Review

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Fibrodysplasia ossificans progressiva: Basic understanding and experimental models

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Summary **Fibrodysplasia ossificans progressive (FOP) is an extremely rare autosomal dominant disorder characterized by congenital malformations of the great toes and progressive heterotopic ossification that can induce a disabling second skeleton. Spontaneously occurring flare-ups can cause inflammatory soft tissue to swell, followed by progressive and disabling heterotopic endochondral ossification. FOP is very rare, with an estimated incidence of one case per two million individuals. There is no definitive treatment for FOP, but the longevity of patients with FOP can be extended by early diagnosis and appropriate prevention of flares-up. Some promising treatment strategies and targets have recently been reported. The current review describes the classical phenotype and genotype of FOP, useful methods of diagnosing the condition, therapeutic approaches and commonly used drugs, and experimental models used to study this disease.**

Keywords: Fibrodysplasia ossificans progressive, phenotype and genotype, disease modeling, induced pluripotent stem cells

1. Introduction

Fibrodysplasia ossificans progressive (FOP), also known as myositis ossificans (*1*), is a rare autosomal dominant disorder with an incidence of one in two million births with no sexual, racial, or regional predisposition (*2*). Most patients are scattered around the world except in instances of familial inheritance (*3*). The earliest reports of FOP by Patin (1692) and Freke (1739) describe its symptoms (*4*). Later, Stonham, Burton-Fanning, and other physicians reported patients of different genders, ages, and even entire families with FOP and their phenotypes (*5*).

Abnormal ossification of the joints and soft tissues such as skeletal muscles, tendons, and ligaments (without myocardium and smooth muscle) and congenital hallux valgus are two typical symptoms of FOP (*6*). Heterotopic ossification (HO) is often associated with disability, such as skeletal deformities (trunk, limb, and facial deformity), chronic pain, growth defects, and stiffness. FOP seriously affects the quality of life and the mental health of patients. The average life expectancy of patients with FOP is no more than 40 years (*7*). The specific pathogenesis of FOP is not yet clear, and the early phenotype of the disease is easily confused with other diseases, including tumors, fibromas, and bursitis, resulting in its misdiagnosis (*8*). Moreover, there is no effective treatment for the disease (*9*).

Here, epidemiological studies on FOP and some common mutations are summarized. Clinically treating the condition is difficult, but diagnosis and treatment of the conditions are making progress. Moreover, experimental models are being used to identify the mechanism of onset of FOP. Greater understanding of the prevalence and symptoms of FOP would facilitate a definitive diagnosis and identify effective precautionary measures. Every step would help to prolong the life-span and improve the quality of life for patients.

2. Prevalence of FOP

FOP is an extremely rare, autosomal dominant disease

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with a prevalence of 1/2,000,000 (*2*). Ninety-five percent of patients manifest HO before the age of 15, and the latest report of the oldest patient with HO involves a patient who was 56 years of age (*10,11*). According to the CEMARA and PMSI databases, the average age of patients with FOP was 25.5 years, the average age of onset was 7.1 years, and the average age at diagnosis was 10.2 years (*11*).

Statistics for Europe indicate that 30 cases have been confirmed in the UK among about 49 million residents, with a prevalence of 0.61 per million. Spain is estimated to have an incidence of 0.36 per million (*12*), and French data indicate a prevalence of 1.36 per million. These figures are roughly similar to the international prevalence of the condition (*11*).

At present, most patients reported are in the United States, accounting for about 25.6% of all registered patients. This is followed by China, which accounts for about 10.8% of registered patients. Patients with FOP in Brazil account for about 8.4%. Compared to European and American patients, Asian patients are younger (*3*). Despite the extremely low incidence of FOP, there are still a large number of patients with FOP in China due to its huge population. Although definite figures for China are still unclear, the prevalence of FOP can be used to estimate the number of patients. Based on the incidence of FOP, there are at least 650 patients with FOP in China (*7*). For various reasons such as the level of medical research into the condition, however, only about 70 cases are reported, accounting for no more than 12% of all such patients in China. Understanding of the symptoms and mutations of FOP needs to be increased and the condition needs to be better diagnosed.

3. Mutations and diagnosis

The types of mutations of FOP in China are the same as those in other countries and regions (*7*). According to that study, 92% patients have the "classic" clinical presentation of FOP with a mutation of the *ACVR1/ALK2* gene (R260H,c.617G>A), while the remaining 8% have atypical symptoms with mutations at other sites of *ACVR1/ALK2* or other bases of R260H.

So far, 13 missense mutations and a 3-base deletion mutation have been found in FOP, and the detailed types and phenotypes of common mutations are shown in Table 1 (*2,7,10,13,14*).

The "classic" clinical presentation of FOP with a mutation of the *ACVR1/ALK2* gene (R260H, c.617G>A) induces structural changes in the GS domain. Eighty percent of patients with this mutation may have a congenital big toe (hallux valgus deformity), and some may exhibit soft tissue swelling leading to the formation of abnormal bone in the first decade of life (*15*). More than 90% of "classic" patients have a tumor in the tibia and more than 80% have a vertebral deformity (*16*). However, 1.5% of patients with this mutation also have a thumb deformity just like those with G356D (G328 R/W/E) mutations, and some patients with R260H will have cataracts, delayed growth, or other atypical symptoms (*14*).

In the early stages, 80% of patients with FOP often only have an obvious phenotype-malformations of the great toes - but trauma and infection may lead to abnormal bone formation from soft tissue swelling (*8*). Trauma, surgery, intramuscular injections, and immune injections cause swelling of soft tissue, and the occurrence of flare-ups is believed to signal the onset of HO (*17*). Inflammation of soft tissue can gradually infect skeletal muscles, tendons, ligaments, fascia, and aponeuroses, causing abnormal bone formation in these areas, and abnormal bone formation ultimately affects the patient's ability to move as well as the patient's lifespan. Though bone formation is episodic,

Table 1. Common mutations of FOP

Codon	Nucleotide	Domain	Features
R206H	605G>T 617G>A	GS	*i*) Characteristic malformations of great toe, *ii*) HO, *iii*) Tibialosteochondromas, *iv*) Spine malformations, *v*)Broad femoral necks
Q207E	c.619C>G	GS	*i*) Characteristic malformations of great toe, *ii*)HO, *iii*) Tibialosteochondromas, *iv*) Spine malformations, *v*) Broad femoral necks
R202I	605G>T	GS	*i*) HO, *ii*)One short great toe
G325A	974G>C	PK	*i*) Characteristic malformations of great toe, *ii*) Late-onset HO
G328W G328E G328R	c.982G>A c.982G>T c.983G>A	PK	*i*) HO, *ii*) Short broad femoral necks, *iii*) Thumb malformations
G356D	1067G>A	PK	*i*) HO, *ii*) Spine malformations, *iii*) Medial tibialosteochondromas
R258S R258G	774G>C 774G>T	PK	*i*) HO, *ii*) Cognitive impairment, *iii*) Diffuse scalp hair thinning
R375P	c.1124G>C	PK	*i*) HO, *ii*) Normal or minimal changes in great toes

FOP, fibrodysplasia ossificans progressiva; GS, glycine-serine-rich domain; HO, heterotopic ossification; PK, protein kinase domain.

disability is cumulative (*17*). Loss of mobility or even chewing ability can be caused by severe osteogenesis abnormalities, so most patients have to rely on wheelchairs to move around by the third decade of life (*18*). Death due to FOP is caused by the complications of thoracic insufficiency syndrome. Deformities of the joints, limbs, and face also place the patient under enormous psychological strain.

Since there is no effective treatment for FOP, diagnosis of the disease needs to be improved and prevention action needs to be taken to delay its progression. Early diagnosis has become the key to extending the life of patients with FOP. However, 90% of patients with FOP are misdiagnosed in the early stages of the disease. Since there are no diagnostic indicators of FOP, doctors and patients lack understanding of FOP and the early symptoms are not taken seriously, causing a delay in treatment. In specific terms, about 90% of patients with FOP worldwide have been misdiagnosed, and 67% of patients have received incorrect or unnecessary treatment. Treatment or diagnostic techniques such as removing excess bone and a biopsy can cause iatrogenic injury that accelerate HO (*13*). Improper treatment has caused irreparable damage or permanent disability to more than 50% of patients (*17*). Differentiating FOP from tumors, fibromas, and bursitis is essential to diagnosis. A typical mutation of R206H, which accounts for the highest proportion of patients overall, causes flare-ups in the first decades of life (*16*). Therefore, pediatricians and parents must be alert to congenital deformities of the great toes and soft tissue swelling in children consider the likelihood of FOP (*19*).

Before HO develops, routine physical examinations, including a radiographic skeletal survey, will not provide sufficient evidence to definitively diagnose FOP. The most authoritative indicator is the detection of the *ACVR1/ALK2* gene. Kaplan *et al*. obtained genomic DNA from 7 children suspected of having FOP after venipuncture (*19*). A genetic analysis confirmed that the 7 patients had an *ACVR1/ALK2* (R206H, c.617G>A) mutation. Single gene detection allows rapid and accurate diagnosis of patients with FOP before the onset of HO. Without a clear goal or obvious disease phenotype, whole genome sequencing or whole-exome sequencing (WES) is an effective means of reducing the trauma caused by a biopsy, improving the accuracy of diagnosis, avoiding a tedious physical examination, and it also equally helps to identify other rare diseases like FOP (*20,21*).

After HO develops, progressive extra-skeletal ossifications become typical deformities of FOP. In addition to clinical manifestations, imaging analysis (CT and MRI) is an important method of diagnosis. CT clearly reveals typical HO (*8*). MRI is also an important tool for diagnosis of FOP because it can reveal pre-osseous lesions, usually appearing as soft tissue swelling, and skeletal malformations. After HO occurs, plain X-rays can reveal abnormal osteogenesis. FOP cannot be diagnosed prenatally (*22*).

Although there is no effective treatment for FOP, prompt diagnosis can allow disease progression to be delayed, because patient can avoid intramuscular injections, tooth modifications which can cause wound (*23,24,25*). Prevention of trauma and infection is crucial before flare-ups occur (*26*). Patients should not enter dangerous areas or participate in strenuous activities. Living arrangements need to be improved and protective devices such as helmets need to be worn. Special attention should be paid to avoiding surgical procedures because trauma resulting from surgery can cause massive HO (*11*).

4. Existing and potential treatments

Patients with FOP are generally normal except for congenital great toe deformities in infancy. Fifty percent of flare-ups are caused by trauma, viral infection, intramuscular injections, muscle strain, and excessive fatigue in the first decade, resulting in swelling of the soft tissue and abnormal ossification of the muscles and ligaments (*20,25*). There is no effective treatment for FOP, but some drugs can be used to relieve initial symptoms.

When flare-ups begin, a brief 4-day course of high-dose corticosteroids such as prednisone can be used to relieve inflammation and tissue edema, but corticosteroids only can be used to relieve inflammation in areas such as the mandibular joint (*27*). The frequent use of corticosteroids to treat swelling in the trunk and neck is not recommended due to the difficulty in assessing the onset of flare-ups (*8*).

When corticosteroids are discontinued, mast cell inhibitors, aminobisphosphonates, non-steroidal anti-inflammatory drugs, and COX-2 inhibitors could be used to treat later flare-ups. A small dose of a muscle relaxant may help to relieve muscle spasms (*27,28*). Non-steroidal anti-inflammatory drugs inhibit the synthesis of prostaglandin, which induces resistant HO in animal models. Clinically, steroids, non-steroids, and anti-inflammatory drugs can mitigate inflammation and pain, but they cannot reduce the frequency of HO. Aminobisphosphonates affect the function and survival of osteoclasts, thus influencing bone formation, but the efficacy and safety of these drugs have not been established (*29*).

At present, effective drugs are a key area of study. The *ACVR1/ALK2* mutation causes partial deletion of the *ACVR1/ALK2* inhibitory protein FKBP12, so *ACVR1/ALK2* remains weakly activated in the absence of stimulation by BMP signals, causing HO (*30*). Therefore, one potential strategy would be to inhibit the activity of pathways related to the *ACVR1/ALK2* gene to inhibit abnormal bone formation (*31*). As an example, LDN-193189, optimized by dorsomorphin,

is a ALK2 protein inhibitor that repairs and maintains abnormal FOP-iPSc cells *in vitro*, and there is evidence of the therapeutic value of this drug in treating FOP (*32*). The other strategy would be to inhibit inflammation or to inhibit of osteoblastic progenitor cell activity (the RAR gamma agonist palovarotene) (*33*). Hindering the microenvironment for HO is a possible strategy. As an example, imatinib has a positive effect on multiple FOP related targets, and a clinical trial has demonstrated that it inhibits *ACVR1/ALK2* signaling, inflammatory triggers, pre-osseous fibro proliferative cells, and stimulatory mast cells. Kaplan *et al*. proved that imatinib significantly reduced the incidence of flare-ups (*9*).

New drug targets have been discovered with the increasing understanding of the pathogenesis of FOP. Many drugs, such as imatinib, are in clinical trials, and appropriate drugs may be available in the near future because of better understanding of the mechanism of onset of FOP.

5. Cells models of FOP

Studies on FOP are mainly focused on the specific mechanism of onset of HO and drug screening (*8,12,34*). The nature of FOP is particularly problematic because of the difficulty in acquiring living tissue to study the mechanisms of the disease. Minor trauma or an infection may cause tissue swelling followed by development of HO in the ligaments and connective tissue (*15*). At present, the main models used to study the pathogenesis of FOP are mouse cells, knockout mice, and induced pluripotent stem cells (*25*).

The cells most often used to model FOP are mouse cells (*35*). When studying the abnormal expression mechanism of pathogenic *ACVR1/ALK2*, different researchers have chosen different cell models, and their results differ. Vectors containing mutated *ACVR1/ALK2* have been transfected into cells such as U-2OS (*36*), MC3T3-E1 (*37*), and C2C12 (*38*), but levels of Smad1/5/8 expression differed. In addition to the transfection process (expression and transfection efficiency), the cell type may account for differences in expression (*39,40*). Patients FOP have endochondral ossification, so many studies have focused on cells related to chondrocytes. The differentiation of mouse embryonic fibroblasts into chondrocytes demonstrates that *ACVR1/ALK2* is a key factor in chondrogenesis. Embryonic fibroblasts, the origin of mouse mesenchymal cells, can be obtained from the head and limbs of mouse embryos. A rat chondrocyte cell line (ATDC5) expressing BMP-responsive luciferase has been used in high throughput drug screening. The cells can differentiate into mature chondrocytes when cultured in differentiation medium, and drugs that down-regulate the *ACVR1/ALK2* gene could be distinguished based on the intensity of the fluorescence signal, providing a basic model for drug screening and retesting of existing drugs (*25*).

ACVR1/ALK2 gene knockout mice are commonly used (*41*). Most knockout mice have the FOP phenotype. Murine cells and mice as models of FOP have indeed made great progress, but mice and murine cells cannot meet more detailed experimental needs. There are certain interspecies differences between mice and humans. For example, some knockout mice die during the perinatal period, so more appropriate models are urgently needed (*42*). Dermal fibroblasts obtained from patients with FOP are more suitable. A 3-mm thick piece of skin is removed from a patient with FOP and then macerated and cultured. Mineralization is then induced to study the role of TGF in osteogenic differentiation. Since trauma can easy trigger flare-ups in patients with FOP, sampling must be performed very carefully and skillfully to avoid trauma or infection (*43*).

In 2006, Takahashiand Yamanaka induced somatic cell reprogramming with a recombinant factor, thus obtaining induced pluripotent stem cells (iPSc) (*44*). This new technique has opened up new avenues and methods of studying the biological characteristics of many diseases (*45*). iPSc have several advantages. First, iPSc have a potent capacity for self-renewal and grow rapidly. *In vitro* experiments have been able to provide large quantities of needed cells, avoiding the tedious process of obtaining primary cells. Second, their potential for differentiation enables iPSc to differentiate into specific cells, providing cells at different stages of differentiation (*46*). iPSc can be obtained from somatic cells without causing ethical issues. With individual specificity, iPSc can carry disease-related pathogenic genes and an individual's specific genetic background (*47*). The induction of iPSc *in vitro* rapidly and effectively indicates the phenotype of disease in an individual specific background. A variety of somatic cells can be

Table 2. Induced pluripotent stem cell models of FOP

Sample	Types	Somatic cell	Vector	*Ref.*
4 patients	R206H and G356D	Skin fibroblasts	Sendai virus	(*32*)
2 patients	R206H	Urine Cell	Episomal vectors	(*48*)
purchased	R206H	Human dermal fibroblasts	Episomal vectors	(*49*)
2 patients	R206H	Urine	Sendai virus vector	(*50*)
5 patients	R206H	Primary human dermal fibroblasts	Retroviruses and integration-free episomal plasmid	(*51*)
5 patients	R260H	Primary human dermal fibroblasts	Retroviruses and integration-free episomal plasmid	(*40,52,53*)
4 patients	R206H	Fibroblasts	Retrovirus or episomal plasmids	(*54*)

FOP, fibrodysplasia ossificans progressiva.

reprogrammed into stem cells, including skin fibroblasts (*32*) and kidney epithelial cells (*48*), and Sendai virus and non-integration vector can be used as programming tools (*49*).

iPSc have been obtained by somatically reprogramming cells from patients with FOP. This provides a new, more accurate and appropriate cell model for the study of the pathogenesis of FOP. Hamasaki *et al*. used incompletely reprogrammed FOP-iPSc as an alternative tool to screen new drugs (*32*). Hino *et al*. induced chondrogenic differentiation of MSC cells from FOP-iPSc and concluded that BMP signals were activated by actinA (*50*). Relevant studies are listed in Table 2.

Surgery is not generally appropriate for patients with FOP, so future studies should focus on drug screening and noninvasive treatment. Intramuscular injections remain a potential risk, so safer dosing schedules should be considered. FOP-iPSc, a strong operational and theoretical basis for elucidation of the pathogenesis of FOP and drug screening, should be the main *in vitro* model used in future experiments. Effective therapies and drugs to treat FOP should be available in the near future.

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