Structure of the Bone Morphogenetic Protein Receptor ALK2 and Implications for Fibrodysplasia Ossificans Progressiva*

Apirat Chaikuad1, Ivan Alfano2, Georgina Kerr2, Caroline E. Sanvitale3, Jan H. Boergermann5, James T. Triffitt6, Frank von Delft3, Stefan Knapp1, Petra Knaus3, and Alex N. Bullock1

Received for publication, March 23, 2012, and in revised form, August 28, 2012. Published, JBC Papers in Press, September 12, 2012, DOI 10.1074/jbc.M112.365932

From the 1Structural Genomics Consortium, University of Oxford, Oxford OX3 7DQ, United Kingdom, the 2Institute for Chemistry/Biochemistry, Freie Universität Berlin, Berlin 14195, Germany, and the 5Botnar Research Centre, University of Oxford, Oxford OX3 7LD, United Kingdom

Background: Mutations in the ALK2 kinase cause extraskeletal bone formation.
Results: We solved the structure of ALK2 in complex with the inhibitor FKBP12.
Conclusion: Disease mutations break critical interactions that stabilize the inactive ALK2-FKBP12 complex leading to kinase activation.
Significance: We offer an explanation for the effects of mutation and a structural template for the design of small molecule inhibitors.

Bone morphogenetic protein (BMP) receptor kinases are tightly regulated to control development and tissue homeostasis. Mutant receptor kinase domains escape regulation leading to severely degenerative diseases and represent an important therapeutic target. Fibrodysplasia ossificans progressiva (FOP) is a rare but devastating disorder of extraskeletal bone formation. BMP-associated mutations in the BMP receptor ALK2 reduce binding of the inhibitor FKBP12 and promote leaky signaling in the absence of ligand. To establish structural mechanisms of receptor regulation and to address the effects of FOP mutation, we determined the crystal structure of the cytoplasmic domain of ALK2 in complex with the inhibitors FKBP12 and dorsomorphin. FOP mutations break critical interactions that stabilize the inactive state of the kinase, thereby facilitating structural rearrangements that diminish FKBP12 binding and promote the correct positioning of the glycine-serine-rich loop and αC helix for kinase activation. The balance of these effects accounts for the comparable activity of R206H and L196P. Kinase activation in the clinically benign mutant L196P is far weaker than R206H but yields equivalent signals due to the stronger interaction of FKBP12 with R206H. The presented ALK2 structure offers a valuable template for the further design of specific inhibitors of BMP signaling.

Bone morphogenetic proteins (BMPs)3 are members of the transforming growth factor-β (TGF-β) superfamily determin-

3 The abbreviations used are: BMP, bone morphogenetic protein; FOP, fibrodysplasia ossificans progressiva; GS, glycine-serine-rich; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-[hydroxymethyl]propane-1,3-diol; BRE, BMP-response element.

This work was supported in part by a Roemex postdoctoral fellowship (to G. K.), the University of Oxford FOP Research Fund (to C. E. S.), and German Research Foundation Collaborative Research Centre Grant SFB 760 (to F. K.).

1 These authors contributed equally to this work.

2 To whom correspondence should be addressed. Tel: 44 1865 617754; Fax: 44 1865 617755; E-mail: alex.bullock@sgc.ox.ac.uk.

3 The abbreviations used are: BMP, bone morphogenetic protein; FOP, fibrodysplasia ossificans progressiva; GS, glycine-serine-rich; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-[hydroxymethyl]propane-1,3-diol; BRE, BMP-response element.

4 The atomic coordinates and structure factors (code 3H9R) have been deposited in the Protein Data Bank (http://wwpdb.org/).

5 These authors contributed equally to this work.

6 This article contains supplemental Fig. S1.

© 2012 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.

Oxford OX3 7LD, United Kingdom
ossification, as well as the extent of skeletal malformation (15–20).

The FOP condition is recapitulated in animal models by transgenic overexpression of BMP4 (21) or caALK2 (22), a classic constitutively active ALK2 receptor containing the artificial mutation Q207D (23). Both proteins can induce osteogenic differentiation in mesenchymal progenitor cells, potentially explaining the origin of heterotopic ossification in FOP (24). Analyses of a subset of ALK2 FOP mutants, including L196P, R206H, and G356D, suggest that FOP mutations are more weakly activating than caALK2 but show similar potential to induce osteogenic differentiation through reduced FKBP12 binding and increased Smad1/5/8 phosphorylation (25–30).

The recent discovery of dorsomorphin, the first chemical inhibitor of BMP receptor signaling, provides hope for future FOP therapies (31). Indeed, the more potent derivative LDN-193189 reduced the effects of FOP in a mouse model expressing FOP therapies (31). Indeed, the more potent derivative LDN-193189 reduced the effects of FOP in a mouse model expressing FOP mutations affecting both the GS and kinase domains to compare their relative activation and sensitivity to FKBP12. To further understand the molecular basis for these effects, we determined the crystal structure of ALK2 in complex with FKBP12 and dorsomorphin. All FOP mutations are predicted to destabilize the inactive receptor conformation promoting a shift toward kinase activation.

**EXPERIMENTAL PROCEDURES**

*Expression Vectors*—Constructs were prepared by ligation-independent cloning. Full-length human FKBP12 (Uniprot ID, P62942) was cloned into pNIC28-Bsa4 for bacterial expression. The GS and kinase domains of ALK2 (residues 172–499; Uniprot ID, Q04771) were cloned into pETLIC-Bse for baculoviral expression. Full-length human ALK2 was cloned into pcDNA3.1, and mutations were engineered using the QuikChange site-directed mutagenesis kit (Stratagene). HA-pcDNA3.1, and mutations were engineered using the QuikChange site-directed mutagenesis kit (Stratagene). HA-FKBP12 plasmid was a gift of C. R. Bertozzi (Addgene plasmid 20220). All constructs were verified by sequencing.

*Luciferase Reporter Gene Assay*—C2C12 cells were co-transfected using Lipofectamine 2000 with BRE-luciferase (51), Renilla luciferase pRLTK (Promega), and the indicated ALK2 constructs, following the manufacturer’s instructions. 16 h after transfection, cells were starved for 7 h in DMEM containing 1% FCS. Cells were then incubated overnight untreated or treated with 1 μM FK506 or 10 ng/ml BMP4 or 50 ng/ml BMP6 before lysis. Luciferase activities were determined according to the Dual-Luciferase® reporter assay system (Promega) using Renilla for normalization of transfection efficiency. Results are the means ± S.E. of at least three independent experiments, each performed in triplicate. Statistical analyses for determination of p values used the Student’s t test. p < 0.05 was considered significant.

*Immunoprecipitation*—C2C12 or HEK293 cells were transfected with FLAG-tagged ALK2 and HA-tagged FKBP12 by FuGENE (Promega), following the manufacturer’s protocol. The following day, cells were lysed for 1 h at 4 °C in buffer containing 150 mM NaCl, 20 mM Tris-Cl, pH 7.5, 0.1% Triton X-100, and protease inhibitors (Roche Applied Science). Lysates were clarified by centrifugation, and the protein concentration was measured using the BCA assay (Pierce). 1 μg of lysate was incubated with anti-HA-agarose beads (Sigma) for 2 h at 4 °C before the beads were washed thoroughly in lysis buffer and resuspended in 20 μl of SDS-PAGE loading dye. Samples were run on a 4–12% BisTris pre-cast gel (Criterion), transferred onto PVDF or nitrocellulose (GE Healthcare) and probed with the relevant antibody; anti-FLAG-HRP (Sigma), or anti-HA (12CA5, Roche Applied Science). Bands were detected by ECL (Pierce) and images acquired on a LAS-4000 image analyzer. Band intensities were quantified using the Kodak ID program.

*Protein Expression*—The FKBP12 plasmid was transformed into *Escherichia coli* strain BL21(DE3)R3-prARE2 for expression. Cultures in LB media were induced with 0.5 mM isopropyl 1-thio-β-D-galactopyranoside overnight at 18 °C, and the cells were harvested and lysed by ultrasonication. ALK2 was expressed in Sf9 insect cells grown at 27 °C. Some 48 h post-infection, cells were harvested and lysed using a C5 high pressure homogenizer (Emulsiflex). Both proteins were initially purified separately by nickel affinity chromatography. The ALK2-FKBP12 complex was prepared by size exclusion chromatography mixing an excess of FKBP12. The eluted complex was stored in 50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM DT. The hexahistidine tags of ALK2 and FKBP12 were cleaved using tobacco etch virus protease.

*Crystallization*—Crystallization was achieved at 4 °C using the sitting-drop vapor diffusion method. The ALK2-FKBP12 complex was preincubated with 1 mM dorsomorphin (Calbiochem) at a protein concentration of 10 mg/ml and crystallized using a precipitant containing 30% PEG3350, 0.25 M ammonium sulfate, and 0.1 M BisTris, pH 6.0. Viable crystals were obtained when the protein solution was mixed with the reservoir solution at 2:1 volume ratio. Crystals were cryoprotected with mother liquor plus 20% PEG400, prior to vitrification in liquid nitrogen.

*Data Collection*—Diffraction data were collected at the Diamond Light Source, station I02 using monochromatic radiation at wavelength 0.9050 Å.

*Phasing, Model Building, Refinement, and Validation*—Data were processed with MOSFLM (32) and subsequently scaled using the program SCALA from the CCP4 suite (33). Initial phases were obtained by molecular replacement using the program Phaser (34) and the structures of FKBP12 (Protein Data Bank code 1A7X) and ALK5 (Protein Data Bank code 1B6C) as search models. Density modification and NCS averaging were performed using the program DM (35), and the improved phases were used in automated model building with the program ARP/wARP (36) and Buccaneer (37). The resulting structure solution was refined using REFMAC5 from the CCP4 suite (38) and manually rebuilt with COOT (39). Appropriate TLS restrained refinement using the tls tensor files calculated from the program TLsMD (40) was applied at the final round of refinement. The complete structure was verified for geometric correctness with MolProbity (41). Data collection and refinement statistics are shown in Table 1.
RESULTS

FOP Mutants Show Gain of Function—To investigate the effects of FOP mutation (Fig. 1A), we tested the functional consequences of these receptors in a Smad-dependent luciferase reporter assay. For this, a BRE-luciferase construct was co-transfected with indicated receptor constructs in C2C12 cells. Signaling from the wild-type ALK2 receptor was observed only upon stimulation with BMP4 (Fig. 1B) or BMP6 (supplemental Fig. S1), whereas the activity of the artificial constitutively active mutant caALK2 was induced independently of added ligand (Fig. 1B). In comparison, the basal activity of FOP mutants was more moderate, demonstrating that their gain of function was weaker (Fig. 1B, white bars). Overall, BMP6 signaling was strongly enhanced by ALK2 transfection, consistent with its preferential signaling through this receptor (supplemental Fig. S1) (42, 43). In contrast, BMP4 signaling was lower in wild-type ALK2-transfected cells than the vector control (Fig. 1B). We speculate that there is some competitive interference of ALK2 with the preferred endogenous BMP4 receptors ALK3 (BMPR1A) and ALK6 (BMPR1B).
The most active FOP mutant contained a deletion in the GS domain, replacing Pro-197/Phe-198 with a single leucine residue that removes part of the FKBP12 interaction site. To assess whether the activity of other FOP mutants was generally restricted by endogenous FKBP12, we treated the cells with the FKBP12 ligand FK506 (also known as the immunosuppressant Tacrolimus). FK506 competes with the GS domain for binding to FKBP12 and thereby dissociates FKBP12 from receptors (44). Under these conditions, the signaling from the wild-type receptor was increased 2.5-fold (Fig. 1B, light gray bars) but remained relatively low, indicating that the mechanism of activation in FOP extends beyond the simple disruption of FKBP12 binding. In comparison, the activity of some FOP mutants was generally increased to a level similar to caALK2, revealing an inherent gain of function in their catalytic domain in the absence of added BMP ligand (Fig. 1B). Significantly, the signaling by R206H was increased by FK506 to a far greater extent than other GS domain mutants, such as L196P (Fig. 1B).

The different responses of L196P and R206H to FK506 may be explained by their different affinities for FKBP12. To test this hypothesis, we transiently co-transfected HA-tagged FKBP12 with wild type, L196P, and R206H ALK2 constructs into C2C12 cells and performed an HA immunoprecipitation. In agreement with their responses to FK506 in the luciferase reporter assay, wild-type ALK2 and the R206H mutant showed significant binding to FKBP12, whereas the interaction of L196P was essentially lost (Fig. 2A). All FKBP12 interactions were inhibited by FK506 (Fig. 2A). Similar results were obtained in HEK293 cells (Fig. 2B), although under less saturating conditions the FKBP12 binding of R206H was notably weaker than wild type. Together, the BRE-luciferase and immunoprecipitation data suggest that FKBP12 remains an important modulator of receptor function in the majority of FOP cases where mutations fall outside the core FKBP12-binding site.

**Overview of the ALK2-FKBP12 Structure**—To understand the structural consequences of FOP mutation, we co-purified the cytoplasmic region of ALK2 containing the GS and kinase domains (residues 172–499) with FKBP12 and determined the crystal structure of the complex at 2.35 Å resolution (Fig. 3A). Parts of the activation loop (A loop) and L45 loop were disordered and resulted in a subtle shift in the packing of the interface and the overall structures of the two complexes are highly conserved (Ca root mean square deviation of 1.5 Å).

The small molecule inhibitor dorsomorphin binds to the ATP-binding pocket with an ATP-mimetic binding mode, while oriented parallel to the hinge (Fig. 3, A and C). The core pyrazolo[1,5-a]pyrimidine ring forms a single hydrogen bond to the hinge amide of His-286. The substituent 4-pyridine ring is accommodated in a hydrophobic pocket formed by Val-222, Leu-263, Leu-281, and Ala-353 and forms a water-mediated hydrogen bond with Glu-248 (αC). On the other side of the inhibitor, the phenyl ring packs below the β strand with its plane stacking between Gly-289 and the side chain of Val-214. Further contact is made with the hinge residue Tyr-285, whereas the piperidinyl-ethoxy group is largely extended away into solvent.

**Structural Changes Determining Receptor Activation**—To understand the structural changes between type I receptors and constitutively active type II receptors, we compared the ALK2 structure with those of the type II activin (ActRIIB) (45) and BMP (BMPRII) receptors (Fig. 4A). Superposition shows that the structures are closely matched with the exception of structural elements linked to the activation state of the catalytic domain. The ALK2 structure displays an inhibited conformation of both the GS and kinase domains (Fig. 4A). The GS loop is protected from phosphorylation by coordination with Arg-258, which buries the GS loop in the kinase N-lobe between αC and β4 (Fig. 4B). As a result, the αC helix is swung away from the catalytic domain at its C terminus and inwards at its N terminus (Fig. 4A). This packing is stabilized by the A loop residue Arg-375 that forms inhibitory salt bridges with the catalytic HRD (Asp-336) and DFG (Asp-354) motifs as well as a hydrogen bond to Ser-244 (αC) (Fig. 4A).
Both aspartates are critically required in all kinases for Mg-ATP binding and catalysis. ATP binding to ALK2 is further inhibited by an inactive conformation of the phosphate-binding loop (P loop) residue Tyr-219 (Fig. 4C). Both aspartates are critically required in all kinases for Mg-ATP binding and catalysis. ATP binding to ALK2 is further inhibited by an inactive conformation of the phosphate-binding loop (P loop) residue Tyr-219 (Fig. 4C).

**FOP Mutations Destabilize the Inactive State**—The ALK2 structure offers molecular explanations for the individual FOP mutations. Overall, the mutations are tightly clustered around the GS domain and ATP pocket (Fig. 5A). As shown in Fig. 4, in the wild-type structure these residues make critical interactions that stabilize the inactive state. FOP mutations are predicted to break these inhibitory interactions promoting a shift toward the active state. The αGS2 helix forms a mutation hot spot and harbors the most frequent FOP mutation R206H alongside the mutations R202I and Q207E (Fig. 5A). All three residues bind the GS domain to the kinase N-lobe and form peripheral interactions that stabilize the inhibitory complex with FKBP12. Gln-207 forms hydrogen bonds with ALK2 Trp-227 (kinase domain) as well as Glu-55 in FKBP12 (Fig. 5B). In the Q207E FOP mutant, the juxtaposition of Glu-207 and Glu-55 would create electrostatic repulsion and weaken FKBP12 binding. Similar repulsion is expected in the caALK2 mutant Q207D. On the
other face of the αGS2 helix, Arg-202 and Arg-206 form hydrogen bonds with Asp-269 (kinase domain β4), and Arg-206 forms a further hydrogen bond with the backbone oxygen of Met-270 (Fig. 5B). FOP mutations at these positions would break these bonds and destabilize the GS domain interactions with FKBP12 as well as the kinase L45 loop (Fig. 5C).

At the opposite end of αGS2, a deletion in FOP replaces Pro-197/Phe-198 with a single leucine residue, removing part of the FKBP12 interaction shown in Fig. 3B. Together with L196P, R258S, and G328R/G328W/G328E, this forms a mutation cluster bordering the buried GS loop. The P197_F198delinsL substitution would dramatically destabilize the inhibitory packing shown in Fig. 4B. Leu-196 is packed against the kinase β4 strand in the hydrophobic core of the GS domain (Fig. 5C). Arg-258 forms three hydrogen bonds to the GS loop. Gly-328 is located inside the neighboring E6 loop where no other side chain can be accommodated (Fig. 5C). Mutations at all three sites break critical bonds, providing freedom for the GS loop to move away from the kinase N-lobe, promoting an active kinase conformation. The remaining mutations G356D (DFG motif) and R375P (A loop) destabilize the inhibitory A loop conformation described in Fig. 4C resulting again in activation of ALK2. Until recently, the glycine position of the DFG motif was considered essential and invariant, but larger residues at this position have recently been identified in active kinases, including Haspin, and are well tolerated (46). In fact, G356D was the most active mutant in the presence of FK506, demonstrating the significant additive effects of structural changes in both the kinase and GS domains.

**DISCUSSION**

Here, we used a large panel of ALK2 constructs to show that weak gain of function is a common and specific disease feature shared by all FOP mutants. This was evidenced by leaky activation in the absence of ligand as well as an enhanced BRE-luciferase response to BMP4 and BMP6. Overall, mild constitutive activation was higher for GS domain mutants than those in the kinase domain. We demonstrated that the low basal signaling of FOP mutants was partly restricted by endogenous FKBP12. Thus, the
full extent of mutant activation was only revealed upon displacement of FKBP12 with FK506. For the first time, this exposed the substantial gain of function inherent in many kinase domain mutants, including G356D.

The GS domain mutant L196P has raised particular interest for its association with a comparatively benign clinical course (18). In agreement with Ohte et al. (30), we found that the basal activity of L196P was surprisingly robust and similar to R206H. However, in contrast to R206H, the activity of L196P was not increased by FK506 and consequently fell below that of all other FOP mutants under this condition. The lower limit for constitutive L196P signaling in the absence of FKBP12 may offer one...
explained for a delayed onset of disease. In support of these data, we observed some binding of FKBP12 to R206H, whereas there was no binding to L196P. These data are consistent and complementary to previous BRE-luciferase assays using FKBP12 overexpression (28–30) as well as in vitro binding data (47).

FOP mutations have the potential to act through multiple (and overlapping) mechanisms, including changes to the kinase domain activation state and changes to the GS domain that diminish inhibition by FKBP12. A key observation from our study is that wild-type ALK2 signaling remains low even upon FK506 treatment. This suggests that FOP does not result from FKBP12 disruption alone. In agreement, Song et al. (28) showed that FK506 induced alkaline phosphatase staining in C2C12 cells stably expressing R206H but not wild-type ALK2. Together, these data support our assertion that FOP mutations act also to induce changes in the activation state of the catalytic domain. The observed level of constitutive activation is then modulated further by differences in FKBP12 affinity.

To understand the molecular basis for these effects, we solved the structure of the ALK2-FKBP12 complex. As observed for ALK5 (8, 9), FKBP12 functions not only to block access to the regulatory GS loop but also inhibits αC movements required for kinase activation. Our data show that the inactive conformation remains relatively stable in wild-type ALK2 even in the absence of FKBP12. Residues associated with FOP map exclusively to sites of structural change and form critical interactions to stabilize this inactive state. FOP mutations thus break bonds in the inhibited receptor that promote activation. The inactive conformation is therefore unstable in FOP mutant kinase domains, facilitating release of FKBP12 and activation of Smad (Fig. 6). This model agrees with earlier predictions for loss of autoinhibition (16) and offers a common mechanism to explain the action of all FOP mutations. The structure of the ternary ALK2-Smad complex remains to be determined and will be an important step to further rationalize the subtle differences between the artificial caALK2 mutant Q207D and the weaker FOP mutants such as Q207E.

In this study we define the structural changes between active and inactive BMP receptors and rationalize how this transition is compromised by FOP mutation. The structure of the ALK2-dorsomorphin complex also provides a new model for structure-based lead optimization of BMP inhibitors. Such inhibitors have many applications in cell signaling and stem cell biology and are proposed as potential therapeutics for heterotopic ossification as well as anemia of inflammation (48).

**Acknowledgments**—We thank Kirsten Petrie and Binesh Shrestra for help with ALK2 expression and staff at the Diamond Light Source for help with diffraction data collection. The Structural Genomics Consortium is a registered charity (number 1097737) that receives funds from the Canadian Institutes for Health Research, Genome Canada, GlaxoSmithKline, Lilly Canada, the Novartis Research Foundation, Pfizer, Takeda, AbbVie, the Canada Foundation for Innovation, the Ontario Ministry of Economic Development and Innovation, and the Wellcome Trust Grant 092809/Z/10/Z.

**REFERENCES**

1. Shi, Y., and Massagué, J. (2003) Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell 113, 685–700
2. Kitisin, K., Saha, T., Blake, T., Golestaneh, N., Deng, M., Kim, C., Tang, Y., Shetty, K., Mishra, B., and Mishra, L. (2007) TGF-β signaling in development. Sci. STKE 2007, cm1
3. Wu, M. Y., and Hill, C. S. (2009) TGF-β superfamily signaling in embryonic development and homeostasis. Dev. Cell 16, 329–343
4. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massagué, J. (1994) Mechanism of activation of the TGF-β receptor. Nature 370, 341–347
5. Heldin, C. H., Miyazono, K., and ten Dijke, P. (1997) TGF-β signaling from cell membrane to nucleus through SMAD proteins. Nature 390, 465–471
6. Derynck, R., and Zhang, Y. E. (2003) Smad-dependent and Smad-independent pathways in TGF-β family signaling. Nature 425, 577–584
7. Wang, T., Li, B. Y., Danielson, P. D., Shah, P. C., Rockwell, S., Lechleider, R. J., Martin, J., Manganaro, T., and Donahoe, P. K. (1996) The immunophilin FKBP12 functions as a common inhibitor of the TGF-β family type 1 receptors. Cell 86, 435–444
8. Huse, M., Chen, Y. G., Massagué, J., and Kuriyan, J. (1999) Crystal structure of the cytoplasmic domain of the type I TGF-β receptor in complex with FKBP12. Cell 96, 425–436
9. Huse, M., Muir, T. W., Xu, L., Chen, Y. G., Kuriyan, J., and Massagué, J. (2001) The TGF-β receptor activation process. An inhibitor–substrate-binding switch. Mol. Cell 8, 671–682
10. Qin, B. Y., Chacko, B. M., Lam, S. S., de Caestecker, M. P., Correia, I. J., and Lin, K. (2001) Structural basis of Smad1 activation by receptor kinase phosphorylation. Mol. Cell 8, 1303–1312
11. Wu, J. W., Hu, M., Chai, J., Seoane, J., Huse, M., Li, C., Rigotti, D. J., Kyin, S., Muir, T. W., Fairman, R., Massagué, J., and Shi, Y. (2001) Crystal structure of a phosphorylated Smad2. Recognition of phosphoserine by the MH2 domain and insights on Smad function in TGF-β signaling. Mol. Cell 8, 1277–1289
12. Kaplan, F. S., Fiovi, I., de la Peña, L. S., Ahn, J., Billings, P. C., and Shore, E. M. (2006) Dysregulation of the BMP-4 signaling pathway in fibrodysplasia ossificans progressiva. Ann. N.Y. Acad. Sci. 1068, 54–65
13. Shore, E. M., and Kaplan, F. S. (2010) Inherited human diseases of heterotopic bone formation. Nat. Rev. Rheumatol. 6, 518–527
14. Shore, E. M., Xu, M., Feldman, G. J., Fenstermacher, D. A., Cho, T. I., Choi, I. H., Connor, J. M., Delai, P., Glaser, D. L., LeMerrer, M., Morhart, R., Rogers, J. G., Smith, R., Triffitt, J. T., Urtizberea, J. A., Zasloff, M., Brown, M. A., and Kaplan, F. S. (2006) A recurrent mutation in the BMP type 1 receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. Nat. Genet. 38, 525–527
15. Furuya, H., Ikezoe, K., Wang, L., Ohyagi, Y., Motomura, K., Fujii, N., Kira,
Structural Insights into ALK2 Activation in FOP

J. and Fukumaki, Y. (2008) A unique case of fibrodysplasia ossificans progressiva with an ACVR1 mutation. G356D, other than the common mutation (R206H). Am. J. Med. Genet. 146, 459 – 463

16. Kaplan, F. S., Xu, M., Seemann, P., Connor, J. M., Glaser, D. L., Carroll, L., Delai, P., Fastnacht-Urban, E., Forman, S. I., Gillessen-Kaesbach, G., Hoo-ver-Fong, J., Köster, 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

17. Petrie, K. A., Lee, W. H., Bullock, A. N., Pointon, J. J., Smith, R., Russell, R. G., Brown, M. A., Wordsworth, B. P., and Triffitt, J. T. (2009) Novel mutations in ACVR1 result in atypical features in two fibrodysplasia ossificans progressiva patients. PloS ONE 4, e5005

18. Gregson, C. L., Hollingworth, P., Williams, M., Petrie, K. A., Bullock, A. N., Brown, M. A., Tobias, J. H., and Triffitt, J. T. (2011) 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. Bone 48, 654 – 658

19. Bocciardi, R., Bordo, D., Di Duca, M., Di Rocco, M., and Ravazzolo, R. (2009) Mutational analysis of the ACVR1 gene in Italian patients affected with fibrodysplasia ossificans progressiva. Conclusions and affirmations. Eur. J. Hum. Genet. 17, 311 – 318

20. Whyte, M. P., Wenkert, D., Demertzis, J. L., DiCarlo, E. F., Westenberg, E., and Kaplan, F. S., Xu, M., Seemann, P., Connor, J. M., Glaser, D. L., Carroll, L., Delai, P., Fastnacht-Urban, E., Forman, S. I., Gillessen-Kaesbach, G., Hoover-Fong, J., Köster, 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