecules, which were initially detected in a screen of ~125,000 compounds, to reduce cAMP production in these cells. Additional experiments show that these compounds have good drug-like properties and could serve as the starting material to create better AC inhibitors to potentially treat FD/MAS.

Dr. Kelly Wentworth, University of California, San Francisco
Elucidating the Role of GNAS Mosaicism in Fibrous Dysplastic Lesions

We are collecting bone samples from patients with FD who are undergoing surgery and then using a technique called single cell RNA sequencing to determine which cell types are present in the FD lesions and which cell types carry the GNAS mutation. We will compare these samples to samples from

healthy human bone and look for differences in gene expression and signaling pathways. If there are unique pathways that are overactive or underactive in FD bone, then we can work towards developing pharmacologic therapies that target these pathways and could help reverse or stop FD bone lesion development.

Dr. Fernando Fierro, University of California, Davis

Single-Cell Transcriptome Analysis of Skeletal Stem Cells Derived from FD/MAS Patients

Bone growth, repair, and maintenance is accomplished by skeletal stem cells (SSCs). Under specific cues, these cells inside the bone proliferate and differentiate into mature bone-producing cells called osteoblasts and osteocytes. It is believed that FD occurs due to genetic mutations in the SSCs that cause them to undergo increased proliferation but inadequate differentiation into mature bone cells. However, current evidence comes mostly from mouse studies, and only provides indirect evidence about the role of SSCs in FD. We have therefore focused on studying SSCs isolated from human FD lesions. Since FD cases are infrequent, we have established a collaboration with the group of Dr. Kelly Wentworth at UCSF, to share patient's biopsies for our studies. We both use the technique called single cell RNA sequencing and compare cells from FD and from normal bone. The group at UCSF looks at multiple cell types, while we focus on the SSCs. Since SSCs are very rare cells, our approach requires that we "zoom in" to see what is happening. So far, we have analyzed bone specimens from three FD patients, which has been key to establishing the proper methodology for understanding the cellular composition of FD lesions at unprecedented resolution. We aim to continue processing samples from both FD and normal bone, because the more samples we can analyze, the more robust our conclusions will be. We expect to determine with precision what is happening in FD with single-cell resolution, not using an animal model, but with a focus on the human clinical condition. This in turn, may translate into new approaches to diagnose and treat FD.

Dr. Mara Riminucci, Sapienza University of Rome

Anti-resorptive drugs in Fibrous Dysplasia of bone: studies on the effects of a RANKL inhibitor and Zoledronic Acid in a murine model of the disease by radiography, histology and genome-wide gene expression analysis (NanoString).

Bisphosphonates such as zolendronic acid (Reclast®) and the anti-RANKL antibody denosumab (Prolia®) are two classes of drugs with different mechanisms that were initially developed to treat osteoporosis and bone destruction associated with metastatic cancer. Because of the way they work, they have subsequently been investigated as treatments for other bone diseases, including FD.

FD lesions are characterized by a very high number of osteoclasts, the cells that resorb bone at the beginning of the normal process of bone regeneration. We have previously shown that in our mouse model of FD, inhibition of osteoclast *formation* by an anti-RANKL antibody arrests the growth of bone lesions, improves the mechanical properties of affected bones, and halts the progression of the disease.
In this project, we have demonstrated that in the same FD mouse model, inhibition of osteoclast *bone resorption activity* by zoledronic acid does not reproduce the same positive effect of the anti-RANKL antibody. This result suggests that osteoclasts

contribute to the establishment and maintenance of FD lesions through multiple mechanisms, some of which are independent of bone resorption. It also helps to explain why drugs that only inhibit bone resorption but do not eliminate osteoclasts do not significantly modify the natural history of the disease. Since FD mice treated with the anti-RANKL antibody show a relapse of the bone disease shortly after the discontinuation of the drug, we have tested a combined treatment of anti-RANKL antibody and zoledronic Acid. We have observed that in our FD mice, zoledronic acid induces a significant delay in the recurrence of FD lesions after the withdrawal of the anti-RANKL antibody. This result may help to develop an improved anti-RANKL-based therapeutic protocol for FD patients. Finally, we have also started to investigate the role of osteoclasts in FD in more detail by applying a method called NanoString to do molecular analysis of gene expression on preserved bone samples from mice and FD patients that were previously used just for histology.

"Developing a better understanding of the disease, identifying improved treatments, and hopefully, a cure for FD/MAS requires ongoing investment in research."

Dr. Andrew Shenker, Chair of the FDF Scientific Advisory Council states, "It is very encouraging to see the nice progress that has been made by all the 2018 MDBR awardees on this complex, mosaic bone disease. Three of the investigators are focusing on a better understanding of the disease at the cellular level, including stem cells, osteoblasts and osteoclasts, and Dr. Hoffman is focusing on finding a novel treatment that may inhibit abnormal signaling downstream of the causative GNAS mutation. The collaboration between Dr. Wentworth and Dr. Fierro to efficiently utilize precious fresh bone samples from patients is exactly the type of cooperation between young investigators that the Team FD/MAS-enabled grants are intended to foster."

Dr. Shenker continued, "Dr. Riminucci's mouse model work utilizes two drugs that have already been studied as treatments for FD in patients. Her findings make significant contributions to our understanding of the pathophysiology of FD that will inform the design of preclinical and clinical studies that could potentially lead to the two drugs being used in concert in a safer and more effective way, as recently highlighted in an editorial from Dr. Collins and his team at the NIH (de Castro et al 2019)." It is also worth highlighting Dr. Riminucci's application of the NanoString technology to preserved patient bone samples, rather than relying only on precious fresh specimens. This approach could provide certain advantages in the way data can be analyzed and facilitate the sharing of samples between laboratories. Dr. Riminucci presented preliminary NanoString results on FD samples from seven patients in a poster at the RARE Drug Development Symposium held by Global Genes in partnership with the Penn Orphan Disease Center in June, 2020.

"It is gratifying that all four of the 2018 MDBR projects have the potential to lead to improved or novel treatments in the clinic someday. It is imperative that this type of research continues to be well-funded by the FD/MAS community, both because of the inherent complexity of FD/MAS compared to many other rare diseases, and the need to attract new investigators and provide them and others a way to explore novel approaches or perform pilot studies in areas that may not be mature enough to merit a highly competitive NIH extramural grant. In fact, Dr. Wentworth recently received an NIH K08 career

development grant that will allow her to continue her research in FD *(Click here to learn more about Dr. Wentworth).* Developing a better understanding of the disease, identifying improved treatments, and hopefully, a cure for FD/MAS requires ongoing investment in research."

2019 Awardees and Securing Future Research Gains

Every grant that FDF facilitates is another opportunity for the FD/MAS community to move closer towards the promise of a better future. Team FD grants funded two projects with 2019 Team FD grants, and FDF looks forward to receiving reports from Dr. Yingzi Yang of the Harvard School of Dental Medicine and Dr. Julia Charles of Harvard Medical School. (Click here to learn more.)

Presently, despite the COVID-19 pandemic, Team FD/MAS was able to rally and raise enough funds to support a grant in excess of $60K to be awarded in the Fall of 2020. This success was in large part due to the generous support of Association MASFD, which gave $20,000 to Team FD/MAS, as well as the UPenn donation matching program.

Additionally, in partnership with the Association MASFD, FDF facilitated the review and approval of the research project "A Murine Model for the Central Nervous System Manifestations of McCune-Albright Syndrome '' to take place in Dr. Ted Abel's laboratory at the Iowa Neuroscience Institute.

We look forward to updating you about these studies as well as two 2019 research grants awarded last December.

About Fibrous Dysplasia Foundation
The Fibrous Dysplasia Foundation (FDF) is a community-led 501(c)3 nonprofit that fights back against fibrous dysplasia, McCune-Albright syndrome (FD/MAS). FD/MAS is a rare, chronic, debilitating disease with no known treatment or cure. FDF serves people affected by FD/MAS through programs of research, education, and advocacy. EIN 02-0715210 To learn more, visit fibrousdysplasia.org.