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UNIVERSITY OF  
**OXFORD**

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## Annual Report from the University of Oxford FOP Research Team



### University of Oxford FOP Research Team

(left to right) Dr. Alex Bullock, Miss Caroline Sanvitale, Dr. Eleanor Williams, Dr. Georgina Mosedale and Professor Jim Triffitt.

**First Anniversary.** The newly expanded FOP research team, pictured above, was activated last year and is about to celebrate its first anniversary. This provides a timely opportunity to share with everyone before the end of 2011 the latest exciting news from Oxford and the lab bench. Jim remains as active and as passionate about FOP research as ever and has been inspiring all the new members of the team. Alex has been collaborating with Jim since the



identification of the FOP gene *ACVR1* in 2006 and brings expertise in how to screen for drug molecules in this particular protein family for possible use in therapy. Alex was helping Jim to supervise the PhD studies of Kirsten Petrie, who was the first student supported by monies raised by FOP Action (through the University of Oxford FOP Research Fund) to study the genetics of FOP. Some of you may well have had the opportunity to meet Kirsten during her tenure. We're delighted to report that Kirsten was awarded her PhD in the summer. Her research work – together with that of many colleagues – was involved with identification of the FOP gene and she subsequently identified three variant FOP mutations in UK FOP patients. This work is now published in two academic papers to disseminate these data to other clinicians and researchers:

- Novel mutations in *ACVR1* result in atypical features in two fibrodysplasia ossificans progressiva patients. Petrie KA, Lee WH, Bullock AN, Pointon JJ, Smith R, Russell RG, Brown MA, Wordsworth BP, Triffitt JT. *PLoS One*. 2009;4(3): e5005.
- A novel *ACVR1* mutation in the glycine/serine-rich domain found in the most benign case of a fibrodysplasia ossificans progressiva variant reported to date. Gregson CL, Hollingworth P, Williams M, Petrie KA, Bullock AN, Brown MA, Tobias JH, Triffitt JT. *Bone*. 2011 **48**: 654-8.

**New members.** Caroline follows in Kirsten's steps as a budding PhD student and again gratefully acknowledges the support of the University of Oxford FOP Research Fund, without which this work on FOP would not be possible. We're also pleased to announce that Caroline has just passed her mid-course examination. With this behind her she is now preparing her first research article (more research news below). Eleanor and Georgina are the newest members, filling the two Roemex postdoctoral fellowship positions described in last year's report that are generously supported and underwritten by Richard Simcox. Both have either studied or worked in the University for several years and we're lucky to have such talented people join the team. They have both been mentoring Caroline as well as making great strides with their own research projects.

**New laboratory.** We've moved to a new state of the art research laboratory facility in the University of Oxford at the Old Road Campus just across the road from the Nuffield Orthopaedic Centre Hospital in the north-east of Oxford. We are hosted here by the SGC (Structural Genomics Consortium). The SGC is an international consortium with the mission to advance the discovery of new medicines by promoting open access collaboration. It solves the 3D structures of important drug targets and releases them freely to allow chemists across the world to design new medicines. In many cases, it also identifies the first drug-like molecules to get things started. The SGC is a subgroup of the Nuffield Department of Clinical Medicine and is a member of the University of Oxford. It is notable for us, in that it is established as a charitable organisation drawing funds from academic sources such as the Wellcome Trust in the UK as well as from drug companies such as GlaxoSmithKline,

Novartis, Eli Lilly and Pfizer. The SGC now forms a strategic partner with us to advance our work on FOP. For example, we are testing the SGC's collection of drug-like molecules to find FOP inhibitors that may be used as potential medicines to stop the disastrous bone formation characteristic of FOP. The SGC has also helped to bring this work to the attention of its many academic and industry partners. In addition, we're grateful to our neighbours, the Oxford Institute of Biomedical Engineering and the Ludwig Institute of Cancer Research, who have provided us with additional access to some essential equipment and cell culture facilities.



The new research building housing the FOP team

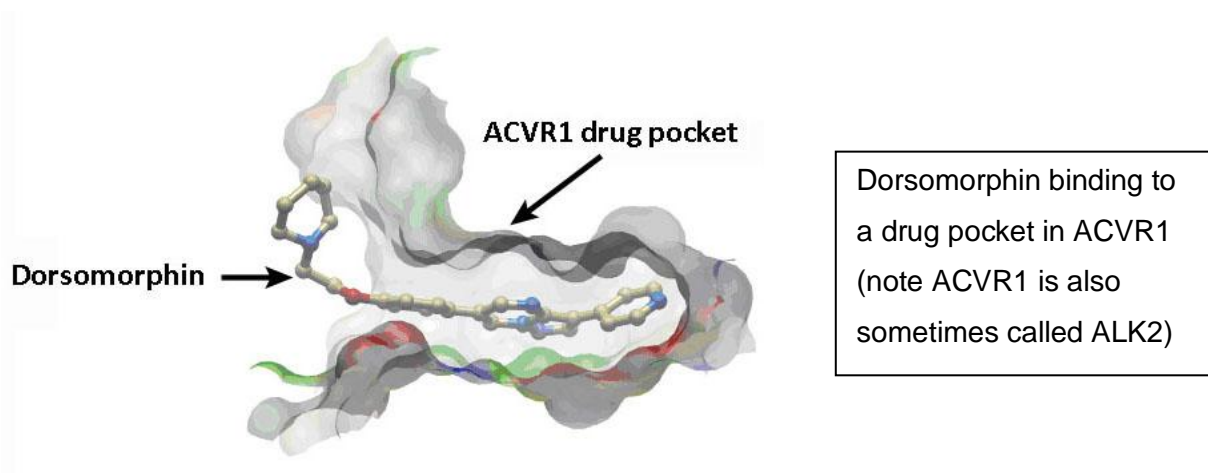
**An introduction to our research.** FOP mutations in the *ACVR1* gene lead to the production of a faulty protein. This protein represents a druggable target and can be regarded as a 500 piece jigsaw, or more accurately a 500 part molecular machine. Due to FOP mutations, one piece of this jigsaw is produced with the wrong shape. Unusually, this wrong piece doesn't stop the machine working, but instead causes it to be hyperactive - leading to the bone formation in FOP. The FOP team at SGC are studying the 3D structure of the ACVR1 protein to answer the important following questions (i) how does the wrong piece activate ACVR1 in this way? (ii) why does the wrong piece cause only muscle bone formation? (iii) how can drugs switch the ACVR1 machine off? (iv) is it possible to make even better and more selective inhibitor drugs for use in patients?

How do you "see" the ACVR1 protein to understand its 3D structure? Proteins cannot be seen under a microscope as they are far smaller than the wavelength of light. Instead we use the same technique used to discover the atomic structure of DNA. This is the technique of X-ray crystallography and as the name suggests targets crystals with finely tuned X-rays. Once obtained pure, many materials like atoms, salts and proteins can be grown into crystals, perhaps the most precious being diamonds! Caroline and colleagues at SGC have been growing crystals of the ACVR1 protein. X-rays are then scattered as they pass through the crystal. Using some clever equations, the structure of the crystallized molecule is deduced from this pattern.

**(i) how does the wrong piece activate ACVR1 in this way?** We have now solved the 3D structure of the ACVR1 protein affected by FOP and released these data to the scientific community. Caroline and Eleanor have also solved very recently the structures of four ACVR1-like proteins that normally work in unison with ACVR1. Together, these 3D structures show how these important cellular molecules, or machines, change shape as they switch between the inactive state, or OFF, and the active state, or ON. We've learnt that the wrong piece in ACVR1 damages the OFF position, but not the ON position. In effect, the brake pedal is broken and the machine continues out of control. However, there is some relief. Other regulatory proteins in our bodies also act as a failsafe brake system, most notably a molecule named FKBP12. Georgina has found that these natural inhibitors retain much of their defence and so reduce the activity of the faulty ACVR1 machine by up to 3-6 fold. Perhaps this explains the periods in between flare ups in FOP? This work should be published shortly.

**(ii) why does the wrong piece cause only muscle bone formation?** Recent studies in bone cells suggest that ACVR1 function is complex, perhaps regulated in normal cells by feedback loops that impose a stop to bone growth when required. Georgina will be establishing cellular assays for ACVR1 activity that may allow us to analyse these pathways in more detail.

**(iii) how can drugs switch the ACVR1 machine off?** Many of you may have read about potential drug-like molecules for FOP which were identified in Harvard University using tiny zebrafish. We've solved additional structures of the ACVR1 protein to understand how these drug molecules, named dorsomorphin and LDN-193189, are working.



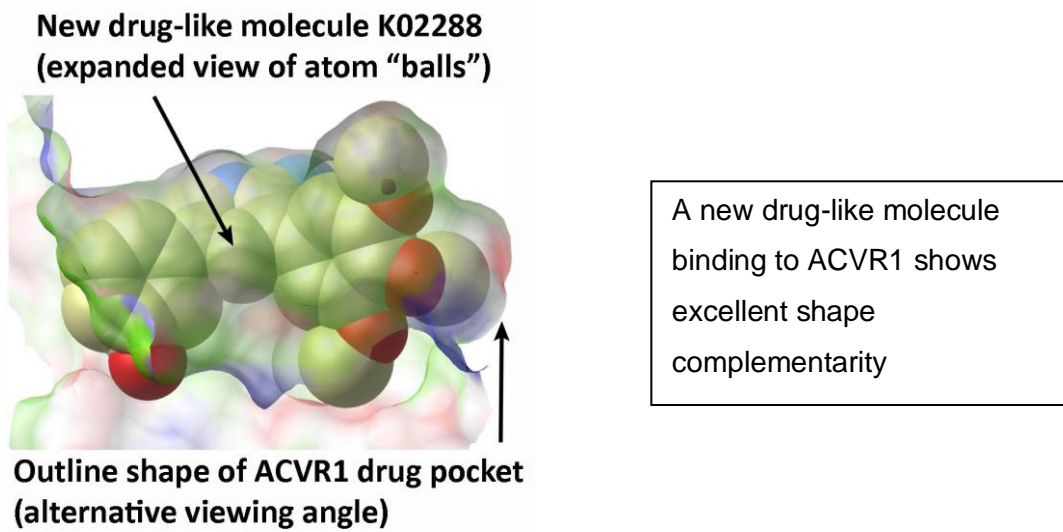


As the image above shows dorsomorphin binds directly to a large pocket in ACVR1. (The atoms and bonds in dorsomorphin are shown in the middle of the pocket as little balls and sticks.) This pocket is the engine room of the ACVR1 machine and so the drug blocks all activity – the machine is forced OFF. Like a lock and key, the art of drug discovery is to design a drug that best fits the pocket. But this is not the end of the story.

We all have two copies of each gene. In a perfect world, we would like a drug to block the activity of the faulty copy of ACVR1, but leave the normal copy alone. At present, we think the two copies of ACVR1 are too similar to achieve this (the locks look the same), but we will keep looking for possibilities. However, a bigger challenge, common to all drug discovery, is making a drug selective so that it binds ACVR1 but not other ACVR1-like genes. In total, humans have about 20,000 genes. ACVR1 belongs to one of the largest gene families (classified as “kinases”). In this family there are 500 genes like ACVR1. The proteins made from these genes all have a drug pocket with a broadly similar shape and size to the one in ACVR1. Our friends at SGC are solving the 3D structures of these other kinases to identify the small differences that can be exploited for drug design (just like a locksmith). In fact, over the last few years the area of drug design for kinases has been quite successful, offering hope for FOP (for example, the drugs “gleevec” for leukaemia and “irressa” for breast cancer). But we should be mindful that these are typically drugs developed to kill cancer cells (where hitting multiple gene targets for cell death can be beneficial). For FOP we must take extra care to minimize any “off-target” effects and any potential toxicity.

**(iv) is it possible to make even better and more selective inhibitor drugs for use in patients?** Caroline, Eleanor and Georgina are examining the selectivity of each ACVR1 inhibitor as well as screening for other drug-like molecules. Dorsomorphin was identified as an inhibitor working in fish. Therefore, it wasn’t selected to be best in humans or specifically to target ACVR1 (although we now know it works). We therefore considered it essential to conduct further drug screens directly against the purified human protein ACVR1, including the altered FOP mutant form. This work has identified a whole series of molecules closely related to dorsomorphin, including some known for activity against leukaemia. This affirms dorsomorphin as a hit molecule, but also highlights the potential for further chemistry.

*A new drug lead.* Caroline’s work has also identified a new drug-like molecule for human ACVR1 that appeared to be very selective against other kinase proteins. This has a completely different chemical structure to dorsomorphin and is therefore a highly complementary discovery. To treat FOP we need as many different leads as possible to ensure one succeeds in the end and we are considering all possibilities. Again, we solved a 3D structure to understand how this molecule targets ACVR1.



The picture above shows the drug-like molecule in a more realistic view compared to the ball and stick model we used for dorsomorphin. It highlights the excellent fit between the drug and the shape of the ACVR1 pocket which likely contributes to its high selectivity. For now, the molecule is simply known by its reference number K02288.

Eleanor and Georgina have also started investigating whether other partner proteins of ACVR1 might also be a drug target in FOP. For example, agonists of retinoic acid receptors are considered in treatment of skin and inflammatory diseases, but show potential to inhibit heterotopic bone formation. Thus, there could be drugs for other diseases that might work in FOP. However, preferably any drug should be suitable for use in children and not inhibit the bone formation in the normal skeleton.

**International effort.** Through the international consortium of the SGC, we're forming collaborations across the world. We are working closely with the Harvard zebrafish team who discovered dorsomorphin. Together we hope to improve on dorsomorphin as well as K02288 by making further chemical derivatives with greater potency and specificity for ACVR1. Each year at various scientific symposia we're also pleased to meet with Eileen Shore and Fred Kaplan and to discuss the latest research developments in FOP. It is important to stress how complementary our approaches are. Our strides towards drug discovery must be mirrored by deeper understanding of the biology of bone formation and FOP. This is critical to tailor strategies for long term treatments which we hope will reduce the symptoms of FOP without causing harmful side effects. We've also started collaborations in Oxford, Cambridge, London and Berlin.

**Public Meetings.** In March this year, Alex and the SGC organized a scientific workshop in Oxford to discuss the science behind a whole host of diseases relating to ACVR1 family members as well as the challenges for drug discovery. This brought together scientists from



seven different countries, including six drug companies and seven university institutions. We were also delighted that Richard Simcox was able to join us and learn more about our research firsthand. Richard tells us he is also keen to arrange quite soon another meeting of FOP Action, where perhaps we can have a similar opportunity to chat more about our work and discuss further ways in which you can contribute to FOP discoveries. In the meantime we wish everyone a safe and pleasant Christmas and look forward to more breakthroughs in the New Year. Once again, we are deeply thankful for all your generosity and financial support. Without your help work on FOP in the UK would not be possible and we are all striving hard to translate this into better clinical care.

Sincerely

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