Bone-specific therapeutic modalities for genetic skeletal diseases

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Skeletal dysplasias are a heritable group of disorders that affect bone and cartilage development. They present in a wide variety of presentations and variable ranges of severity affecting the quality of life of these patients. They also represent a therapeutic challenge owing to poor understanding of the underlying pathological mechanisms. Currently, the treatment is mainly symptomatic, including medical, surgical, and physical therapy. Recent advances in the genetic technology have helped to identify new causative genes and better understanding of the pathological mechanisms of genetic skeletal diseases, and this opened the doors for innovative discoveries of new therapies that are disease specific. This review outlines the normal bone development process and factors involved in bone growth and demonstrates current and recent therapeutic modalities in a wide group of genetic bone diseases, including osteogenesis imperfecta, mucopolysaccharidosis, hereditary rickets, hypophosphatemia, hypophosphatasia, achondroplasia, fibrodysplasia ossificans progressiva, and osteopetrosis. Some of these therapeutic targets have been approved and others are currently under clinical trial investigations. Remarkable advances and availability of new therapeutic options would give hope to clinicians and a wide variety of patients with skeletal dysplasia for better chances of management in the future and amelioration of their skeletal disabilities.

Keywords:
bone development, genetic technology, genetic, new therapies, pathological mechanism, skeletal dysplasia, therapeutic modalities

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Introduction

The genetic skeletal diseases (GSDs) are a broad and heterogenous group of disorders that affect primarily bone development, growth, and bone homeostasis, and most of the GSDs are caused by monogenic defect. Although individually rare, more than 450 GSDs have been described, with overall incidence around 1 : 3000–1 : 5000 live births (Barbosa-Buck *et al.*, 2012; Stevenson *et al.*, 2012; Bonafe *et al.*, 2015).

Advances in the molecular diagnostic techniques have paved the way to identify new genes and better characterize different GSDs with more defined clinical–molecular correlation. The last revision nosology and classification of skeletal dysplasias in 2019 added new conditions to the list to reach 461 disorders under 42 groups, which are differentiated by their characteristic clinical, radiological, and molecular background. The list of incorporated genes expanded dramatically from 80 genes in 2001 classification to 226 genes in the 2011 revision classification to 364 involved genes in the 2015 revision to reach 437 in the latest nosology of 2019. However, the molecular pathways have not been elucidated for many of these disorders (Mortier *et al.*, 2019).

Genetic testing is very important not just because it helps in definite diagnosis and improved counseling for patients and families but also provides a very important clue for the therapeutic intervention based on the understanding of the underlying pathophysiology. Molecular testing also solves a lot of dilemma in diagnosis of different skeletal dysplasias in the presence of a wide range of phenotypic variability, severity, and variations with age and sometimes atypical presentations.

Management of skeletal diseases is complex and depends on the underlying etiology and usually needs a multidisciplinary team approach. Currently, the treatment is symptomatic, including physical therapy, surgical corrections for bone deformities, and regular follow-up for other emerging complications over different stages of life in addition to psychological and social support groups (Nikkel, 2017).

The growing knowledge in the field of hereditary skeletal disorders has changed the management approaches over the past decade. Different therapeutic targets have been elucidated after identification of the underlying pathological mechanisms in some diseases (Semler *et al.*, 2019).

Literature review

Normal bone development and factors involved in growth

Bone development

Bone development in the embryo is initiated by the mesenchymal stem cells (MSC), mesoderm, and neural crest cells, and bone grows by either intramembranous ossification, as in flat bones, or endochondral ossification, as in tubular bones in the appendicular skeleton, base of the skull, and vertebrae. Intramembranous ossification begins with the condensation of MSCs, which differentiate into osteoprogenitors and become mature osteoblasts, which will change later into osteocyte or undergo apoptosis. Endochondral ossification also starts with condensation of the MSCs, which differentiate into chondrocytes and form cartilage models (anlagen) of the future bones (Hall and Miyake, 1992; Berendsen and Olsen, 2015). The proliferating chondrocytes secrete and deposit cartilage extracellular matrix, including collagen type II and the proteoglycan components, then become nonproliferative and hypertrophic and secrete type X collagen and alkaline phosphatase, and then, osteoclasts, osteoblast progenitors, blood vessels, endothelial cells, and hematopoietic cells invade the model from the perichondrium and proceed to form the primary center of ossification. The hypertrophic chondrocytes become degraded and removed with remnants of extracellular matrix by osteoclasts, bone matrix becomes deposited by the osteoblasts, and bone marrow is formed by hematopoietic and endothelial cells (Mackie *et al.*, 2011; Berendsen and Olsen, 2015).

Bone composition

Bone is composed of 50–70% mineral, 20–40% organic extracellular bone matrix (the osteoid), 5–10% water, and 3% lipids. Only 2% of the overall organic bone mass is cells from which the entire structure is built and remodeled (Luhmann *et al.*, 2012).

Overall, 90% of the organic matrix of bone is composed of type I collagen interspersed with a complex mixture of noncollagen proteins and proteoglycans (Gelse *et al.*, 2003).

Glycosaminoglycans (GAGs) are linear polysaccharides composed of repeating disaccharide monomers that covalently bind with proteins to form proteoglycan. Proteoglycans act as excellent lubricants and shock absorbers owing to their water retention and strength. Abnormal breakdown of the complex proteoglycan [mucopolysaccharide (MPS)] molecules produces a large variety of disorders known as the MPS (Smith, 2009).

Most bone mineral is in the form of hydroxyapatite [$Ca_{10}(PO_4)6(OH)_2$], with mainly calcium and pyrophosphate, which provides rigidity to the bone. The hydroxyapatite also consists of very small crystals and contains many impurities, including carbonate and magnesium. These impurities and imperfections in bone apatite may be quite important in rendering the apatite more soluble, thus permitting the apatite to release ions when needed for homeostasis (Shea and Miller, 2005). Bone is the most important reservoir for body calcium, containing ~99% of total body calcium and the rest in plasma. Ionized plasma calcium, which represents ~50% of the total plasma calcium pool, is essential for countless metabolic functions and is therefore tightly controlled through two major circulating hormones: parathormone hormone and 1, 25-dihydroxyvitamin D (calcitriol) (Clarke, 2008).

Bone-forming cells are osteoblasts that arise from osteoprogenitor cells, which are derived from primitive mesenchymal cells. They produce the bone matrix and become entrapped within it to become osteocytes and have a variable life span that ranges from a few days up to 100 days. Osteoclasts are phagocytic bone-resorbing cells that arise from hematopoietic stem cells in the bone marrow and have 12-day life span, after which they die by apoptosis. They break down old matrix, so that new matrix can replace it through a process called bone remodeling (Beyens and Van Hul, 2007; Ross and Pawlina (2011)).

Bone modeling and remodeling

Modeling is the formation of bone at one site and removal of bone at another site within the same bone, allowing for bone growth in size and length and change in bone shape by independent action of osteoblasts and osteoclasts in response to biomechanical forces. Modeling mainly takes place during growth of bones (Clarke, 2008).

Remodeling is the process of old bone resorption by osteoclasts and new bone formation by osteoblasts to prevent accumulation of bone microdamage. Bone remodeling unit is composed of a tightly coupled group of osteoclasts and osteoblasts, and their activity is strictly regulated to retain a healthy skeleton, and any imbalance between bone formation and bone resorption leads to skeletal dysplasias characterized by increased bone mass owing to defect in bone resorption like in osteopetrosis or decreased bone mineral density (BMD) like in osteoporosis (Bachrach and Sills, 2011; Feng and McDonald, 2011).

Growth factor involvement in skeletal development

The fibroblast growth factors (FGFs) regulate embryonic development and cell growth at the epiphyseal growth plate. One of these factors is FGF3 acting through FGF receptor 3 (FGFR3). Activating mutations in its coding gene cause achondroplasia and other disorders of FGFR3 chondrodysplasias, including milder hypochondroplasia or more severe lethal thanatophoric dysplasia.

Other factors include bone morphogenetic proteins (BMPs). Mutations in *activin A receptor type I* gene, which encodes activin receptor-like kinase-2 protein, lead to increase in signaling of BMP type I receptor as in fibrodysplasia ossificans progressiva (FOP) with subsequent extraskeletal bone formation in skeletal muscle, fascia, tendons, and ligaments, leading to progressive immobility and respiratory failure because of thoracic stiffness (Shore *et al.*, 2006).

Based on the composition of bone and steps involved in its development and the available and emerging target therapeutic modalities, defects of bone development and therapeutic targets are classified as in Table 1.

Various genetic skeletal diseases and their available treatment modalities

Osteogenesis imperfecta

Osteogenesis imperfecta (OI) is a genetically and phenotypically heterogenous group of hereditary bone dysplasias affecting primarily the skeleton, with decreased BMD and bone fragility, with increased liability to long bone fractures and vertebral compression with subsequent deformities in long bones, ribs, and spine deformities and substantial short stature (Marini *et al.*, 2017). Overall, 90% of the cases are caused by dominant mutations in the genes encoding type 1 collagen (*COL1A1* and *COL1A2*), leading to quantitative or qualitative defects in type 1 collagen. Since 2006, an expanding list of other genes, mostly recessive, dominant, and x-linked encoding proteins involved in type I collagen synthesis, post-translational modification, secretion and processing, in addition to proteins regulating differentiation and activity of osteoblasts, has been described to cause OI (Barnes *et al.*, 2006; Forlino and Marini, 2016; Lim *et al.*, 2017).

Management of OI needs a multidisciplinary approach, including orthopedic management, physical therapy, and medications. Many experts agree that muscle forces applied to the bones stimulate osteoblasts to produce osteoid and have recently published a consensus statement on physical rehabilitation in OI (Mueller *et al.*, 2018).

Of different skeletal dysplasias, OI currently has the most pharmacologic treatment options, and the main goal is to reduce the fracture rate and increase BMD. Medications include antiresorptive treatments mainly bisphosphonates. Additional therapeutic targets, including anabolic drugs and MSC therapy, are currently under investigations (Jelin *et al.*, 2017).

Bisphosphonates

Bone strength depends on the quality (bone material properties), quantity (bone mass), and distribution (bone architecture). Bisphosphonates are the mainstay

Table 1 Steps of bone development, defects, diseases, and therapeutic modalities

Steps of bone development	Defect	Disease	Therapeutic modality
Bone matrix formation	Defect in collagen synthesis	Osteogenesis imperfecta	Bisphosphonates, denosumab, anabolic treatment, cell therapy, and gene therapy
	Glycosaminoglycans accumulation owing to enzyme deficiency	Mucopolysaccharidosis	ERT, HSCT, SRT, PCT, and gene therapy
Mineralization	Calcipenic rickets	VDDR I (1 alfa hydroxylase deficiency) VDDR II (receptor resistance)	1-Alpha-Hydroxycholecalciferol IV calcium
	Hypophosphatemic rickets	X-linked hypophosphatemic rickets (increased FGFR23)	Oral phosphorous and calcitriol Burosumab
	Defect in synthesis of ALP	Hypophosphatasia	Asfotase alpha
Bone growth factors	FGFR3 activating mutations	Achondroplasia	CNP analog (Vosoritide) Meclizine, P3 peptide, decoy receptor, and statins
	BMPs signaling pathway overactivation	Fibrodysplasia ossificans progressiva Multiple hereditary exostoses	Palovarotene
Modeling and remodeling	Defect in osteoclast function or differentiation	Osteopetrosis	HSCT Gene therapy Interferon γ-1b

ALP, alkaline phosphatase; BMPs, bone morphogenetic proteins; CNP, C-natriuretic peptide; ERT, enzyme-replacement therapy; FGFR23, fibroblast growth factor receptor 23; HSCT, hematopoietic stem cell transplantation; IV, intravenous; PCT, pharmacological chaperone therapy; SRT, substrate reduction therapy; VDDR, vitamin D-dependent rickets.

of pharmacological treatment prescribed for patients with OI (Marini *et al.*, 2017).

Bisphosphonates adhere to mineralized bone, leading to inactivation and apoptosis of osteoclasts with subsequent inhibition of bone resorption, preserving more bone to compensate for the poor quality but cannot induce new bone formation (Harrington *et al.*, 2014; Marini *et al.*, 2017).

Oral and intravenous bisphosphonates have been used for many years for treatment of children with OI in clinical trials and have consistently reported improvements in BMD, decrease in fractures and chronic pains, and improved mobility and quality of life in patients with OI (Dwan *et al.*, 2016; Marini *et al.*, 2017). Advantages of intravenous over oral route are less gastrointestinal upsets, better bioavailability, and more compliance; however, no significant difference in efficacy was reported (George *et al.*, 2015).

Bisphosphonates are usually initiated when children with OI experience two or more fractures per year. Intravenous pamidronate is mostly used and given in cycles over 3 days every 2–4 months, whereas zoledronic acid is generally given intravenously with more convenient shorter course of single-day infusion twice per year (Palomo *et al.*, 2015). Moreover, there is reduced cost of treatment with zoledronic acid compared with pamidronate, but there are more data on pamidronate regimens than zoledronic acid (Saraff *et al.*, 2018). Otaify *et al.* (2016) studied the effect of biannual use of zoledronic acid over 2 years on 33 Egyptian patients with OI and five patients with Bruck syndrome and showed a reduction in fracture rates and pain and improvement in BMD and motor development. Another Egyptian study by El Sobky *et al.* (2006) compared the efficacy of intervention in two groups of OI by surgery alone versus combined surgery and pamidronate. Best results were obtained with combined approach. They advised preoperative and postoperative pamidronate therapy.

Oral bisphosphonates (e.g. risedronate and alendronate) have also been studied in patients with mild OI and showed an increase of BMD and appeared to decrease fractures (Bishop *et al.*, 2013; Dwan *et al.*, 2016).

The maximum effect was reached after 3 years of treatment based on BMD and bone histology, but many children continue treatment till closure of the growth plates outside of research protocols (Marini *et al.*, 2017). Although bisphosphonates are widely used in treatment of patients with OI, no treatment protocol is universally agreed upon, and no consensus is available on the drug, dose, and optimal duration of treatment in children. Furthermore, there is a knowledge gap about long-term effects on bone remodeling in treated patients (Morello, 2018).

Generally, patients with OI can tolerate bisphosphonates very well. Fever and malaise can occur with initial doses, and some may develop hypocalcemia. Additionally, it was reported that bisphosphonates can delay the healing of osteotomies (Munns *et al.*, 2004; Otaify *et al.*, 2016; Thomas and DiMeglio, 2016).

Denosumab

Patients with OI type VI have mutations in *SERPINF1* gene, which was identified in 2011, and it elucidated a new pathophysiological mechanism for OI. *SERPINF1* codes for the pigment epithelium-derived factor, which inhibits osteoclast differentiation and hence bone resorption via osteoprotegerin/receptor activator of nuclear factor κ-ligand (OPG/RANKL) pathway. Decoy receptor OPG, receptor activator of nuclear factor κ (RANK), and the ligand RANKL are pivotal regulators of osteoclast differentiation and function. Mutations in *SERPINF1* gene have no effect on collagen synthesis but rather increase the number and activity of osteoclasts with high bone turnover and an increase in bone resorption (Becker *et al.*, 2011).

Denosumab is a monoclonal antibody that acts by inactivating RANKL (mimicking the effect of OPG) and inhibits osteoclast formation and bone resorption with a subsequent increase in bone mass (Semler *et al.*, 2019).

Patients with OI type VI who were treated with denosumab after 12 months showed an increase in BMD and reduced chronic pain, whereas treatment outcomes for this type with bisphosphonates were poor (Semler *et al.*, 2012). Success in treatment of type VI promoted further testing in patients with OI with collagen mutations, which also showed positive response without severe adverse effects (Hoyer-Kuhn *et al.*, 2014, 2016). Currently, a phase III trial is underway, aiming to approve denosumab as the first choice for treatment of OI in children (NCT02352753).

It is worth noting that denosumab has a considerable effect on calcium homeostasis. Immediately following subcutaneous injection with denosumab, osteoclasts are blocked with subsequent hypocalcemia, which needs supplementation with oral calcium and vitamin D. Some months later, antibodies become resorbed and osteoclasts begin to differentiate causing bone resorption and rebound hypercalcemia and hypercalciuria, which may lead to nephrocalcinosis or calcification of vessels (Trejo *et al.*, 2018). Additionally, during rebound activation of osteoclasts, some patients

experienced chronic pain at joints, lasting for 1–2 weeks, and some vertebral fractures were reported after cessation of denosumab in adults with osteoporosis (Bandeira *et al.*, 2019; Florez *et al.*, 2019).

Bone anabolic treatments

The idea of this treatment modality came from understanding the pathological mechanism behind one of the sclerosing bone disorders. Sclerosteosis is AR sclerosing bone dysplasia caused by mutations in *SOST* gene that codes for sclerostin causing high bone mass and skeletal overgrowth. Sclerostin is mainly produced by osteocytes and involved in bone formation through inhibition of osteoblast differentiation via the WNT pathway. Understanding the pathological mechanism behind this disease opened the door for a new anabolic target drug named romosozumab.

Antisclerostin (romosozumab)

Human monoclonal antibodies against sclerostin targeting the WNT pathway were developed and tested in phase I and II clinical trials on postmenopausal women with osteoporosis treated with subcutaneous injections of romosozumab at 3-month intervals. Results were favorable and showed increases in BMD and decreased fracture risk after 12 months. Then, it was tested in multiple mouse models of OI with a good anabolic bone response at all ages tested (McClung *et al.*, 2014; Cosman *et al.*, 2016; Glorieux *et al.*, 2017; Faienza *et al.*, 2018). Similar response was reported in another phase II trial with blosozumab in patients with postmenopausal osteoporosis (Recker *et al.*, 2015).

Together, these data support the use of sclerostin antibodies for the future treatment of patients with OI to increase bone mass and strength.

Combined anabolic and antiresorptive therapies may add a synergistic effect to improve bone mass more than each therapy alone. Preliminary data from preclinical studies on mouse model of moderately severe dominant OI revealed an increase in BMD and bone strength when combining sclerostin-antibody and zoledronic acid therapies more than either drug alone (Marom *et al.*, 2016).

Cell therapy, gene targeting, and gene editing

The idea of stem cell therapy is to infuse stem cells that would successfully graft into the marrow of patients with OI and can differentiate into osteoblasts and produce normal/healthy collagen. Bone marrow-derived stem cells were transplanted into three toddlers with severe OI from compatible siblings and showed a good response regarding bone formation, growth, and fracture rate (Horwitz *et al.*, 1999; Le Blanc *et al.*, 2005).

Case series of prenatal and postnatal transplantation showed transient improvement for several months with increased linear growth and improvement of mobility and fracture. Second transplantation postnatally resumed the positive effects without alloreactivity to the donor MSCs (Chan and Götherström, 2014; Götherström *et al.*, 2014). A larger clinical trial is currently running (NCT03706482) (Chitty *et al.*, 2016).

Gene therapy approaches, using various silencing techniques (i.e., ribozymes, small interfering RNA, and short hairpin RNA), have been attempted *in vitro* and await final proof of principle *in vivo* (Besio and Forlino, 2015).

The rationale is that pathogenic variants that affect the structure of collagen produce a dominant negative effect by disrupting the triple helix structure of the collagen molecule. Gene therapy by inactivation of the mutated allele or silencing of its transcription would translate to production of only healthy collagen from the normal allele and transform a qualitative collagen defect with severe OI phenotype to a quantitative defect with a milder phenotype (Morello, 2018). Safety of genetic manipulation, efficient delivery to bone tissue, and maintaining long-term effects *in vivo* remain to be addressed (Marom *et al.*, 2016).

Gene editing although considered the most hopeful future treatment of OI has not yet been tried (Morello, 2018).

Mucopolysaccharidosis

MPS are inborn errors of metabolism produced by a deficiency of one of the enzymes involved in the degradation of GAGs, leading to their accumulation in different body tissues with progressive deterioration in skeletal, visceral, and neurological functions (Regier and Tanpaiboon, 2016). The enzyme deficiency is specific to the MPS type. Current treatments for the MPS are focused mainly on enzyme-replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) (Clarke, 2011; Jelin *et al.*, 2017).

Enzyme-replacement therapy

ERT started since 2003 with FDA approval for treatment of three types (MPS I, II, and VI). Currently ERT is approved by the FDA and the European Medicines Agency for the treatment of MPS type I (laronidase), type II (idursulfase), type IV (elosulfase alfa), type VI (galsulfase), and type VII (vestronidase

alfa) by intravenous infusion (Qi *et al.*, 2019). On the contrary, clinical trials are running on intrathecal therapy for types I, II, and IIIA [owing to the inability of these molecules to cross the blood–brain barrier (BBB)]. ERT trials showed a significant effect on most visceral organs, physical performance (6-min walk test, 3-min stair climb test), pulmonary function tests, and decrease in urinary GAGs. Nevertheless, some challenges remain unsolved. As the enzymes are incapable of crossing the BBB, they show no effect on the neurological symptoms. Moreover, treatment cannot reach lesions in avascular cartilage, heart valves, and corneas (Sawamoto *et al.*, 2019). Additionally, anti-ERT antibodies have been noticed in most of treated cases with continuous administration (weekly or biweekly), which affects the efficacy and final outcome (Xue *et al.*, 2016). Furthermore, high cost of the available ERTs adds more burden on the health system, especially in low-income and developing countries where these diseases are common (Sawamoto *et al.*, 2019).

Therapy should, ideally, be initiated presymptomatically to ensure the best outcome. This may be possible for cases screened before manifestations appear when there is positive family history; however, most patients who are diagnosed clinically will be symptomatic by the time therapy is initiated (Heese, 2008).

Hematopoietic stem cell transplantation

HSCT and bone marrow transplantation represent a gold standard therapeutic target for MPS, by introducing unaffected healthy matched donor cells into the body that will eventually secrete the deficient enzyme permanently and correct the lysosomal disease; however, the patient needs close monitoring for immune response (Haneef and Doss, 2016). The efficacy of HSCT depends on many factors, including the patient's age, disease stage at the time of transplantation, availability of matched donors, and specialized facilities and preparative regimen (Wang *et al.*, 2016; Barth *et al.*, 2017).

HSCT is recommended for the treatment of severe forms of MPS type I (Hurler syndrome), combined with and before ERT for better results (Clarke, 2011; Muenzer, 2014). HSCT is also useful but less effective in severe forms of types II and III and in types VI and VII (Muenzer, 2014; Haneef and Doss, 2016; Motas *et al.*, 2016).

Substrate reduction therapy

Substrate reduction therapy (SRT) is a therapeutic target that acts by decreasing the synthesis of GAGs to correct the imbalance between the formation and degradation of GAGs. It has been approved in the treatment of other lysosomal storage disorders, like Gaucher type 1 and Niemann–Pick type C diseases, but not in MPS yet. The most advantage of SRT is its liability to cross the BBB owing to its small size and charge, which is why it is potentially useful in treating CNS symptoms in neuropathic types of MPS, especially MPS type III (Ahluwalia *et al.*, 2018).

Early studies with oral genistein (which is a soy isoflavone), at the dose of 5 mg/kg/day, showed reduction in GAG excretion with neurocognitive improvements in MPS III treated patients (Delgadillo *et al.*, 2011). Further studies with a dose of 10 mg/kg/day in 30 patients showed only temporary reduction in GAG excretion but no clear neurological benefit in treated patients (de Ruijter *et al.*, 2012). A phase III clinical trial for MPS III with high-dose oral genistein was completed in July 2018, and it showed that treatment is safe, but clinical efficacy remains uncertain, and no significant improvement of intellectual function was detected (Mumal, 2018; Sawamoto *et al.*, 2019).

Despite the failure of genistein to improve the neurological outcome as expected, SRT remains an attractive therapeutic target for MPS trying to find novel drugs inhibiting certain steps in GAG synthesis, which might improve the results (Fecarotta *et al.*, 2018).

Pharmacological chaperone therapy

Chaperones are small-molecule ligands that bind with the defective protein, favor correct folding and intracellular trafficking of the protein at the endoplasmic reticulum, stabilize the mutant enzymes, and improve lysosomal activity. Compared with other approaches, pharmacological chaperone therapy has some advantages, as it can cross BBB, can be orally administered, has a broad biodistribution, and has a positive effect on the clinical phenotype. After preclinical *in vitro* and *in vivo* studies, pharmacological chaperone therapy has been studied as a potential therapy in clinical trials, either as monotherapy or in combination with ERT for MPS II, MPS IIIC, and MPS IVA (Boyd *et al.*, 2013; Olarte *et al.*, 2014; Parenti *et al.*, 2015; Hoshina *et al.*, 2017).

Gene therapy

Gene therapy approach is to introduce normally functioning gene into the cells of patients to correct the expression of defective genes. Gene therapy for MPS has been studied, and recently, some successful clinical trials have been carried out to overcome the weakness of ERT and HSCT (Chen *et al.*, 2018).

Two main approaches are available for gene therapy: *in vivo* and *ex vivo*. *In vivo* approach involves direct delivery to the brain by intravenous, intrathecal, and intraventricular injection of viral vector, mainly using adeno-associated viral vector (Kobayashi, 2019).

Ex vivo gene therapy uses the HSCs of patients with MPS, which become transduced with a therapeutic gene using viral vectors like retrovirus or lentivirus, and then are infused back into the patients. With *ex vivo* gene therapy, there is no risk of GVHD, which usually occurs following allogeneic HSCT (Sawamoto *et al.*, 2019).

Adeno-associated viral vector-mediated gene therapy has been reported for MPS III, in which neurological symptoms are profound and there is no indication of ERT or HSCT (Chen *et al.*, 2018).

Ex-vivo gene therapy using lentiviral vectors is currently under investigation through running clinical trials on animal models of MPS I, II, and III (Wakabayashi *et al.*, 2015; Sawamoto *et al.*, 2019).

Disorders with defective mineralization

Calcium and phosphate are necessary for mineralization of bone matrix. Defective mineralization causes rickets and/or osteomalacia. Rickets can be classified broadly as calcipenic or phosphopenic. Calcipenic rickets is caused by calcium deficiency, which usually is owing to insufficient intake or metabolism of vitamin D (Misra *et al.*, 2008; Sahay and Sahay, 2012). Phosphopenic rickets usually is caused by renal phosphate wasting (Sahay and Sahay, 2013).

Hypophosphatasia (HPP) is a hereditary metabolic bone disorder characterized by defect in bone and tooth mineralization and low serum and bone alkaline phosphatase (ALP) activity.

Most cases of hypocalcemic rickets are not inherited. Calcium or vitamin D deficiency can be corrected with supplements.

Genetic disorders of vitamin D metabolism (calcipenic rickets):

(1) Vitamin D-dependent rickets type I, also referred to as vitamin D 1α-hydroxylase-deficiency rickets, can respond well to active form of vitamin D (1-alpha-hydroxycholecalciferol or calcitriol) in the dose range of 0.5–2.0 μg/day (Ramasamy, 2008)
(2) Vitamin D-dependent rickets type II, also known as hereditary vitamin D-resistant rickets (OMIM 277440), is caused by mutations in vitamin D receptor. Treatment is more difficult owing to receptor resistance. Although some patients may respond to high dose of 1-alpha-hydroxycholecalciferol, many require intravenous calcium infusions via a central line to treat the rickets (Pai and Shaw, 2011).

Hypophosphatemic rickets

Hypophosphatemic rickets is a group of diseases that are characterized by impaired bone mineralization OWING to defects in renal tubular reabsorption of phosphate. X-linked hypophosphatemic rickets (XLH) is the most common form of heritable rickets owing to mutation in the *PHEX* gene causing elevation of FGF23 promoting renal phosphate leak and impaired bone mineralization. FGF23 is expressed by osteocytes and is a key regulator of phosphate reabsorption and 1, 25-dihydroxyvitamin D synthesis in the proximal renal tubules (Quarles, 2012). Patients with XLH present with typical rachitic manifestations, short stature, and bone deformities, and they have an additional risk of developing nephrocalcinosis (Shimada *et al.*, 2004a, 2004b).

Conventional treatment

Most children with XLH are treated currently with oral phosphorus and calcitriol from the time of diagnosis until growth is complete. Owing to poor store in the circulation, oral phosphorus needs multiple administrations (three to five doses daily), and this leads to poor compliance to treatment, and many patients develop limb deformities and need surgical correction (Makitie *et al.*, 2003).

There is wide range of doses from 10 to 80 ng/kg/day of calcitriol and 30–180 mg/kg/day of elemental phosphorus, reflecting uncertainty toward the optimal dosage and also regarding the adverse effects, especially nephrocalcinosis and secondary hyperparathyroidism in addition to the patient poor compliance to oral treatment (Carpenter *et al.*, 2011; Linglart *et al.*, 2014). Recommended dose is calcitriol 20–30 ng/kg/day (two to three divided doses) and elemental phosphorus with dose of 20–40 mg/kg/day (in –three to five divided doses), which can be started with gradual titration over days to avoid gastrointestinal upsets and can be adjusted upon follow-up with clinical radiological and biochemical response (Carpenter *et al.*, 2011; Linglart *et al.*, 2014).

Burosumab

Burosumab is a human monoclonal antibody against FGF23, which was approved by European Medicines Agency in 2018. It acts by inhibition of

the phosphaturic effect of FGFR23 that is increased in patients with *PHEX* mutations. In a clinical trial, it was administered by subcutaneous route every 2 weeks. It improved phosphate homeostasis and bone mineralization in children aged 5–12 years with XLH. In a comparative trial between burosumab and conventional therapy with phosphate and vitamin D, the former was associated with a significantly greater improvement regarding the severity of rickets, growth, and biochemistries in children with XLH (Imel *et al.*, 2019a).

Hypophosphatasia

HPP is a rare metabolic bone disorder caused by deficiency of tissue-nonspecific alkaline phosphatase owing to loss-of-function mutation in the ALPL gene leading to insufficient mineralization of bone and teeth with subsequent bone pain, high risk of fractures, premature loss of teeth, and osteomalacia in adults (Yap and Savarirayan, 2016; Whyte, 2017). HPP is clinically heterogeneous with six clinical forms of variable severity classified according to the age of onset and severity from perinatal lethal type, infantile, childhood, adult type, odontohypophosphatasia, and pseudohypophosphatasia (Whyte, 2010).

The tissue-nonspecific alkaline phosphatase protein transforms inorganic pyrophosphate (PPi) into phosphate (Pi) through hydrolysis (Orimo, 2010). Pi is important for hydroxyapatite formation, whereas PPi inhibits bone mineralization. Affected patients experience defective mineralization with typical rachitic manifestations clinically and radiologically. Accumulation of PPi and other metabolites in the kidneys leads to nephrocalcinosis. Additionally, patients with HPP may have chronic pain, rheumatoid arthritis inflammation-like manifestations, and skull deformities owing to premature closure of sutures, sometimes requiring neurosurgical intervention (Whyte, 2017).

Asfotase alfa

Asfotase alfa is a human recombinant TSNALP recently approved as an ERT for patients with HPP to increase ALP level for proper degradation of inorganic pyrophosphate. A study on 10 patients with severe types of HPP using a subcutaneous form of Asfotase alfa showed dramatic improvement of mineralization and increased perinatal and postnatal survival rate immensely (Whyte *et al.*, 2012). The drug must be administered by subcutaneous injection two to three times per week. It showed efficacy and safety in Japanese clinical trial with improved mineralization and improved clinical outcome, especially respiratory problems and failure to thrive (Kitaoka *et al.*, 2017). Treatment is recommended for severe cases and also approved for milder types if affected children present with skeletal symptoms. Clinical improvement sustained up to 6 years in phase II open-label study (Hofmann *et al.*, 2019).

Achondroplasia

Achondroplasia is an autosomal dominant disorder representing the most common cause of short-limb dwarfism with prevalence around 1 in 25 000–30 000 live births (Ireland *et al.*, 2014). Overall, 90% of the cases result from gain-of-function mutation (G380R) in the FGFR3, which encodes for the FGFR3 receptor on the chondrocytes in growth plates, leading to inhibition of chondrocyte proliferation and impairment of endochondral ossification in the long bones with subsequent short-limb dwarfism. Additional symptoms include midface hypoplasia, macrocephaly, and joint hypermobility (Pauli, 2019).

Currently, treatment approaches are symptomatic and directed for prevention of complications like recurrent otitis media and its effect on hearing and neurosurgical complications that may arise from spinal cord compression or foramen magnum stenosis. Additionally, orthopedic interventions include either to correct limb deformities or lengthening operations through osteotomies and gradual distraction using external fixators, which is a long process with high rate of complications (Donaldson *et al.*, 2015). Growth hormone therapy in patients with achondroplasia shows transient increase of height in the first 2 years of treatment, which then diminishes in subsequent years (Miccoli *et al.*, 2016).

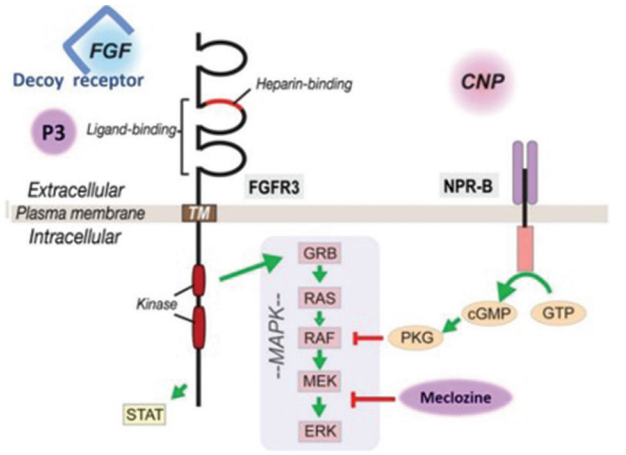
Increased knowledge and understanding of molecular processes underlying achondroplasia have promoted efforts in pharmacologic strategies targeting the FGFR3-mediated signaling pathway, as represented in Fig. 1.

FGFR3 consists of an extracellular domain (ligand-binding) with three immunoglobulin-like regions, a transmembrane domain, and an intracellular tyrosine kinase. Binding of FGF to the extracellular domain of FGFR3 induces kinase activation followed by activation of downstream signaling pathways involving STAT (signal transducer and activator of transcription) and MAPK (mitogen-activated protein kinase) cascades.

C-natriuretic peptide analog

C-natriuretic peptide (CNP) is highly expressed in the growth plate. It counterbalances the effects of FGFR3

Figure 1



Therapeutic targets for treatment for achondroplasia. P3 blocking peptide, decoy receptor, CNP analog-mediated antagonism of downstream FGFR3 signaling, and meclizine interrupting downstream signal. Diagram is modified from Klag and Horton (2016). CNP, C-natriuretic peptide; FGF, fibroblast growth factor; FGFR3, fibroblast growth factor receptor 3; MAPK, mitogen-activated protein kinase; NPR-B, natriuretic peptide receptor-B; STAT, signal transducer and activator of transcription.

on the growth plate. Binding of CNP to its receptor natriuretic peptide receptor-B induces the generation of the second messenger cGMP, which antagonizes the MAP kinase pathways downstream of FGFR3 at the RAF level (Yasoda *et al.*, 2009). The balanced relation between the FGFR3 and CNP systems is very crucial for endochondral ossification and linear growth (Yap and Savarirayan, 2016).

Increasing CNP activity to counteract the excess negative signal of *FGFR3* mutations was the idea of the new therapeutic target CNP analog, which proved to improve the growth in mouse models and in normal cynomolgus monkeys. Most CNPs become degraded rapidly in the body before 3 min by neutral endopeptidase (Wendt *et al.*, 2015). CNP analog, which is BMN-111 (now called Vosoritide), was developed to resist digestion by neutral endopeptidase with a subsequent longer half-life in the body. Vosoritide is the only target treatment that proceeded to human clinical trials (NCT03197766). It is administered once daily subcutaneously (Klag and Horton, 2016).

Early results revealed favorable safety profile and efficacy even with higher doses. There was an increase in growth velocity not only in first year of treatment but also after 2–3 years, still there was no decline in growth rates as noticed in children treated with growth hormone. CNP also seems to have a beneficial effect on midfacial development and increase in size of lumbar vertebral foramina (Yamanaka *et al.*, 2017).

No results are available yet about final height, and the effect on the foramen magnum is also unknown because the cohort of studied children is more than 4 years, and the clinical trial is still in phase 2, with active recruitment for phase 3 (Pauli, 2019; Semler *et al.*, 2019).

Meclizine

Meclizine (an oral antihistamine used for motion sickness) has been shown to improve linear bone growth in a mouse model of achondroplasia, possibly by inhibiting downstream FGFR3 signaling pathway at the MEK-ERK level (Matsushita *et al.*, 2015; Klag and Horton, 2016). Soon, a phase I clinical trial of meclizine will start (Pauli, 2019).

Statins

The effect of gain-of-function mutation of *FGFR3* on induced pluripotent stem cells generated from skin fibroblasts of patients with thanatophoric dysplasia type I on differentiation to chondrocytes was limited cartilage formation or cartilage degradation.

Statins are lipid-lowering agents that were noticed to have anabolic effects on chondrocytes. Statin rescued chondrogenically differentiated thanatophoric dysplasia type I induced pluripotent stem cells and a mouse model of achondroplasia from an increased amount of phosphorylated MAPK downstream signaling of FGFR3 (with significant recovery of bone growth) (Yamashita *et al.*, 2014).

Fibroblast growth factor receptor 3 isoform (decoy)

Decoy is a drug currently under investigation in phase I/II trials. It is a soluble FGFR3 isoform that lacks the transmembrane component and acts as decoy receptor decreasing binding to FGFs and consequently reducing FGFR3 signaling. Documented data on animal models after subcutaneous injection twice weekly appeared promising especially regarding the shape of the skull and increased skeletal growth (Garcia *et al.*, 2013). As the drug targets young children, so it is expected to have a gainful effect on the size of foramen magnum. Phase II/III trials were planned to begin in 2019 (Semler *et al.*, 2019).

Blocking peptide

Blocking FGFR3 with designed 12-amino acid P3 peptide interferes with ligand binding and inhibits receptor activation and signaling pathway (Jin *et al.*, 2012).

Fibrodysplasia ossificans progressiva

FOP is a rare autosomal dominant disorder with an incidence of one per two million people. It is

characterized by congenital malformation of great toes and progressive extraskeletal ossification. Heterotopic ossification begins in childhood and is sometimes triggered by trauma, intramuscular injections, or surgery, leading to restricted mobility, spine deformity, respiratory problem from thoracic stiffness, and trismus from jaw muscle involvement.

FOP is caused by mutations in *activin A receptor type I* gene, which codes for activin receptor-like kinase-2 protein leading to overactivation of the BMP signaling pathway that induces ectopic chondrogenesis, which later mineralizes and will be converted to solid bone (Shore *et al.*, 2006).

Most pharmacologic therapies for FOP aim at preventing the progression of the disease by avoiding trauma and intramuscular injections. In case of trauma, corticosteroids in high doses and retinoic acid were tested for reducing new ossification in patients with FOP in late 1990s; however, the results were inconclusive (Brantus and Meunier, 1998; Kaplan *et al.*, 2008).

Palovarotene

Previous studies showed that exogenous retinoid agonists can block chondrogenesis rapidly and effectively. Palovarotene is an orally active, gamma selective retinoic acid receptor agonist (RARγ) that was tested in murine models for subcutaneous and intramuscular injury-induced heterotopic ossification and proved to be effective in inhibition of heterotopic ossification in addition to restore long bone growth and musculoskeletal functions (Chakkalakal *et al.*, 2016). This brought palovarotene into clinical trial, which is now in phase III trial ongoing on children and adults (NCT03312634) (Semler *et al.*, 2019). Previous trials showed reduction of new ossifications and improvement of chronic pain in 40 studied patients with age above 6 years (Kaplan *et al.* 2018). It is expected to receive approval in the near future, as it was approved in adults with emphysema (Hind and Stinchcombe, 2009).

Interestingly, palovarotene was used in a mouse model of multiple hereditary exostoses, and it suppressed osteochondroma formation. This supports further studies of palovarotene as a potential therapeutic target for multiple hereditary exostoses (Inubushi *et al.*, 2018).

Osteopetrosis

Osteopetroses are a rare heterogeneous group of inherited skeletal disorders with increased bone mass and high bone fragility. They can be inherited by either autosomal dominant (usually benign adult type) or more severe recessive malignant type, which is usually lethal early in life, or rarely x-linked form. Most forms of osteopetrosis are caused by osteoclast dysfunction (osteoclast rich osteopetrosis), whereas a smaller proportion is owing to impaired osteoclastogenesis (osteoclast-poor osteopetrosis) (Sobacchi *et al.*, 2013). Osteoclast impairment leads to bone sclerosis, bone marrow failure with extramedullary hematopoiesis, and dental abnormalities. The resulting thick calvarial bones lead to increased intracranial tension, choanal stenosis, and cranial nerve compression with progressive blindness, deafness, and nerve palsies (Penna *et al.*, 2019).

Hematopoietic stem cell transplantation

As severe osteopetroses are caused by osteoclast dysfunction and osteoclasts are derived from myeloid lineage, so HSCT is the treatment of choice as successful HSCT leads to engraftment of matched donor-derived osteoclast precursors that further differentiate into mature functioning osteoclasts allowing bone remodeling and recovery of hematopoiesis and extramedullary hematopoiesis but fail to rescue secondary neurological deficits, which necessitate doing HSCT as early as possible (Orchard *et al.*, 2015; Natsheh *et al.* 2016). Nevertheless, HSCT is contraindicated in patients with primary neurodegenerative disease and osteoclast-poor osteopetrosis owing to the defect in pro-osteoclastogenic cytokine, RANKL, as HSCs fail to replace osteoblast cells that produce the mutant RANKL that are derived from MSCs. Furthermore, the engraftment of MSCs met some difficulties, which limit their current use for therapies in humans (Zhao and Liu, 2016).

Experience with HSCT was published by different groups and has improved over the years, but it is still considered a risky procedure with lots of complications; therefore, it should be reserved for patients with severe disease. The most encountered posttransplantation complications are GVHD and engraftment failure owing to limited bone marrow space causing delayed hematological recovery in addition to posttransplant hypercalcemia that can be treated with denosumab (Shroff *et al.*, 2012; Sobacchi *et al.*, 2013; Wu *et al.*, 2017). One Egyptian report studied the effect after HSCT on a boy with malignant severe osteopetrosis. Stem cells were derived from healthy identical mother. The transplantation was done at 2 years, and follow-up after 1 year revealed improvement in radiographic bone abnormalities. The skull base and periorbital sclerosis persisted. The patient had short stature and dolichocephaly. No recurrent infections were reported, but abnormal dentition and anemia persisted (El-Sobky *et al.*, 2017).

Gene therapy

Gene therapy represents an alternative option for patients lacking matched donors where genetically modified HSCs, isolated from peripheral blood of the patients with osteopetrosis, are used. The efficacy was tested in oc/oc murine model to study the neonatal transplantation of genetically corrected HSC using retroviral vectors that were able to improve their survival as well as bone resorption but showed risk of leukemia, which later was avoided with the use of lentiviral vectors (Naldini, 2011; Sessa *et al.*, 2016).

Other strategies involve the use of subcutaneous implants releasing soluble RANKL to stimulate osteoclastogenesis in a RANKL-knockout mouse model, showing promising future therapeutic target (Cappariello *et al.*, 2015). Recently, another novel approach using biomimetic scaffold, seeded with Tnfsf11 knockout MSC, overexpressing human soluble RANKL after transduction with lentiviral vector has been produced and then implanted subcutaneously and was able to restore secretion of soluble RANKL and restore the formation of mature osteoclasts in the bone tissue (Menale *et al.*, 2019).

Interferon γ-1b

Owing to the same origin of leukocytes and osteoclasts from hematopoietic stem cells, a study of superoxide generation was found to be dysfunctional in leukocytes, raising the susceptibility to infections in osteopetrosis patients. Additionally, it was found that superoxide is also important for osteoclasts to resorb bone. Trials of interferon γ-1b on ARO showed improvement of leukocyte function as well as decrease in the trabecular bone volume after up to 18 months of therapy (Key *et al.*, 1995); however, the desired benefits are not always seen and limited to use in malignant severe osteopetrosis. Recent clinical trial on the use of interferon γ-1b on patients with adult osteopetrosis type 2 proved to be ineffective, as it could not increase bone turnover markers and was poorly tolerated (Imel *et al.*, 2019b).

Conclusion

GSDs are heterogenous group of disorders that need a multidisciplinary team for management. Progress in genetic technology and groundbreaking research studies in the pathological mechanisms of genetic disorders have paved the way and fueled a lot of unprecedented discoveries for target and new therapies that are disease specific, which are expected to improve the outcome and change the natural history of these chronic disabling disorders and reduce the need for surgical interventions. Further studies and long-term effects are yet to be determined. This review provides a collective guide for clinicians about available and new emerging therapeutic modalities for a wide range of hereditary skeletal disorders.

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There are no conflicts of interest.

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