Fibrodysplasia ossificans progressiva (FOP) is a disease that causes excessive and irreversible bone formation at soft tissue sites. In FOP, muscles, ligaments and connective tissue can transform into bone upon soft tissue damage.1,2,3,4 Moreover, soft tissue damage from surgery, intramuscular immunizations or even a bump can induce bone growth at joints that permanently restricts local movement. 4,5,6 FOP is caused by a single missense mutation in the 206th amino acid (c.617 G>A; R206H) within a transmembrane protein called activin A receptor type I (ACVR1). 3,4 The mutation resides in the TGF-beta domain of the ACVR1 protein. ACVR1 is a receptor in the bone morphogenetic protein family (BMP).2 Under normal conditions, the BMP pathway causes changes in gene expression that induce the formation of bone and cartilage.7 ACVR1 is an integral component of BMP pathway by initiating signaling when bound to the ligand, ultimately causing the downstream changes that initiate bone development. In the pathological state, it is thought that the mutation perturbs normal signaling in soft tissue by making the inhibitor FKBP12 unable to bind to the TGF-beta domain in the absence of the ligand, which confers the pathway to be “leaky” and facilitate signaling in tissues that are not supposed to be forming bone.1,3,7 Ultimately, this aberrant signaling of the BMP turns endothelial cells into mesenchymal stem cells and subsequently into bone. Although it is agreed in literature that the improper signaling by ACVR1 is responsible for FOP, it is still murky as to how soft tissue trauma causes the overstimulation of this pathway that leads to the eventual transforming mesenchymal cells into bone.

The **hypothesis** is that the missense mutation in the TGF-beta domain confers ACVR1 signaling to be constitutively active therefore leading to changes in gene expression, and subsequently, transdifferntiation of the soft tissue into bone. This is supported by protein modeling studies which demonstrates the protein binding domain is likely possess differential folding with the single amino acid change. The **primary goal** of this research is to better understand the genes involved in the conversion of soft tissue to bone as well as the role of the inhibitor. The **long-term** **goal** is to understand this mechanism and gain a better insight into FOP to ultimately develop treatments.

**Aim 1:** Perform an expression comparison of muscular tissue in the normal and pathological state.

**Approach:** Using microarray, compare transcripts and gene expression between normal mouse muscle tissue and FOP mouse muscular tissue after being damaged. This will be used to determine which genes are active in the pathological vs. normal state and what genes are involved in the transdifferentiation.

**Aim 2:** Compare protein activities in pathological state compared to normal state in muscle tissue.

**Approach:** Use liquid chromatography and mass spectrometry to look at protein abundance and protein activity via phosphorylation in the normal and the pathological state. This discerns how the leaky receptor causes changes in protein expression and activity that confers the disease phenotype.

**Aim 3:** Determine if using chemical genetics to inhibit the aberrant receptor enables the rescue of normal gene expression.

**Approach:** Screen the inhibitors found on the Pubchem database. Then apply these inhibitors to a diseased mouse and determine if it helps diminish some of the uncontrolled activation to restore similar gene expression to the normal conditions using microarray.

This project is crucial due to the fact that it is not clear how these cells are undergoing their metamorphosis. It is expected that the outcome of this study will help identify which genes are unregulated, try to restore normal signaling through investigating alternative inhibitors, and to discover more clues as to how this disease works. In the long term, the hope is that this information will allow for insight into potential genes and transcripts to target for the development of future treatments.

1. Shore, E.M. et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. Nat. Genet. 38, 525–527 (2006). <http://www.bio.davidson.edu/molecular/restricted/02bone/FOP_cause.pdf>
2. Clinical Reviews in Bone and Mineral Metabolism, vol. 3. Humana Press Inc. 2005. [file:///C:/Users/User/Downloads/Clinical%20Reviews%20in%20Bone%20and%20Mineral%20Metabolism.pdf](file:///C%3A%5CUsers%5CUser%5CDownloads%5CClinical%20Reviews%20in%20Bone%20and%20Mineral%20Metabolism.pdf)
3.  Kaplan, F. S., Xu, M., Seemann, P., Connor, J. M., Glaser, D. L., Carroll, L., Delai, P., Fastnacht-Urban, E., Forman, S. J., GillessenKaesbach, G., Hoover-Fong, J., Koster, B., Pauli, R. M., Reardon, W., Zaidi, S. A., Zasloff, M., Morhart, R., Mundlos, S., Groppe, J., and Shore, E. M. (2009) Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1, Hum. Mutat. 30, 379–390. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2921861/>
4. Chakkalakal, S. A., Zhang, D., Culbert, A. L., Convente, M. R., Caron, R. J., Wright, A. C., Maidment, A. D. A., Kaplan, F. S. and Shore, E. M. (2012). An Acvr1 R206H knock-in mouse has fibrodysplasia ossificans progressiva. J. Bone Miner. Res. 27, 1746-1756.. 7. "Fibrodysplasia Ossificans Progressiva." Genetics Home Reference. U.S. National Library of Medicine, 9 Feb. 2015. Web. 11 Feb. 2015. <http://onlinelibrary.wiley.com.ezproxy.library.wisc.edu/doi/10.1002/jbmr.1637/pdf>
5.   Pignolo RJ, Shore EM, Kaplan FS. Fibrodysplasia ossificans progressiva: clinical and genetic aspects. Orphanet J Rare Dis. 2011;6:80. <http://www.ojrd.com/content/pdf/1750-1172-6-80.pdf>
6. "FOP Fact Sheet." FOP Fact Sheet. International Fibrodysplasia Ossificans Progressiva Association, 2009. Web. 12 Feb. 2015. <[http://www.ifopa.org/fop-fact-sheet.html](http://www.ifopa.org/fop-fact-sheet.htm)>.
7. "Fibrodysplasia Ossificans Progressiva." Genetics Home Reference. U.S. National Library of Medicine, 9 Feb. 2015. Web. 11 Feb. 2015. <http://ghr.nlm.nih.gov/condition/fibrodysplasia-ossificans-progressiva>
8. Dinther et al. (2010). "ALK2 R206H mutation linked to fibrodysplasia ossificans progressiva confers constitutive activity to the BMP type I receptor and sensitizes mesenchymal cells to BMP-induced osteoblast differentiation and bone formation". Journal of Bone and Mineral Research <http://onlinelibrary.wiley.com/doi/10.1359/jbmr.091110/abstract>