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When one skeleton is enough: approaches and strategies for the treatment of fibrodysplasia ossificans progressiva (FOP)

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Abstract

A heterozygous missense mutation in activin receptor IA/activin-like kinase-2 (ACVR1/ALK2), a bone morphogenetic protein (BMP) type I receptor, is responsible for fibrodysplasia ossificans progressiva (FOP), the most catastrophic disorder of skeletal metamorphosis in humans. The discovery of the FOP gene establishes a crucial milestone in understanding FOP, reveals a highly conserved target in the BMP signaling pathway for drug development and specifically stimulates therapeutic approaches for the development of inhibitors for ACVR1/ALK2 signaling. Effective therapies for FOP, and possibly for more common conditions of heterotopic ossification, will be based on interventions that selectively block promiscuous ACVR1/ALK2 signaling, and/or themolecular triggers, responding cells and tissue microenvironments that facilitate aberrant skeletal metamorphosis in a permissive genetic background of increased BMP pathway activity.

Introduction

Fibrodysplasia ossificans progressiva (FOP; MIM#135100), the most disabling condition of extraskeletal ossification in humans, is a rare autosomal dominant disorder that causes progressive immobility through metamorphosis of skeletal muscle and soft connective tissue into a second skeleton of heterotopic bone [1,2]. At the present time, there is no effective treatment or prevention.

The recent discovery of the missense point mutation in the gene encoding activin receptor A, type I/activin-like kinase 2 (ACVR1/ALK2), a bone morphogenetic protein (BMP) type I receptor, that causes classic FOP in all affected individuals, suggests four approaches that hold promise in arresting the progression of the condition [3]:

1. Blocking the ACVR1/ALK2 signaling pathway.
2. Blocking the inflammatory triggers.
3. Blocking the responding connective tissue progenitor (CTP) cells.

4. Altering the inductive and/or conducive microenvironments that promote heterotopic ossification.

In this article, we will briefly review the clinical and biological features of FOP and place them in context of these four approaches for identifying more effective treatments for FOP and related conditions of heterotopic ossification.

Clinical features of FOP

Two clinical features define classic FOP: congenital malformations of the great toes and progressive heterotopic ossification in characteristic anatomic patterns [2] (Fig. 1). Individuals with FOP appear normal at birth except for malformations of the great toes which are present in all classically affected individuals. In the first decade of life, children with FOP develop painful inflammatory soft tissue swellings (or flare-ups) that progressively transform soft connective tissues into an armament-like encasement of heterotopic bone [4,5]. Ribbons, sheets and plates of heterotopic bone replace skeletal muscles and other connective tissues through a process of endochondral ossification that leads to the formation of a highly ramified second skeleton with resultant permanent immobility [6,7]. Minor trauma such as intramuscular immunizations, mandibular blocks for dental work, muscle fatigue, blunt muscle traumas from bumps, bruises, falls or influenza-like illnesses can trigger painful new flare-ups of FOP that lead to progressive heterotopic ossification [8–14]. These crucial observations indicate a common requirement for inflammatory triggers (Fig. 2).

Surgical attempts to operatively remove heterotopic bone commonly lead to episodes of explosive new bone growth [2,14]. Heterotopic ossification in FOP progresses in characteristic anatomic and temporal patterns that mimic the patterns of normal embryonic skeletal formation [4,5]. FOP involvement is seen first in the dorsal, axial, cranial and proximal regions of the body and later in the ventral, appendicular, caudal and distal regions [4,5]. Several skeletal muscles characterized by slow-twitch fibers that are generally resistant to fatigue (including the diaphragm, tongue and extraocular muscles) are spared from the ravages of FOP. Cardiac and smooth muscles are not involved in the FOP process [2,4].

Bone formation in FOP is episodic, but disability is cumulative [5]. Most patients are confined to a wheelchair by the third decade of life, and require lifelong assistance in performing activities of daily living [2,4,5]. Severe weight loss often results from ankylosis of the jaw [11], and pneumonia or right-sided congestive heart failure complicates rigid fixation of the chest wall [15,16]. The severe disability of FOP results in low reproductive fitness [17]. The median age of survival is approximately 41 years, and death often results from complications of thoracic insufficiency syndrome [16].

Diagnosis and misdiagnosis of FOP

FOP is commonly misdiagnosed, because clinicians often fail to associate the rapidly developing soft tissue swellings with the malformed great toes. FOP can be diagnosed clinically even before radiographic evidence of heterotopic ossification is seen if soft tissue lesions are associated with symmetrical malformations of the great toes. When such associations are not made, FOP is commonly misdiagnosed as progressive juvenile fibromatosis, lymphedema or soft tissue sarcomas. Children often undergo unnecessary and harmful diagnostic biopsies that exacerbate the progression of the condition [18,19].

Additional skeletal anomalies in FOP

In addition to the malformations of the great toes, early developmental anomalies are frequently observed in the cervical spine, thumbs and femoral necks. Proximal medial tibial osteochondromas are a prominent feature of the condition [1,19–21].

Epidemiologic, genetic and environmental factors in FOP

FOP is extremely rare with a worldwide prevalence of approximately one in two million individuals. There is no ethnic, racial, gender or geographic predisposition. Most cases arise as a result of a spontaneous new mutation. Fewer than ten multigenerational families have been identified worldwide. When observed, genetic transmission is autosomal dominant and can be inherited from mothers or fathers. Penetrance is complete [17].

Both genetic and environmental factors affect the phenotype of FOP. The study of three pairs of monozygotic twins with FOP found that within each pair, congenital malformations of the great toes were identical. However, postnatal heterotopic ossification varied greatly depending on life history and environmental exposure indicating that genetic factors strongly influenced disease phenotype during prenatal development and that environmental factors strongly influenced postnatal progression of heterotopic ossification [22].

FOP and the BMP signaling pathway

The classic and invariable FOP phenotype of great toe malformations and progressive heterotopic endochondral ossification suggested that the primary molecular pathology in FOP involves both skeletal patterning and heterotopic bone formation. The association of these two processes strongly suggested that the BMP signaling pathway might be involved in the pathogenesis of the disease [23]. Several discoveries provided compelling evidence of profound dysregulation of the BMP signaling pathway in cells from patients who had FOP and in early attempts at animal models [24–33].

The pathobiology of skeletal metamorphosis in FOP

FOP exemplifies the pathological process of metamorphosis, the promiscuous transformation of one normal tissue or organ into another. Metamorphosis does not involve the transdifferentiation of one mature cell into another, but rather a pathological process in which the normal structure and function of one tissue or organ is destroyed and replaced by that of another tissue or organ [34].

The pathologic stages of skeletal metamorphosis in FOP have been well described [6,7,27,35]. The process begins with a destructive stage, frequently associated with a soft tissue injury that triggers an inflammatory mononuclear cell infiltrate, proceeds to muscle cell injury and death and replacement with a highly angiogenic fibroproliferative lesion that matures through an endochondral process and culminates in the formation of a new heterotopic skeletal element. As the process of metamorphosis continues and spreads at contiguous and adjacent sites, the new skeletal elements ramify to form a disabling second skeleton of heterotopic bone [36,37].

Discovery of the FOP gene

FOP was mapped by genome-wide linkage analysis to chromosome 2q23-24, a region that includes the gene encoding activin receptor IA/activin-like kinase 2 (ACVR1/ALK2) [3]. ACVR1/ALK2 was a prime candidate gene, and a large body of work over the past decade has supported dysregulated BMP signaling in the pathogenesis of FOP [24–33,38]. ACVR1/

ALK2 is one of seven activin-like kinases (ALKs) in the human genome that encodes TGF-beta/BMP type I serine-threonine kinase transmembrane receptors involved in the specification of cell fate in a wide variety of tissues during embryonic development and postnatal life (reviewed in [39]).

In all classically affected FOP patients worldwide, an identical heterozygous missense mutation (c.617G > A; R206H) was identified in the glycine serine (GS) activation domain of ACVR1/ALK2 [3]. This single nucleotide mutation transforms a morphogen receptor gene into a metamorphogene and provides a permissive genetic background for progressive skeletal metamorphosis [3,34]. The resultant mutant protein appears to alter the basal set-point and ligand-dependent sensitivity for BMP signaling in a cell-autonomous manner in CTP cells. The identification of the mutant receptor (caused remarkably by a single substituted amino acid residue) provides the basis for elucidating the molecular pathophysiology of dysregulated BMP signaling and resultant skeletal metamorphosis in FOP [34,34].

Although ACVR1/ALK2 has been recognized as a BMP receptor, the investigations of its function in embryonic development and in regulating cell differentiation have been limited (reviewed in [3]). ACVR1/ALK2 is expressed in many tissues including skeletal muscle and chondrocytes, providing support for its phenotypic effects in FOP. Constitutive activation of ACVR1/ALK2 induces alkaline phosphatase activity in C2C12 cells, upregulates BMP4, downregulates BMP antagonists, expands cartilage elements, induces ectopic chondrogenesis and stimulates joint fusions, findings nearly identical to those seen in FOP. Constitutive ACVR1/ALK2 expression (similar to that seen in FOP) in embryonic chick limbs induces expansion of chondrogenic anlage and leads to joint fusions, suggesting that promiscuous ACVR1/ALK2 signaling alters cell fate and induces undifferentiated mesenchyme to form cartilage and bone [3,40].

Recent preliminary *in vitro* and *in vivo* data using the classic FOP mutation support that enhanced ACVR1/ALK2 activation occurs in FOP. The mutant ACVR1/ALK2 protein increases pathway-specific SMAD phosphorylation and expression of BMP transcriptional targets in FOP cells, as well as reversal of an ACVR1/ALK2 loss of function phenotype in mutant zebrafish embryos [41,42].

Protein homology mapping of ACVR1 (FOP)

Protein homology modeling of the mutant ACVR1/ALK2 receptor in FOP predicts changes in both ligand-independent BMP signaling and in ligand-stimulated BMP signaling in FOP cells [3,43]. The canonical FOP mutation (ACVR1 c.617G > A; R206H) which replaces an arginine residue with a histidine residue at amino acid 206 in the GS domain of ACVR1/ALK2, is predicted to affect the binding and downstream function of coregulatory proteins [3–43].

The GS domain of all TGF-beta/BMP type I receptors is a crucial site for the activation of pathway-specific SMAD signaling proteins by constitutively active TGF-beta/BMP type II receptors. FKBP12 binds and stabilizes the inactive conformation of all type I TGF-beta/BMP receptors including ACVR1/ALK2. When bound to the GS domain, FKBP12 prevents leaky activation of type I receptors in the absence of ligand. Importantly, FKBP12 also serves as a docking protein for SMAD/SMURF complexes that mediate ubiquitination, internalization and degradation of ACVR1/ALK2 and is predicted to regulate the concentration of ACVR1/ALK2 and its BMP type I receptor oligomerization partners at the cell membrane [44–49].

The FOP mutation is predicted to impair FKBP12 binding and/or activity with resultant increased basal activity of BMP signaling in the absence of ligand as well as hyper-responsiveness of BMP signaling following ligand binding, two features of BMP signal dysregulation that have recently been demonstrated in FOP cells [25,41–43]. Thus, it is probable that FKBP12 interaction with the GS domain may be altered in FOP leading to promiscuous ACVR1/ALK2 activity. Recent preliminary data support this hypothesis [41].

Historical descriptions of FOP and the treatment imperative

While references to likely cases FOP date back to antiquity, FOP was first described by John Freke of St. Bartholomew's Hospital, London in a letter to *The Royal Society* dated 14 April 1736 (published in 1740). Although the descriptions of the characteristic great toe malformations in FOP would follow nearly a century later, this first description of heterotopic ossification in FOP contains the challenge of developing definitive treatments and a cure [50].

Dr Freke wrote: 'There came a boy of healthy look, and about 14 years of age, to ask of us at the Hospital, **what should be done to cure him** of many large swellings on his back, which began about three years since, and have continued to grow as large in many parts as a penny-loaf particularly on the left side. They arise from all the vertebrae of the neck, and reach down to the os sacrum; they likewise arise from every rib of his body, and joining together in all parts of his back, as the ramifications of coral do, they make as it were, a fixed boney pair of bodice' [50].

Nearly 200 years later in 1918, Jules Rosenstirn of Mount Zion Hospital in San Francisco, once again took up the treatment challenge and wrote, 'One does not wonder that a disease, so baffling in its form from the first causes to its ultimate state, should invite the speculative as well as the patiently investigating observer to lift the obscuring veil and solve this embarrassing puzzle' [51].

While definitive treatments and cures are not yet available, the recent discovery of the FOP gene and the emerging insights into its mechanism of action reveal at least four approaches to the treatment and/or prevention of FOP. These approaches include:

1. Blocking the overactive ACVR1/ALK2-mediated BMP signaling pathway (through four possible strategies which include: monoclonal antibodies, signal transduction inhibitors (STIs), inhibitory RNA and secreted antagonists).
2. Blocking the requisite inflammatory triggers.
3. Blocking the responding CTP cells.
4. Altering inductive and/or conducive microenvironments that promote heterotopic ossification.

Approach #1: blocking the ACVR1/ALK2 signaling pathway

In an article entitled 'A New Grammar for Drug Discovery,' Mark Fishman and Jeffrey Porter asked, 'Why has the deciphering of the 25,000 or so genes in the human genome not swelled the ranks of new drug targets? The major reason, of course, is that targets are of value for drug discovery only if they can be convincingly related to disease. Of the 6000 or so illnesses with a Mendelian pattern of inheritance, the gene responsible has been identified in approximately 1200. Historically, pharmaceutical companies have not concentrated on these diseases, in some cases because the affected proteins are not tractable to pharmaceutical approaches, in others perhaps, because the number of people affected is

small. But the powerful role of a single gene in Mendelian disease can provide insight into complex diseases where same gene accounts for part of the phenotype' [52].

Fishman and Porter go on to explain, 'If we hope to come to grips with the heterogeneity of common disease and its consequences for the choice of targets for drug discovery, clinical manifestations of disease must be causally related to a molecular definition. A further step of integration and interpretation is needed. Ideally, this would provide a grammar that would apply right through from the discovery of a drug target to its testing in animal models, and finally to the treatment of patients. Intracellular signaling pathways provide such a grammar for the genome. These pathways are triggered by extracellular molecules that bind to the receptors in the cell membrane, thereby switching on relay systems inside the cell. The upshot is gene activation or inactivation that affects a cell's behavior – its ability to grow or differentiate, for example, or to undergo division or self-destruction. The link between disease and signaling pathways is best validated for genetic disorders' [52].

FOP is a prime example of this new mode of thinking outlined by Fishman and Porter, and is tractable to approaches that investigate signaling pathways in cells for relevant drug targets. The discovery of the FOP gene specifically identifies ACVR1/ALK2, a BMP type I receptor, as a target for pharmaceutical development [2,39,53].

The identification of the recurrent heterozygous point mutation that causes FOP in all classically affected individuals provides a specific pharmaceutical target and rational point of intervention in a highly conserved signaling pathway [39]. Plausible therapeutic strategies to inhibiting BMP signaling in FOP include: monoclonal antibodies directed against ACVR1/ALK2, orally available small molecule STIs of ACVR1/ALK2, small inhibitory RNA (siRNA) directed against mutant ACVR1 messenger RNA, and secreted antagonists to block ligand binding to the putatively overabundant ACVR1/ALK2 receptor.

Signal transduction inhibitor (STI) strategy

Small molecule STIs have proven to be invaluable for investigating signal transduction pathways, and have the potential for development into powerful therapeutic agents. The development of specific STIs for promiscuous ACVR1/ALK2 signaling in FOP has the potential to modify the natural history of the disease. Residues close to the ATP-binding site of ACVR1/ALK2 could be exploited to achieve selectivity, even among closely related receptor serine threonine kinases such as ALK3 (BMPRIA) and ALK6 (BMPRII). Small soluble molecular inhibitors designed to specifically block ACVR1/ALK2 signaling intracellularly will need to have sufficient specificity, efficacy, tolerance to resistance, as well as acceptable safety profiles [39].

Recently, dorsomorphin, a small molecular STI of BMP signaling which was identified in a screen for compounds that perturb dorsoventral axis formation in zebrafish has shown promise [54,55]. Dorsomorphin selectively inhibits type I BMP receptors ALK2, ALK3 and ALK6, and thus blocks BMP-mediated SMAD 1, 5, 8 phosphorylation, target gene transcription and osteogenic differentiation. This was shown in both *in vitro* and *in vivo* studies. Dorsomorphin is able to inhibit SMAD activation without compromising the ability of BMPs to activate p38MAP kinases, a finding that suggests that this function may be independent of SMAD activation [54,55].

Recent data show that dorsomorphin inhibits ALK2-mediated BMP signaling in FOP cells and in *in vivo* models of heterotopic ossification [42,56]. Compounds such as dorsomorphin derivatives, as well as other even more specific ALK2 STIs, offer tremendous promise as pharmaceutical agents in the treatment of FOP and related disorders of heterotopic ossification. However, FOP cells also signal robustly through the p38 MAPk pathway,

especially in the presence of ligand. This contribution to BMP signaling needs to be evaluated in *in vivo* models of classic FOP to determine potential efficacy of this class of molecules in preventing trauma-induced flare-ups in FOP.

Monoclonal antibody strategy

A series of recent studies provide the rationale for considering a monoclonal antibody strategy in the treatment of FOP [25,26,32]. FOP cells express higher levels of BMP receptors at the cell surface associated with decreased internalization and degradation of those receptors [32]. FOP cells from multiple sources also exhibit a dramatic ligand-stimulated increase in signaling in addition to a basal leakiness of BMP signaling in the absence of ligand [25,26,32]. These findings strongly suggest that FOP cells are not only constitutively active at a basal level, but also hyper-responsive to ligand stimulation (possibly because of increased receptor levels at the cell surface).

Several recent studies strongly suggest that the BMP type I receptor ALK3 is a possible signaling partner of ALK2 in a heterotetrametric signaling complex that may be involved in the pathogenesis of FOP [25,26,32]. Presently available research antibodies lack specificity to assess the role of ALK2 in stimulating downstream signaling in the BMP pathway and in endogenous (nontransfected) FOP cells. Effective research antibodies as well as therapeutic blocking antibodies will be helpful in assessing the specific role of mutant ALK2 in heterotopic ossification. While further studies are necessary to characterize the density and activity of ACVR1/ALK2 receptors at the cell membrane in FOP cells, preliminary data strongly suggest that a strategy to block ligand-dependent signaling might prove effective in modulating disease-causing BMP signaling in FOP.

Attempts to develop monoclonal antibodies against various extracellular epitopes of the ALK2 receptor may help block ligand-stimulated hyper-responsiveness to signaling and will be necessary to assess the role of such signaling in the induction of postnatal heterotopic ossification. Such antibodies are presently being developed for *in vitro* and *in vivo* studies.

Small inhibitory RNA (siRNA) strategy

Classic FOP is an autosomal dominant disorder and results from a missense mutation in one of the two alleles of ACVR1/ ALK2 [3]. Thus, a strategy to inhibit messenger RNA from the mutant allele while leaving messenger RNA from the normal allele unperturbed is plausible. Presently, studies are underway to investigate the potential utility of an siRNA strategy for treating FOP. Present studies are investigating the role of siRNA against normal and mutant ALK2 in downregulating BMP signaling in FOP cells and eventually in knock-in mouse models (currently under development) of the classic disease genotype.

BMP antagonist strategy

The formation of the skeleton requires inductive signals that are balanced with their antagonists in a highly regulated negative feedback system [24]. BMP-induced heterotopic ossification was used as a model for testing the ability of the BMP antagonist noggin to block de novo bone formation, either by local or systemic delivery [56]. Because noggin acts locally in tissues as a paracrine mediator, a noggin mutein, human NOG Δ B2, was engineered to circulate systemically. The ability of human NOG Δ B2 to block heterotopic ossification was tested in a mouse model of heterotopic ossification with the use of adenovirus-mediated somatic cell gene transfer [57].

Systemic delivery of NOG Δ B2 adenovirus resulted in systemic levels of noggin that persisted for more than two weeks in a mouse model and were sufficient to block BMP4-induced heterotopic ossification. The study provided proof-of-concept that a naturally

occurring factor can be engineered for systemic delivery for a desirable pharmacological outcome. Blocking bone formation is clinically relevant to many nonhereditary as well as inherited disorders of heterotopic ossification in humans [57].

Although potentially effective in the context of FOP, gene transfer of endogenous antagonists such as noggin have many associated problems associated with *in vivo* use. Although gene transfer is not likely to be a practical therapeutic strategy for FOP, such work provides important insight into pathway-specific inhibition of promiscuous BMP signaling.

Approach #2: block the inflammatory triggers

While dysregulation of the BMP signaling pathway can explain many features of skeletal metamorphosis in FOP, other puzzling features of the disease strongly implicate an underlying inflammatory trigger [35,58–60]. A recent study showed that heterologous bone marrow transplantation does not cure FOP, and that even normal donor-derived hematopoietic inflammatory cells are capable of stimulating heterotopic ossification in genetically susceptible individuals [60]. The study demonstrated that at least two populations of cells, one of hematopoietic lineage and the other of a CTP lineage (that gives rise to the preosseous skeletal anlagen) are necessary to form an ectopic skeleton [60]. Although, the specific identity of the hematopoietically derived inflammatory cell that triggers heterotopic ossification in FOP has not yet been confirmed, preliminary findings suggest that it probably arises from the monocyte/ macrophages lineage [60]. Therapeutic regulation of inflammatory cells involved in triggering FOP lesions holds promise for the treatment of FOP and possibly other disorders of heterotopic ossification.

Approach #3: blocking the responding connective tissue progenitor (CTP) cells

Traditional approaches to preventing post-traumatic heterotopic ossification use cytotoxic radiation to destroy CTP cells [61]. However, such approaches have generally been unsuccessful in preventing or treating FOP, possibly because tissue injury (which exacerbates the process) occurs at the boundaries of the radiation fields.

Stromal cells of early FOP lesions have been hypothesized to be locally recruited vascular cells that maintain the potential to differentiate along an endochondral ossification pathway in a permissive BMP environment [58]. Recent studies in a transgenic model of progressive heterotopic ossification strongly support this hypothesis [62].

The prospect of targeted therapies for heterotopic ossification propels the characterization of cellular events enabled by dysregulated BMP signaling pathways. The development of a *bona fide* knock-in animal model for FOP [63] will enable lineage tracing studies to be performed to specifically identify CTP cell populations involved in transducing inflammatory-induced lesions in FOP [63]. The identification of such cells and their specific cell surface markers may provide a therapeutic target for a cytotoxic-monoclonal antibody-based strategy to eliminate those cells.

Approach #4: altering the inductive and/or conducive microenvironments that promote heterotopic ossification

To better understand the molecular constraints and physiological implications of the FOP mutation in ACVR1/ALK2, *in silico* modeling of wild type and mutant ACVR1 has been performed. In both the wild-type ACVR1 model and the template crystal structures, the conserved arginine at codon 206 in the GS domain appears to form a salt bridge with an

invariant aspartate residue at codon 269 in the kinase domain [43]. Although lysine, a conservative substitution in BMPRIA and BMPRIB, can be readily accommodated, histidine at residue 206 (as in FOP) would participate in a salt bridge with the aspartate only at decreased intracellular pH and with extensive structural rearrangement. Protein modeling predicts that substitution with histidine and only histidine, creates a pH-sensitive switch within the activation domain of the receptor that leads to ligand-independent activation of ACVR1 in FOP [43].

In the event that the salt bridge forms in ACVR1 (R206H) and functions as an aberrant switch that induces ligand-independent BMP signaling, the pH sensitivity of the histidine side chain could then serve as a tractable therapeutic target [43]. Flare-ups of FOP might result from physiologic changes such as trauma-associated hypoxia that lower intracellular pH, and turn the switch on. If so, perhaps the intercellular pH of affected connective tissues could be modified to suppress flare-ups of FOP or diminish the extent of heterotopic ossification. Interestingly, generation of a hypoxic environment (triggered by BMP2 in muscle) has recently been shown to be a crucial step in the formation of heterotopic bone in a murine model [64]. Thus, the development of therapeutic strategies that eliminate hypoxic stress may serve to block heterotopic bone formation in general, whether initiated by BMP ligand or by the aberrant ACVR1 R206H receptor that is hypothesized to activate under hypoxic conditions.

Conclusion

To physicians and scientists, FOP is one of medicine's most elusive mysteries. To patients who suffer from FOP, it is a painful metamorphosis into progressive immobility and a lifelong obstacle to physical freedom.

The ultimate goal of FOP research is the development of treatments that will prevent, halt, or even reverse the progression of the condition. As Bridget Hogan wrote: 'There is so much being discovered about how the BMPs act, it might be possible to develop drugs that would block some part of the BMP pathway, and therefore prevent the progression of what is a horrible nightmare disease' [65].

The identification of the heterozygous missense point mutation that causes FOP in all classically affected individuals worldwide provides a specific pharmaceutical target and a rational point of intervention in a crucial signaling pathway. Plausible therapeutic approaches to inhibiting BMP signaling in FOP include: blocking the inflammatory triggers, inhibiting the responsive CTP cells, inhibiting the inductive or conducive microenvironment and most plausibly inhibiting or blocking the promiscuous ACVR1/ALK2 signaling pathway. In the latter approach, plausible strategies include small molecule selective STIs of ACVR1/ALK2, monoclonal antibodies directed against ACVR1/ALK2, inhibitory RNA technology, or the use of BMP antagonists. It is still too early to determine which one of these approaches or strategies will be most effective, and all are being studied intensively in the laboratory.

The prevention and treatment of skeletal metamorphosis in FOP, as in any of the more common forms of heterotopic ossification, will probably be based on at least one of four approaches and/or several strategies noted above. The mission to establish better treatments and a cure for FOP is not just a matter of scientific inquiry; it is nothing less than a declaration of physical independence and personal freedom for those afflicted with one of nature's most catastrophic and disabling conditions.

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References

1. Kaplan FS, et al. The phenotype of fibrodysplasia ossificans progressiva. *Clin Rev Bone Miner Metab.* 2005; 3:183–188.
2. Kaplan FS, et al. Fibrodysplasia ossificans progressiva. *Best Pract Res Clin Rheumatol.* 2008; 22:191–205. [PubMed: 18328989]
3. Shore EM, et al. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. *Nat Genet.* 2006; 38:525–527. [PubMed: 16642017]
4. Cohen RB, et al. The natural history of heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. *J Bone Joint Surg.* 1993; 75-A:215–219. [PubMed: 8423182]
5. Rocke DM, et al. Age and joint-specific risk of initial heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res.* 1994; 301:243–248.
6. Kaplan FS, Zasloff MA. The histopathology of fibrodysplasia ossificans progressiva: an endochondral process. *J Bone Joint Surg.* 1993; 75-A:220–230. [PubMed: 7678595]
7. Pignolo RJ, et al. The fibrodysplasia ossificans progressiva lesion. *Clin Rev Bone Miner Metab.* 2005; 3:195–200.
8. Glaser DL, et al. Catastrophic falls in patients who have fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res.* 1998; 346:110–116.
9. Janoff HB, et al. Submandibular swelling in patients with fibrodysplasia ossificans progressiva. *Otolaryngol Head Neck Surg.* 1996; 114:599–604. [PubMed: 8643271]
10. Lanchoney TF, et al. Permanent heterotopic ossification at the injection site after diphtheria–tetanus–pertussis immunizations in children who have fibrodysplasia ossificans progressiva. *J Pediatr.* 1995; 126:762–764. [PubMed: 7752003]
11. Luchetti W, et al. Severe restriction in jaw movement after routine injection of local anesthetic in patients who have fibrodysplasia ossificans progressiva. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1995; 81:21–25. [PubMed: 8850477]
12. Moriatis JM, et al. Limb swelling in patients who have fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res.* 1997; 336:247–253.
13. Scarlett RF, et al. Influenza-like viral illnesses and flare-ups of fibrodysplasia ossificans progressiva (FOP). *Clin Orthop Rel Res.* 2004; 423:275–279.
14. Shah PB, et al. Spinal deformity in patients who have fibrodysplasia ossificans progressiva. *J Bone Joint Surg.* 1994; 76-A:1442–1450. [PubMed: 7929490]
15. Kussmaul WG, et al. Pulmonary and cardiac function in advanced fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res.* 1998; 346:104–109.
16. Kaplan FS, Glaser DL. Thoracic insufficiency syndrome in patients with fibrodysplasia ossificans progressiva. *Clin Rev Bone Miner Metab.* 2005; 3:213–216.
17. Shore EM, et al. The genetics of fibrodysplasia ossificans progressiva. *Clin Rev Bone Miner Metab.* 2005; 3:201–204.
18. Kitterman JA, et al. Iatrogenic harm caused by diagnostic errors in fibrodysplasia ossificans progressiva. *Pediatrics.* 2005; 116:654–661.
19. Kaplan FS, et al. Early diagnosis of fibrodysplasia ossificans progressiva. *Pediatrics.* 2008; 121:e1295–e1300. [PubMed: 18450872]
20. Schaffer A, et al. Developmental anomalies of the cervical spine in patients with fibrodysplasia ossificans progressiva are distinctly different from those in patients with Klippel-Feil syndrome. *Spine.* 2005; 30:1379–1385. [PubMed: 15959366]
21. Deirmengian GK, et al. Proximal tibial osteochondromas in patients with fibrodysplasia ossificans progressiva. *J Bone Joint Surg Am.* 2008; 90:366–374. [PubMed: 18245597]

22. Hebela N, et al. Three pairs of monozygotic twins with fibrodysplasia ossificans progressiva: the role of environment in the progression of heterotopic ossification. *Clin Rev Bone Miner Metab*. 2005; 3:205–208.
23. Kaplan FS, et al. Fibrodysplasia ossificans progressiva: a clue from the fly? *Calcif Tissue Int*. 1990; 47:117–125. [PubMed: 2117991]
24. Ahn J, et al. Paresis of a bone morphogenetic protein-antagonist response in a genetic disorder of heterotopic skeletogenesis. *J Bone Joint Surg*. 2003; 85-A:667–674. [PubMed: 12672843]
25. Billings PC, et al. Dysregulated BMP signaling and enhanced osteogenic differentiation of connective tissue progenitor cells from patients with fibrodysplasia ossificans progressiva. *J Bone Miner Res*. 2008; 23:305–313. [PubMed: 17967130]
26. Fiori JL, et al. Dysregulation of the BMP-p38 MAPK signaling pathway in cells from patients with fibrodysplasia ossificans progressiva (FOP). *J Bone Miner Res*. 2006; 21:902–909. [PubMed: 16753021]
27. Gannon FH, et al. Bone morphogenetic protein 2/4 in early fibromatous lesions of fibrodysplasia ossificans progressiva. *Hum Pathol*. 1997; 28:339–343. [PubMed: 9042799]
28. Kan L, et al. Transgenic mice overexpressing BMP4 develop a fibrodysplasia ossificans progressiva (FOP)-like phenotype. *Am J Pathol*. 2004; 165:1107–1115. [PubMed: 15466378]
29. Kaplan FS, et al. Dysregulation of BMP4 receptor trafficking and signaling in fibrodysplasia ossificans progressiva. *Clin Rev Bone Miner Metab*. 2005; 3:217–223.
30. Kaplan FS, et al. Dysregulation of the BMP4 signaling pathway in fibrodysplasia ossificans progressiva. *Ann N Y Acad Sci*. 2006; 1068:54–65. [PubMed: 16831905]
31. Olmsted EA, et al. Bone morphogenetic protein 4 regulation in fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res*. 2003; 408:331–343.
32. Serrano de la Peña L, et al. Fibrodysplasia ossificans progressiva (FOP), a disorder of ectopic osteogenesis, misregulates cell surface expression and trafficking of BMPRIA. *J Bone Miner Res*. 2005; 20:1168–1176. [PubMed: 15940369]
33. Shafritz AB, et al. Over-expression of an osteogenic morphogen in fibrodysplasia ossificans progressiva. *N Engl J Med*. 1996; 335:555–561. [PubMed: 8678932]
34. Kaplan FS, Shore EM. Morphogen receptor genes and metamorphogenesis: skeleton keys to metamorphosis. *Ann N Y Acad Sci*. 2007; 1116:113–133. [PubMed: 17872396]
35. Gannon FH, et al. Acute lymphocytic infiltration in an extremely early lesion of fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res*. 1998; 346:19–25.
36. Kaplan FS, et al. Radiographic and scintigraphic features of modeling and remodeling in the heterotopic skeleton of patients who have fibrodysplasia ossificans progressiva. *Clin Orthop Rel Res*. 1994; 304:238–247.
37. Mahboubi S, et al. Fibrodysplasia ossificans progressiva. *Pediatr Radiol*. 2001; 31:307–314. [PubMed: 11379597]
38. Jiao X, et al. Heparan sulfate proteoglycans (HSPGs) modulate BMP2 osteogenic bioactivity in C2C12 cells. *J Biol Chem*. 2007; 282:1080–1086. [PubMed: 17020882]
39. Kaplan FS, et al. A new era of fibrodysplasia ossificans progressiva (FOP): a druggable target for the second skeleton. *Exp Opin Biol Ther*. 2004; 7:705–712.
40. Zhang D, et al. ALK2 functions as a type I BMP receptor and induces Indian Hedgehog in chondrocytes during skeletal development. *J Bone Miner Res*. 2003; 18:1593–1604. [PubMed: 12968668]
41. Shen Q, et al. Activation of BMP signaling by the FOP ACVR1 R206H mutation. *J Bone Miner Res*. 2007; 22 (Suppl 1):S43.
42. Fukuda T, et al. Constitutively activated ALK2 and increased smad 1/5 cooperatively induce BMP signaling in fibrodysplasia ossificans progressiva. *J Biol Chem*. 2008 Aug 6. (Epub ahead of print).
43. Groppe JC, et al. Functional modeling of the ACVR1 (R206H) mutation in FOP. *Clin Orthop Rel Res*. 2007; 462:87–92.
44. Chen YG, et al. Mechanism of TGF- β receptor inhibition by FKBP12. *EMBO J*. 1997; 16:3866–3876. [PubMed: 9233797]

45. Ebisawa T, et al. Smurf 1 interacts with transforming growth factor- β type I receptor through Smad7 and induces receptor degradation. *J Biol Chem.* 2001; 276:12477–12480. [PubMed: 11278251]
46. Huse M, et al. Crystal structure of the cytoplasmic domain of the type I TGF- β receptor in complex with FKBP12. *Cell.* 1999; 96:425–436. [PubMed: 10025408]
47. Huse M, et al. The TGF- β receptor activation process: an inhibitor-to-substrate binding switch. *Mol Cell.* 2001; 8:671–682. [PubMed: 11583628]
48. Wang T, et al. The immunophilin FKBP12 functions as a common inhibitor of the TGF- β family type I receptors. *Cell.* 1996; 86:435–444. [PubMed: 8756725]
49. Yamaguchi T, et al. FKBP12 functions as an adaptor of the Smad7–Smurf1 complex on activin type I receptor. *J Mol Endocrinol.* 2006; 36:569–579. [PubMed: 16720724]
50. Kaplan FS. Fibrodysplasia ossificans progressiva: an historical perspective. *Clin Rev Bone Miner Metab.* 2005; 3:179–181.
51. Rosenstirn JA. A contribution to the study of myositis ossificans progressiva. *Ann Surg.* 1918; 68:468–520. 591–637.
52. Fishman MC, Porter JA. A new grammar for drug discovery. *Nature.* 2005; 437:491–493. [PubMed: 16177777]
53. Kaplan FS. The key to the closet is the key to the kingdom: a common lesson of rare diseases. *Orphan Dis Update.* 2006; 24:1–9.
54. Yu PB, et al. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat Chem Biol.* 2008; 4:33–41. [PubMed: 18026094]
55. Anderson GJ, Darshan D. Small-molecule dissection of BMP signaling. *Nat Chem Biol.* 2008; 4:15–16. [PubMed: 18084273]
56. Yu PB, et al. BMP type I receptor inhibition reduces heterotopic ossification. *Nat Med.* 2008; 14(12):1363–1369. [PubMed: 19029982]
57. Glaser DL, et al. In vivo somatic cell gene transfer of an engineered noggin mutein prevents BMP4-induced heterotopic ossification. *J Bone Joint Surg.* 2003; 85-A:2332–2342. [PubMed: 14668502]
58. Hegyi L, et al. Stromal cells of fibrodysplasia ossificans progressiva lesions express smooth muscle lineage markers and the osteogenic transcription factor Runx2/Cbfa-1: clues to a vascular origin of heterotopic ossification. *J Pathol.* 2003; 201:141–148. [PubMed: 12950027]
59. Kaplan FS, et al. Immunological features of fibrodysplasia ossificans progressiva and the dysregulated BMP4 pathway. *Clin Rev Bone Miner Metab.* 2005; 3:189–193.
60. Kaplan FS, et al. Hematopoietic stem-cell contribution to ectopic skeletogenesis. *J Bone Joint Surg Am.* 2007; 89:347–357. [PubMed: 17272450]
61. Kaplan FS, et al. Heterotopic ossification. *J Am Acad Orthop Surg.* 2004; 12:116–125. [PubMed: 15089085]
62. Lounev V, et al. Identification of progenitor cells that contribute to heterotopic skeletogenesis. *J Bone Joint Surg Am.* in press.
63. Chakkalakal SA, et al. ACVR1 knock-in mouse model for fibrodysplasia ossificans progressiva. *J Bone Miner Res.* 2008; 23 (Suppl 1):S57.
64. Olmsted-Davis E, et al. Hypoxic adipocytes pattern early heterotopic bone formation. *Am J Pathol.* 2007; 170:620–632. [PubMed: 17255330]
65. Roush W. Protein builds second skeleton. *Science.* 1996; 273:1170. [PubMed: 8787120]

Figure 1. Characteristic clinical features of classic FOP. Extensive heterotopic bone formation typical of classic FOP is seen by three-dimensional reconstructed tomography (CT) scan of the back of a 12-year-old child (a). Anteroposterior radiograph of the feet of a three-year-old child shows classic symmetrical toe malformations (b). (From Shore *et al.* (2006) *Nat. Genet.* 38, 525–527; Copyright held by authors.)

Figure 2.

Hypothetical approaches to preventing and treating FOP. Key: Y, ACVR1; →, stimulatory pathway; ⊥, inhibitory blockade; CTP, connective tissue progenitor cell; EC, extracellular; MABs, monoclonal antibodies; siRNA, small inhibitory RNA; STI, signal transduction inhibitor.