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Fibrodysplasia Ossificans Progressiva (FOP): A Disorder of Osteochondrogenesis

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Abstract

Fibrodysplasia Ossificans Progressiva (FOP) is an ultra-rare genetic disorder of extraskeletal bone formation, but could appropriately be viewed as a seminal disorder of osteochondrogenesis. Many, if not most, of the musculoskeletal features of FOP are related to dysregulated chondrogenesis including abnormal articular cartilage formation, abnormal diarthrodial joint specification, growth plate dysplasia, osteochondroma formation, heterotopic endochondral ossification (HEO), and precocious arthropathy. In FOP, causative activating mutations of Activin receptor A type I (ACVR1), a bone morphogenetic protein (BMP) type I receptor, are responsible for the osteochondrodysplasia that impacts developmental phenotypes as well as postnatal features of this illustrative disorder. Here, we highlight the myriad developmental and postnatal effects on osteochondrogenesis that emanate directly from mutant ACVR1 and dysregulated bone morphogenetic protein (BMP) signaling in FOP.

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Keywords

Fibrodysplasia ossificans progressiva; bone morphogenetic protein signaling pathway; ACVR1; heterotopic ossification; osteochondrodysplasia; osteochondrogenesis; osteochondroma; arthropathy; growth plate

1. INTRODUCTION

The first verifiable description of fibrodysplasia ossificans progressiva (FOP; MIM 135100) was published in the *Philosophical Transaction of the Royal Society of London* by John Freke in 1740. One hundred twenty-eight years later, in 1868, the name myositis ossificans progressiva was ascribed to the condition by von Dusch in recognition of the episodic inflammatory flare-ups involving skeletal muscle. During the following decade, the malformation and microdactyly of the great toes were recognized by Frankel and Helferich. For nearly a century, the condition was commonly known as myositis ossificans progressiva (MOP). The name Fibrodysplasia Ossificans Progressiva (FOP) was proposed by Bauer & Bode in 1940 and adopted by McKusick in 1960 in recognition of the primary connective tissue involvement of tendons, ligaments, fascia and aponeuroses [1].

The discovery in 2006 of activating mutations in Activin receptor A type I (ACVR1), a bone morphogenetic protein (BMP) type I receptor, as the proximate genetic cause of FOP in all affected individuals ushered in a myriad of scientific discoveries that are leading to an understanding of the molecular basis of FOP as well as the development of biologically and genetically faithful animal models and the rapid expansion of genotype-phenotype insight into the condition [2]. A more robust understanding of the molecular basis of the developmental and post-natal phenotypes of FOP has highlighted the role of disordered osteochondrogenesis in the temporal and spatial progression of the disease and in evaluating the veracity of relevant molecular targets for clinical trials.

Our understanding of this complex and illustrative disorder continues to evolve as genotypic insight and phenotypic characterization converge around the seminal molecular defect of the condition - activating mutations of ACVR1 receptor - and the resulting downstream dysregulated BMP signaling pathway. This brief review focuses on the concept that the musculoskeletal phenotypes of FOP represent a primary disorder of osteochondrogenesis.

2. Mutant ACVR1 & the Dysregulated BMP Pathway

Most FOP patients have the same mutation in *ACVR1* (c.617G>A; R206H) and a characteristic clinical presentation (described as ‘classic’ FOP). Phenotypic and genotypic variants of FOP patients and the *ACVR1* gene occur in ~3% of all FOP patients worldwide. Classic and variant forms of ACVR1 in FOP all exhibit loss of normally-regulated signaling activity of ACVR1 and ligand-independent and ligand-stimulated BMP signaling activity [3, 8].

All of the ACVR1 mutations identified in individuals with classic or variant FOP occur in highly evolutionarily conserved amino acids, indicating their functional importance. Many

studies have supported that signal transduction through the BMP pathway is altered in cells from individuals with FOP, with decreased expression of BMP antagonists, increased phosphorylation of BMP pathway signaling mediators (BMP-specific SMAD proteins and p38MAPK), dysregulated trafficking of BMP receptors, and increased expression of BMP transcriptional targets in the absence of exogenous BMP ligand [3,4–11].

FOP is caused by heterozygous activating mutations of ACVR1 [generally referred to as muACVR1 in this review], a bone morphogenetic protein (BMP) type I receptor [2, 3]. Patients who have classic FOP have the identical single nucleotide mutation in ACVR1 (c.617G>A), which results in the substitution of histidine for arginine at codon 206 (p.R206H) in the intracellular GS domain of the receptor. Protein structural homology modeling predicts that this amino acid substitution results in a conformational change in the receptor, which alters its sensitivity and activity, leading to a loss of usual regulatory constraints. This loss causes mild basal activation in the absence of ligand as well as ligand-dependent hyperactivity of the downstream BMP signaling pathway [4–11].

The interaction of the GS domain of muACVR1 with FKBP12, a glycine/serine domain-binding protein that has been shown to prevent leaky BMP type I receptor activation in the absence of ligand has been investigated [6, 9, 10]. Mutant ACVR1 exhibits reduced binding to FKBP12 in *in vitro* assays, suggesting that increased BMP pathway activity in cells with muACVR1 may be due, at least in part, to decreased binding of this inhibitory factor [9–12].

Despite the loss of regulation of muACVR1 in FOP, the mutant receptor is responsive and highly sensitive to BMP family ligands. Recent studies highlighted the differential response to ligands by wild type (wt) ACVR1 and muACVR1 and showed that muACVR1 was significantly more sensitive than wtACVR1 to BMP4-induction or Activin A-induction of the BMP-Smad1/5 pathway [13–16]. The basis for the increased sensitivity of the mutant receptor kinase activity to the BMPs and other canonical or non-canonical ligands is an area of intense study.

The development of *Acvr1* knock-in mouse (*Acvr1*^{R206H/+}) models has propelled *in vivo* research on FOP [14, 17–19]. Although germline transmission of *muAcvr1* in the mouse leads to perinatal lethality, mice with 70–90% *muAcvr1* cells exhibit clinical features of FOP including embryonic skeletal malformations and postnatal heterotopic ossification, recapitulating these characteristic features of the human condition [18]. Importantly, knock-in *muAcvr1* mice also develop spontaneous and injury-induced lesions that progress to mature heterotopic bone through a cellular cascade of events exactly like the progression identified in humans with FOP [17]. These mouse models validate that *muACVR1* is a direct genetic cause of FOP and that mild basal signaling activity and increased ligand sensitivity of muACVR1 reproduce the heterotopic endochondral ossification (HEO) of the human disease [14, 17–19].

ACVR1^{R206H} has been shown to regulate early chondrogenic fate in FOP [20]. Importantly, chondrogenic differentiation was accelerated and Sox9 expression increased in *muACVR1* cells due, at least in part, to enhanced sensitivity to low levels of BMP ligand [20]. BMP4 has been detected in patient lesions prior to the appearance of chondrocytes [21], suggesting

that the mutation, together with endogenous BMPs and perhaps other non-canonical ligands, may direct lineage decisions towards cartilage, a critical scaffold of HEO in FOP.

Two studies reported an unexpected role for Activin A (Act A) as a non-canonical ligand in the pathogenesis of HEO in FOP [13, 14]. Hino and colleagues [13] showed that Act A, a member of the TGF β /BMP family of ligands, enhanced chondrogenesis in muACVR1 induced pluripotent stem cells (FOP-iMSCs) and aberrantly activated BMP pathway signaling (pSmad1/5) in addition to TGF β pathway signaling (pSmad2/3); further, HEO was induced *in vivo* by implanted FOP-iMSCs. In the second study [14], investigators showed that inhibition of Act A with a fully humanized monoclonal antibody completely blocked formation of HEO *in vivo* in a conditional knock-in *Acvr1*^{R206H/+} model of FOP. Thus, muACVR1 appears to sensitize FOP cells not only to BMP ligands but also to Act A in promoting HEO [14]. Clinical trials using an anti-Activin A antibody are currently underway (<http://www.clinicaltrials.gov>). The molecular, physiologic, and structural basis for the increased sensitivity of muACVR1 to Act A is presently unknown but may involve an altered role of type II receptors in the signaling complex.

3. Developmental Phenotypes of Dysregulated Osteochondrogenesis

Dysregulated osteochondrogenesis is at the center of the musculoskeletal phenotype of FOP which can logically be seen through the lens of muACVR1 and the resulting dysregulated BMP signaling pathway. In this section, we will examine the developmental phenotypes of dysregulated osteochondrogenesis in FOP. These include: abnormal articular cartilage formation, abnormal diarthrodial joint formation, precocious arthropathy, dysplastic growth plates, and osteochondroma formation. These topics are categorized below under joints, growth plates and osteochondromas.

3.1 Joints

ACVR1 is widely expressed in most tissues, and increased signaling activity by muACVR1 is expected to impact the development and function of these tissues as well as its more readily noted clinical impact of heterotopic ossification in FOP. The clearest example of this can be seen in characteristic FOP-associated skeletal malformations, most notably of the great toes [8, 22, 23].

To investigate the role of muACVR1 in malformation of the joints, a genetic mouse model of FOP was used in which the mutation was specifically expressed in cells of the developing skeletal elements of the limbs [24]. These mice not only spontaneously form HEO after birth, as occurs in human FOP, but also recapitulate the characteristic FOP great toe malformation and provide an *in vivo* model to investigate the activity of ACVR1 and the downstream BMP pathway signaling in skeletal development.

While BMPs are needed to form skeletal elements, down-regulation of the BMP signaling pathway in joint progenitor cells is critical for the initiation and progression of joint formation. Thus, while BMPs are necessary for the formation of skeletal bones, they are inhibitory for the formation of the joints. Ongoing studies in mouse models suggest that muACVR1 in FOP leads to delayed and disrupted joint formation in the digits and altered

development of cartilage and bone at sites of joint formation. These findings support a critical role for ACVR1-mediated BMP signaling in the regulation of joint formation and show a direct effect of failure to restrict BMP signaling at sites of joint formation. These studies further suggested that the joint malformations in patients are not restricted to the great toe and led to investigation of the skeleton in a large cohort of FOP patients [22]. This study identified widespread joint malformations, severe degenerative joint disease, and intra-articular joint fusion in a high percentage of FOP patients at multiple sites of the axial and appendicular skeleton [22].

Baseline whole body computed tomographic (CT) scans of 113 individuals with classic clinical features of FOP and the classic ACVR1 (R206H) mutation who were enrolled in a non-interventional natural history study were analyzed for skeletal malformations, atypical bone and joint structure, intra-articular synovial osteochondromatosis, developmental joint disease, and associated degenerative joint disease. Individuals were evaluated in three age groups: 4–13, 14–25, and 25–56 years old, based on historical models of FOP disease progression [22].

Widespread evidence of developmental joint disease was found throughout the FOP skeleton in all age groups, with no evidence of sex as an influencing factor (61M, 52F). Asymmetric narrowing and subchondral sclerosis, which are classic findings of degenerative arthritis, were present throughout the joints of the axial and appendicular skeleton of individuals who have FOP in all age groups. Bone spurs were additionally common in the hips and knees. Further, these degenerative changes were observed at extremely early ages, particularly in the spine, sacro-iliac joints, and lower limbs. The costovertebral joints, intervertebral facet joints, and proximal tibio-fibular joints frequently showed partial or total intra-articular fusion, particularly after age 13. The hip joints of individuals with FOP were frequently malformed. Intra-articular synovial osteochondromas were noted in the appendicular joints (especially in the hips and knees) of 10% of individuals 14 years of age and older [22].

Although the mechanism of the intra-articular fusions is undetermined, the specific sites and modes of joint fusion are nonetheless enlightening. Costovertebral joints were fused in almost half of all subjects, which may lead to rapidly progressive scoliosis, restrictive chest wall disease, and restricted breathing independent of HEO. In some subjects, facet joints of the thoracic and lumbar vertebra were fully fused, and the pattern of joint fusion suggested possible ossification of the associated tendons and ligaments. Upper limbs were rarely affected, suggesting load-bearing may have an impact on the progression of joint degeneration, but the knees were also less affected, suggesting the specific architecture of joints may also be a contributing factor [22, 25].

The study in FOP patients not only validates widespread structural changes at sites of joint formation, but also confirms that signs of degenerative joint disease become more frequent with age in the FOP population at much higher rates than the general population, supporting that both inhibited joint formation and dramatically decreased joint health in adulthood occur in FOP.

Thus, the effects of muACVR1 on the joints of individuals who have FOP extend far beyond malformation of the great toes and include both structural skeletal defects and developmental joint disease. Associated degenerative joint disease occurring at multiple sites starts in adolescence and progresses throughout life. These findings appear to be uncoupled from HEO, indicating a role for ACVR1 in the development and progression of degenerative joint disease independent of HEO. Thus, FOP is a disease of not only progressive HEO, but also widespread and extensive developmental joint disease and associated degenerative joint disease [22].

Several drugs are in current development for the treatment of heterotopic ossification in FOP [26]. While these drugs are mainly evaluated for efficacy in preventing or reducing the extra-skeletal bone formation, it will be of great interest to determine whether these treatments would have additional protective benefits in preventing or reducing degenerative joint disease in FOP patients over time, or whether the observed progressive joint disease has been pre-determined through aberrant joint formation during embryonic development. Likely, additional benefits to FOP patients from these drugs will depend on the direct drug target and mechanism of action.

3.2 Growth Plate Dysplasia

Clinical observations have suggested that disproportionate skeletal growth occurs in some FOP patients. An *in vivo* study was undertaken in a FOP mouse model in which expression of *muACVR1* was limited to a population of skeletal progenitor cells (*Prrx1*⁺) in order to investigate effects on growth plates and local skeletal long bone development. Investigators discovered that these mice mimicked skeletal defects found in FOP patients, including skeletal growth retardation, which likely resulted from muACVR1 and enhanced BMP signaling on growth plate physiology [24]. Of interest was that some long bones appeared to be more affected than others, suggesting differences in ACVR1 expression and/or ACVR1 responsiveness in various skeletal elements.

ACVR1 appears to play a seminal role in early steps of chondrocyte differentiation and commitment [20, 27]. ACVR1 was recently shown to be required for chondrogenesis during development and expressed in growth plates, with its conditional ablation in cartilage causing growth plate dysfunction and reduced chondrocyte proliferation [28]. *Acvr1*^{R206H/+} growth plates showed expansion of proliferating chondrocytes, with failure or significant delay in differentiation to hypertrophic chondrocytes [24]. Growth plates of *Acvr1*^{R206H} knock-in mice at day 14 were examined for several markers of chondrogenesis [24]. In addition to increased BMP signaling (pSmad1/5/8) and Sox9 expression, high levels of PTHrP and collagen II were detected throughout the mutant growth plates and the region of PTHrP expression was expanded. The overall effects of enhanced signaling through ACVR1 led to compromised bone growth, supporting the hypothesis that the appropriate magnitude and distribution of BMP receptor signaling are critical for growth plate functioning and normal bone growth.

3.3 Osteochondromas

Osteochondromas are associated with dysregulated BMP signaling and are a common phenotypic feature of FOP [29, 30]. Ninety percent of all patients with FOP have been diagnosed with an osteochondroma of the proximal tibia [29]. FOP-associated osteochondromas usually were asymptomatic and were most commonly bilateral. Seventy-five percent were pedunculated, and 25% were sessile. The common occurrence of osteochondromas in FOP expands the recognized consequences of *muACVR1* to include not only congenital skeletal malformations and HEO, but also orthotopic lesions of skeletal modeling [29, 30].

Hereditary multiple exostosis (HME) is a rare disorder characterized by extensive formation of osteochondromas throughout the skeleton in response to heterozygous loss-of-function mutations in the *EXT1* or *EXT2* genes which regulate the synthesis of heparan sulfate (HS), a component of cell matrix-associate proteoglycans (reviewed in [31]). One function of HS is to regulate the activities of signaling proteins including BMPs, hedgehogs, and fibroblast growth factors (FGFs). Extensive data support that decreased HS, as occurs in MHE, increases BMP pathway signaling and that inhibition of BMP pathway signaling prevents osteochondroma formation. Other signaling pathways have important roles in regulating chondrogenesis, including decreased signaling by FGFs and increased hedgehog signaling, both of which occur in MHE perichondrial cells from which osteochondromas arise [31, 32]. Constitutively active *ACVR1* mutations have also been associated with increased hedgehog signaling in the perichondrium [27]. Although induced by different primary causes, in both FOP and MHE, cellular mechanisms that normally restrict chondrogenic cell differentiation are perturbed, with elevated BMP pathway signaling as a common mechanism that appears to stimulate a network of signaling pathways regulating chondrogenesis and, in FOP and MHE osteochondroma formation.

4. Predominant Postnatal Phenotype of Dysregulated Osteochondrogenesis - HEO

The main histopathologic phenotype of FOP, and a signature of this condition, is progressive post-natal HEO [33]. During the first decade of life, episodic soft tissue swellings (or flare-ups) arise in the neck and back. While some flare-ups regress spontaneously, most undergo pathological metamorphosis into mature heterotopic bone through an endochondral pathway [8, 23, 33]. Minor trauma such as intramuscular immunizations, mandibular blocks for dental work, muscle fatigue, blunt muscle trauma including through bumps, bruises, and falls, or influenza-like viral illnesses can trigger new flare-ups of FOP leading to progressive HEO [8, 23, 34, 35]. FOP lesion formation is associated with inflammatory triggers that suggest participation of the innate immune system in the pathophysiology of the disease [17, 36–38].

In a recent study, investigators used murine mesenchymal progenitor cells to investigate the contribution of *Acvr1* during chondrogenic differentiation and HEO [20]. *Acvr1*^{R206H/+} (gain-of-function), *Acvr1* knock-out (loss-of-function), and wild-type *Acvr1*^{+/+} mouse embryonic fibroblasts were evaluated for chondrogenic potential *in vitro*. Chondrogenic

differentiation was accelerated in *Acvr1^{R206H/+}* cells, due in part to enhanced sensitivity to BMP ligand. *In vivo*, implanted *Acvr1^{R206H/+}* cells initiated robust HEO and recruited wild-type cell contribution. Despite expression of other type I BMP receptors (Alk3/BMPRI1A and Alk6/BMPRI1B), chondrogenesis of *Acvr1* loss-of-function cells was severely impaired by absence of *Acvr1* during early differentiation stages. ACVR1 is therefore a direct regulator of cartilage formation and mediates chondrogenic commitment of progenitor cells. These data establish that at least one effect of *ACVR1* activating mutations in FOP is enhanced chondrogenic differentiation that supports formation of HEO. This finding established ACVR1 itself as a plausible therapeutic target during early chondrogenic stages of lesion formation in preventing HEO in FOP [20].

As an obligatory step in endochondral ossification [39], chondrogenesis is an appealing target for inhibiting HEO in FOP. Suppression of the retinoid signaling pathway is required to promote chondrogenesis [40, 41], and the first drug to enter clinical trials for FOP targeted chondrogenic cell differentiation by activating RAR γ receptors with the agonist palovarotene (www.clinicaltrials.gov). An important consideration is that inhibiting chondrogenesis and endochondral ossification as a strategy to prevent HEO has the added risk of blocking endochondral ossification in growth plates, with the potential for premature growth plate closure and growth retardation in children [24, 42]. Palovarotene treatment of *Acvr1^{R206H}* knock-in mice, which have impaired long bone growth due to the mutation, showed a partial restoration of growth plate function [24] suggesting interactions between BMP and RAR γ pathway signaling in chondrogenesis. However, achieving a balance between these pathways to prevent growth plate closure during palovarotene treatment is likely to be difficult, making this approach potentially problematic for children.

Two recent studies demonstrate that biomechanical signals from the FOP tissue microenvironment and subsequent responses of FOP cells to these physical cues, such as tissue stiffness, are important determinants of cell differentiation and are modulated by BMP signaling [43, 44]. An FOP mouse model of injury-induced HEO was used to examine the lesional fibroproliferative tissue that occurs prior to heterotopic ossification. Investigators unexpectedly identified pathologic stiffening of the tissue at the preosseous phase of the FOP lesion. Assays showed that cells with the *Acvr1^{R206H}* FOP mutation inappropriately sense their environment, responding to soft substrates similarly to wild-type cells on stiff substrates, and identical to cells undergoing cartilage and bone formation. *Acvr1^{R206H}* stimulated increased activation of RhoA, a component of a biomechanical signaling pathway, and increased mechanosignaling in the FOP cells [43, 44]. Biomechanical signaling through the YAP/TAZ pathway is also activated by *Acvr1^{R206H}* [44]. The data support that increased BMP signaling in FOP cells alters the tissue microenvironment in a fundamental way that results in misinterpretation of mechanical stimuli and dysregulation of biomechanical signaling pathways, lowering the threshold for forming heterotopic cartilage and bone [43, 44].

Utilizing mouse embryonic fibroblasts (MEFs) derived from the *Acvr1^{R206H/+}* FOP mouse model, the authors further explored the effects of the abnormal mechanosensing and determined that FOP cells are poised for differentiation into bone [44]. These cells express increased levels of cartilage and bone markers compared to control cells even when on a soft

substrate that does not normally promote chondro/osteo differentiation and in the absence of BMP ligands. This work supports both that changes in the tissue microenvironment and that the ability of FOP cells to sense their microenvironment are altered by muACVR1 in FOP. Importantly, these studies provide the first direct experimental evidence that FOP progression towards chondrogenesis and osteogenesis may be influenced by the tissue microenvironment of an evolving FOP lesion. Understanding the mechanism by which FOP progenitor cells are directed to cartilage and bone formation by a cue of improper mechanosignaling may have relevance in identifying novel treatment approaches for FOP. Such treatment strategies to prevent HO formation in FOP patients, and potentially more common non-hereditary forms of HO, may reside in the regulation of mechanotransduction pathways during tissue repair and may combine inhibitors of mechano-signaling and BMP signaling pathways [43, 44].

The tissue resident cells that are induced to chondro-osteogenic differentiation and HEO remain incompletely defined [45, 46], however, a population of $PDGFR\alpha^+$ cells known as fibroadipogenic progenitors (FAPs), a mesenchymal cell population that resides in muscle tissue but are not muscle progenitor cells, was identified as a major cell-of-origin of HEO in a mouse model of classic FOP [47, 48]. Expression of *Acvr1*^{R206H} in $PDGFR\alpha^+$ cells was shown to recapitulate the full spectrum of HEO observed in FOP patients. $PDGFR\alpha^+$ cells expressing muACVR1 activate BMP signaling in response to Act A and BMP [48]. Understanding the progenitor cells that drive HEO may facilitate the development of targeted cell-specific therapeutic approaches to inhibit HEO in FOP.

5. Summary

The identification of activating mutations in ACVR1 and the downstream dysregulation of the BMP signaling pathway as the cause for classic and variant FOP have propelled scientific research into the myriad developmental and postnatal features of FOP.

This review focused on the developmental and post-natal skeletal phenotypes of osteochondrodysplasia of FOP, however many other physiologic systems are impacted by muACVR1 and dysregulated BMP pathway signaling including the immune system, neurologic system, and cardiopulmonary system, to name a few [17, 25, 36–38, 49]. This review highlights just a few of the challenges for ongoing and future research in FOP and for the search for relevant pathway-associated treatments and cures [50–52]. However, the roadmap is becoming more clear. A complete definition of this complex disease will undoubtedly encompass many tissues and organ systems that are impacted by muACVR1 and dysregulation of the BMP signaling pathway.

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HIGHLIGHTS

- Fibrodysplasia Ossificans Progressiva (FOP) is a genetic disease of heterotopic ossification that can be considered a disorder of osteochondrogenesis.
- The activating mutations in ACVR1, a BMP type I receptor, that cause FOP alter normal skeletal development in addition to inducing extra-skeletal bone formation.
- Dysregulated chondrogenesis in FOP includes abnormal articular cartilage formation, abnormal diarthrodial joint specification, growth plate dysplasia, osteochondroma formation, precocious arthropathy, and heterotopic endochondral ossification (HEO).

Figure 1.

A hypothetical schema of FOP includes the broad developmental features of joint dysplasia, growth plate dysplasia and osteochondroma formation as well as the predominant post-natal feature of FOP: heterotopic endochondral ossification (HEO).