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Osteopetrosis

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CONTENTS

Abstract

The osteopetroses are a heterogeneous group of sclerosing bone dysplasias with an increased bone density due to an osteoclast defect, with consequently recurrent fractures. Different forms have been described with radiological and clinical features varying from mild to severe with a very poor prognosis. Thus far, six genes have been associated with osteopetrosis and these will be discussed in this review. Except for one gene with a function in osteoclast differentiation, all identified genes encode proteins that play a role in the resorption process. This review additionally focuses on the current treatments for osteopetrosis. In this light, it is crucial that a precise diagnosis be made before therapy is attempted. Recent advances in the understanding of the molecular mechanisms involved in the pathogenesis of these conditions greatly contribute to making the exact diagnosis and selecting the most appropriate type of treatment.

Introduction

Throughout life, bone tissue is continuously undergoing active remodeling. In this tightly coordinated and dynamic process, osteoclasts resorb old bone from the bone surface and new bone is subsequently formed by osteoblasts. Many factors influence this complex interplay between osteoclasts and osteoblasts, which in healthy bone tissue is maintained in a state of dynamic homeostasis. Conversely, an imbalance in the regulation of bone remodeling can result in bone dysplasias with an aberrant bone density. On one side of the clinical spectrum are conditions with a decrease in bone density, osteoporosis and osteogenesis imperfecta being well-known examples. On the other side of the spectrum we find skeletal diseases, including the osteopetroses, in which bone density is increased.

The heterogeneous group of osteopetroses consists of heritable sclerosing bone disorders that are characterized by failure of the osteoclast to resorb bone. The osteoclast is a specialized multinucleated cell arising from the fusion of mononuclear cells from the hematopoietic lineage. This cell type has a typical ruffled border and attaches to the bone surface through the sealing zone, resulting in the formation of an extracellular lacuna where active bone resorption takes place. During resorption, osteoclasts release hydrogen ions and proteases into this sealed microenvironment, thereby acidifying it in order to dissolve and subsequently degrade the mineralized bone matrix (1).

A diversity of osteopetrosis types can be distinguished, all of which are associated with increased skeletal mass and abnormally dense bones, but also with an increased risk of fracture. This apparent contradiction can be explained by the fact that in the osteopetroses the bone is not of optimal structure, with the presence of remnants of unresorbed cartilage. Based on inheritance, age of onset, severity and secondary clinical features, they can be classified into three major groups: 1) autosomal recessive infantile or malignant osteopetrosis; 2) the milder autosomal recessive intermediate osteopetrosis; and 3) autosomal dominant benign osteopetrosis with adult onset (2).

In recent years, significant progress has been made in the understanding of the pathogenesis of these types of sclerosing bone dysplasias. Molecular genetic studies resulted in the identification of osteopetrosis-associated mutations in different genes. Thus far, the majority of these genes encode proteins that play a role in the process of bone resorption, while only one osteopetrosis gene was found to encode a protein involved in the differentiation of osteoclasts.

This review focuses on the current knowledge of the molecular genetics of the osteopetroses. We describe the

radiological and clinical features of the different forms and elaborate on the disease genes known for osteopetrosis at this moment. Additionally, we summarize the current therapies available for these types of skeletal dysplasias.

Different forms of osteopetrosis

Due to their increased bone mass, the osteopetroses are a subgroup of the sclerosing bone dysplasias. They can be differentiated from the other members by a shared mechanism, *i.e.,* impaired osteoclastic bone resorption. Therefore, the presence of remnants of unresorbed cartilage is considered to be a typical histological hallmark for the osteopetroses. Despite a shared pathogenic mechanism, the group is clinically and radiologically very heterogeneous, as shown in Table I. In most human forms, the impaired bone resorption is clearly not due to a defect in the proliferation or differentiation of osteoclasts, as an increased number of osteoclasts is seen, indicating a defect intrinsic to the osteoclast. On the contrary, a few rare cases are reported with a significant decrease in osteoclast number and are therefore called the osteoclast-poor osteopetroses.

Osteoclast-poor osteopetrosis

Only a few osteopetrotic cases with a markedly reduced number of osteoclasts (MIM #259720) have been described in the literature (3, 4). These patients have dense but fragile bones with a severely reduced bone marrow cavity, which contain virtually no hematopoietic cells, including osteoclasts. This suggests a defect in the proliferation or differentiation of osteoclasts rather than a defect intrinsic to the osteoclast itself. This was confirmed by performing bone marrow transplantation in these patients without any improvement of the bone structure despite a successful engraftment (5). One study reported that the patient's' osteoclasts failed to differentiate *in vitro* and neither macrophage colony-stimulating factor (M-CSF, MCSF) nor receptor activator of nuclear factor κ B ligand (RANKL), two crucial factors required for osteoclast differentiation, were able to reverse this defect (3). However, very recently Sobacchi *et al.* (6) were able to find loss-of-function mutations in the gene encoding RANKL in 6 such patients, thereby explaining the impaired differentiation of osteoclasts (Table II).

Osteopetrosis with renal tubular acidosis

This form of osteopetrosis (also known as Guibaud-Vainsel syndrome or marble brain disease; MIM #259730) is associated with renal tubular acidosis and has an autosomal recessive inheritance. The clinical course is not entirely benign. Although the increased bone density begins during childhood, it does not lead to severe bone marrow failure. Other clinical manifestations include cerebral calcifications, short stature, increased frequency of fractures, dental abnormalities, cranial nerve compression and developmental delay (reviewed in Refs. 14 and 15).

A considerable clinical heterogeneity is observed. Patients from the Middle East are most severely affected by mental retardation and acidosis, but do not fracture frequently, whereas those from Europe and North America have mild mental retardation but break bones frequently (16). Loss-of-function mutations in the carbonic anhydrase II (*CAII*) gene have been shown to be responsible for this form of osteopetrosis (7) (Table II). This enzyme catalyzes the intracellular conversion of CO_2 and H_2O to HCO_3^- and H^+, providing a source of protons to acidify the extracellular resorption lacuna. All patients with this form discovered so far have been found to have mutations in the coding sequence or the splice site junctions of the *CAII* gene (17). The most common, known as the Arabic mutation, leads to loss of the splice site junction of intron 2. It accounts for almost all cases in the Arab populations of the Middle East and Maghreb, which comprise almost 75% of those reported (16). One interesting study reported that 14 Tunisian families with *CAII* deficiency were all descended from a common ancestor in the Maghreb in the 10th century (18).

Malignant autosomal recessive osteopetrosis

Malignant or infantile autosomal recessive osteopetrosis (ARO; MIM #259700) is a rare but severe disorder with an average incidence of 1:300,000. The highest incidence is found in Costa Rica (3.4:100,000) (19, 20). It

Table I: Overview of the different forms of osteopetrosis.

Type of osteopetrosis	Clinical and radiological features
Autosomal recessive osteopetroses	
Osteoclast-poor osteopetrosis	Severe osteopetrosis, reduced number of osteoclasts
Osteopetrosis with renal tubular acidosis	Mild osteopetrosis, renal tubular acidosis, cerebral calcifications, short stature, mental retardation, fractures
Malignant osteopetrosis	Severe osteopetrosis, bone marrow failure, fractures, infections, neurological symptoms, early death
Intermediate osteopetrosis	Mild osteopetrosis, fractures, short stature
Autosomal dominant osteopetrosis	
Type II	Fractures, Rugger-Jersey spine, bone-in-bone appearance

Table II: Overview of the identified genes involved in human osteopetrosis.

Gene	Protein function	Type of osteopetrosis	Mechanism	Reported patients	Ref.
Osteoclast differentiation					
RANKL	Osteoclast differentiation	Osteoclast-poor osteopetrosis	Loss of function	Six patients	6
Osteoclast function					
CAII	Production of protons and carbonic acid	Osteopetrosis with renal tubular acidosis	Loss of function	More than 100 families	7
TCIRG1	Proton pump	Malignant osteopetrosis	Loss of function	About 50% of patients with malignant osteopetrosis	8
CLCN7	Chloride channel	• Malignant osteopetrosis	• Loss of function	• About 10% of patients with malignant osteopetrosis	9-11
		• Intermediate osteopetrosis • Autosomal dominant osteopetrosis type II	• Partial loss of function • Dominant-negative effect	• A few families • Majority of patients with ADOII	
OSTM1	β-Subunit of *CLCN7*	Malignant osteopetrosis	Loss of function	Five patients	12
PLEKHM1	Involved in GTPase signaling	Intermediate osteopetrosis	Loss of function	One family	13

typically presents during childhood, and if untreated, most patients die during the first decade of life due to recurrent infections. Patients with this form are characterized by a diffuse sclerotic skeleton. Typical radiological findings include the mask-like appearance of the skull, the typical bone-in-bone feature and club-like shape of the metaphyses of long bones. The bone marrow spaces are severely narrowed, resulting in anemia, extramedullar hematopoiesis and hepatosplenomegaly. Other symptoms include multiple fractures and loss of hearing and sight due to narrowing of the foramina. Other complications occur with variable severity and outcome, including nystagmus, failure to thrive, hydrocephalus, sleep apnea, cerebral ischemia, arterial stenosis, developmental delay, mental retardation, osteomyelitis of the mandible, cerebral atrophy and dysarthria (19-22). Increased osteoclast numbers were observed in most biopsies from malignant ARO patients (23).

So far, three genes (*TCIRG1, CLCN7* and *OSTM1*) are known to be involved in the pathogenesis of malignant osteopetrosis (Table II). However, not all malignant ARO cases could be explained by mutations in any of these three genes, highlighting the existence of at least one other gene involved in this type of osteopetrosis. About 50% of patients with malignant ARO have inactivating mutations in the *TCIRG1* gene (also called the *ATP6i* gene), encoding the A3 subunit of the vacuolar proton pump adenosine triphosphatases (8, 24-27). This protein is located in the ruffled border, where it releases protons in the resorbing lacuna, acidifying this microenvironment and solubilizing the hydroxyapatite crystals. Most of these homozygous and compound heterozygous mutations are predicted to disrupt the protein product. In Costa Rica, where the incidence of malignant ARO is much higher than the expected incidence, there is evidence for a founder effect with two missense mutations accounting for all the defects in 9 unrelated families (8). *In vitro* cultured osteoclasts from 2 patients with compound heterozygous *TCIRG1* mutations could attach to the bone matrix, but were not able to secrete acid into the resorption lacuna, confirming the functional relevance of the *TCIRG1* gene (28).

Loss-of-function mutations in the *CLCN7* gene, encoding chloride channel 7, are responsible for about 10% of cases of malignant osteopetrosis (9, 11, 12). *CLCN7* is located in the lysosomes, late endosomes and the ruffled border of osteoclasts, and provides the chloride conductance necessary for an efficient proton pump. However, recent work on other members of the CLC family suggests that CLC channels rather act as electrogenic Cl^-/H^+ exchangers (29).

The clinical spectrum of malignant ARO patients harboring *CLCN7* mutations is broader than patients with *TCIRG1* mutations. The phenotype of patients in this latter group is homogeneous; their nervous system involvement is secondary to the compression of the foramina. On the other hand, there is increasing evidence that patients with recessive *CLCN7* mutations also show primary neurological defects (including primary retinal degeneration) in addition to the cranial nerve compression (9, 12). Moreover, severe lysosomal storage dysfunctions have been described. Studies using *Clcn7*- and *Tcirg1*-null mice confirmed these observations (9, 27, 30). The prognosis of this *CLCN7*-dependent form is extremely poor, having implications for treatment (see below).

A third gene found to be mutated in some malignant ARO patients is the *OSTM1* gene (12). So far, only 5 malignant ARO patients have been described carrying loss-of-function mutations in this gene (26, 31, 32). All patients suffered from an extremely severe osteopetrotic phenotype and died before the age of 6 months. These

patients also had lysosomal storage disease and abnormalities in the central nervous system, including defective myelination, hypoplasia of corpus callosum and cerebral atrophy —a phenotype that is similar to that observed in the *grey-lethal* mouse carrying a deleterious mutation in this gene (12). These findings suggest that malignant ARO with *OSTM1* mutations defines a new subset of patients with a very poor prognosis (26).

The *OSTM1* gene encodes a type I transmembrane protein with E3 ubiquitin ligase activity, suggesting a role for *OSTM1* in the protein degradation machinery (33). Recent work identified *OSTM1* as a β-subunit for *CLCN7* and demonstrated that it requires *CLCN7* to localize to lysosomes. The stability of *CLCN7* depends on its association with *OSTM1*, and the authors speculated that the highly glycosylated OSTM1 protein shields CLCN7 from lysosomal proteases. The osteopetrosis and the neuronal degeneration upon loss of OSTM1 may be largely explained by reduced levels of CLCN7 (34).

Intermediate autosomal recessive osteopetrosis

Patients with the intermediate form of osteopetrosis (IARO; MIM #259710) present with the typical radiological features of the other forms, including a generalized increase in bone density with metaphyseal modeling defects and a bone-in-bone appearance. The inheritance is autosomal recessive. Other clinical manifestations may include short stature, osteomyelitis, dental problems and fractures. In general, no severe hematological manifestations are observed. Intermediate osteopetrosis can be differentiated from malignant osteopetrosis because the outcome is less severe and the life expectancy is much greater (35-37). So far, two genes (*CLCN7, PLEKHM1*) have been identified that play a role in the pathogenesis of intermediate osteopetrosis (Table II). It still needs to be established whether additional genes are also involved in this form. Homozygous mutations in the *CLCN7* gene in families with this intermediate type of osteopetrosis were reported. These mutations are predicted to lead to only partial loss of function and hence to a less severe phenotype (10, 12). However, functional studies will be necessary to make proper genotype-phenotype correlations.

Recently, we demonstrated that the *PLEKHM1* gene has a crucial role in bone resorption. We first identified this gene by positional cloning studies in the *incisors absent* rat. The mild osteopetrosis seen in this rat model is caused by a loss-of-function mutation in the *Plekhm1* gene. We also identified an inactivating mutation in the *PLEKHM1* gene in 2 members of a family with intermediate autosomal recessive osteopetrosis. The clinical outcome in this family is mild. The oldest patient suffers from Erlenmeyer flask deformities of the distal femora and a chondrolysis of the hip. The youngest brother, homozygous for the *PLEKHM1* mutation, has not yet developed any clinical symptoms; however, radiological examination revealed the presence of dense metaphyseal bands in the long bones. *In vitro* cultured osteoclasts from the 2 patients failed to form ruffled borders and showed little evidence of bone resorption. The *PLEKHM1* gene encodes a cytosolic protein without any transmembrane helices. It contains a RUN and two pleckstrin homology domains, indicating that it might be involved in the small GTPase signaling pathway. Indeed, overexpression studies implicated *PLEKHM1* as a component of Rab7-regulated late endosomal trafficking in osteoclasts (13).

Autosomal dominant osteopetrosis type II

The autosomal dominant form of osteopetrosis type II (ADOII, Albers-Schönberg disease; MIM #166600) generally presents with a milder phenotype and is therefore also known as the benign form. It is more common than the recessive forms, with a prevalence of up to 5.5:100,000. However, since many patients are asymptomatic and are only detected by coincidental radiographic examination, the prevalence could be underestimated. This form is characterized by a sandwich-like appearance of the spine due to thickening of the endplates of the vertebral bodies (Rugger-Jersey spine), endobone structures in the iliac wings and sclerosis of the skull base. The main clinical features are multiple fractures, osteomyelitis and cranial nerve involvement (38-41). Although this type is generally accepted as mild, there are reports of families with a high intrafamilial clinical variability ranging from anemia in the neonatal period to asymptomatic increased bone density in adults (10, 42, 43). In addition, the penetrance of this type is incomplete and ranges from 75% to 94% (40, 41, 44, 45). Both genetic and environmental factors may likely be involved in the mechanism of this incomplete penetrance and highly variable phenotype. Chu *et al.* (46) found potential evidence of linkage for a modifier gene on chromosome 9q21-22 that may affect the autosomal dominant osteopetrosis disease status and severity. No differences in differentiation were observed between *in vitro* osteoclasts from ADOII patients and controls. However, osteoclasts from a few ADOII patients show a more motile phenotype, as demonstrated by the appearance of lammelipodia and membrane ruffling (23). Heterozygous mutations in the *CLCN7* gene, which most likely act in a dominant-negative way, underlie most cases with autosomal dominant osteopetrosis (11, 47) (Table II). However, in a few typical ADOII cases, no mutations in the coding region of the *CLCN7* gene were found (our unpublished observations, Ref. 23).

Treatment

Osteopetrosis patients can suffer from a broad range of clinical symptoms that require pain relief and symptomatic treatment. Obviously, this only can improve the quality of life of the patients treated, but not reverse the real causes of these symptoms. Attempts to address the causes of different forms of osteopetrosis have mainly focused on hematopoietic stem cell transplantation (HSCT), which could induce the differentiation of functional osteoclasts and therefore represent a real cure for the disease.

HSCT as a treatment for osteoclast-poor osteopetrosis has been tried in some patients with no or only limited success (6). Nicholls *et al.* (5) reported a limited rescue of this form after successful hematopoietic engraftment. This was not unexpected since it is now known that, at least in some cases, the genetic defect is outside the osteoclast lineage, but rather in loss of functional RANKL. Therefore, attempts to treat these cases with RANKL protein will definitely be performed in the future with high expectations.

Patients with malignant ARO have a poor prognosis. They are anemic due to bone marrow failure, and together with the fact that the peripheral blood leukocytes of these patients are unable to produce superoxide in response to bacterial and viral stimuli, they are also highly susceptible to infections. For these patients, HSCT, with all its risks, is definitely an option. A retrospective study of 69 children with malignant osteopetrosis who received allogeneic HSCT demonstrated a 79% probability of 5-year survival with osteoclast function for HLA-matched transplants (48). Graft failure rates are much higher when no HLA-compatible donor is available and T cell-depleted transplants are used to prevent complications of graft-*versus*-host disease (49). Also, conservation of vision is better in children transplanted before the age of 3 months (50). The underlying genetic defect appears to be of prognostic relevance concerning HSCT in malignant ARO cases. In the case of *TCIRG1* defects, the neural defects are secondary to the compression of the foramina because of skull deformities, and HSCT can rescue these secondary features (26). On the other hand, mutations in *CLCN7* and the β-subunit *OSTM1* also cause neurodegeneration and lysosomal storage disease. These primary neurological defects cannot be rescued, which leads to death despite the transplantation (9, 34).

The phenotype of osteopetrosis with renal tubular acidosis and cerebral calcifications varies considerably. Only where the risks are justified should HSCT be considered. Otherwise, symptomatic treatment should be sought. Although HSCT restores normal osteoclast function and bone remodeling, it fails to provide a self-replenishing source of the carbonic anhydrase enzyme to renal tubular cells, and it may prevent cerebral calcifications only if the donor is homozygote wild type for *CAII* (51).

ADOII is characterized by a broad spectrum of clinical and radiographic features. It varies from asymptomatic to severely affected patients with multiple fractures, osteomyelitis, cranial nerve deficits and bone marrow failure (52). Intermediate osteopetrosis includes mandibular prognathism, occasional osteomyelitis, genu valgum, hepatosplenomegaly and a tendency to fracture (10, 13). When needed, ADOII and intermediate osteopetrosis are treated symptomatically. In severe forms of ADOII with bone marrow failure, HSCT might be considered.

In cases where HSCT is not designated, medical treatment with interferon gamma, calcitriol and prednisone may be favorable. Interferon gamma, a cytokine produced by T lymphocytes and natural killer (NK) cells, enhances the capacity to generate superoxide by white blood cell phagocytes (53). In addition, interferon gamma is believed to promote osteoclast superoxide generation, which contributes to the bone resorption process by fragmentation of bone matrix collagen and proteins (54). These positive effects have been reported in a small number of patients, but more studies are warranted and awaited (55). High doses of calcitriol and prednisone have also been reported to be helpful in the treatment of osteopetrosis (56, 57). Calcitriol is the active form of vitamin D and promotes osteoclastogenesis, whereas prednisone, a corticosteroid, improves blood counts in patients with anemia (57, 58). The positive effect of both medical treatments seems unwarranted and further investigations are needed (21).

Discussion

Clinical and radiological data already indicated that the osteopetroses are a heterogeneous group of conditions. The molecular genetic findings on the primary causes of these conditions confirmed this, with six genes having been identified at this point; most likely several others remain to be identified. Furthermore, these genes confirm that osteopetroses have a pathogenic mechanism impairing osteoclastic bone resorption. Autosomal dominant osteopetrosis type I is one exception to this rule; it was considered to be an osteopetrosis based on the osteoclastopenia seen in these patients (40). However, as these patients lack the typical hallmark of cartilage remnants, the inclusion of this form with the osteopetroses has long been debated. Recently, we were able to show that this type of osteopetrosis is due to a gain-of-function mutation in the low-density lipoprotein receptor-related protein 5 (*LRP5*) gene, which plays a role in Wnt signaling (59). Since there is currently plenty of evidence that this is a key pathway in bone formation, the involvement of *LRP5* in ADOI supports the removal of autosomal dominant osteopetrosis type I from the group of osteopetroses (60).

It is clear that identification of the molecular genetic cause of cases of osteopetrosis is of major relevance for performing genetic counseling in osteopetrosis families. It can also provide strong indications for the prognosis of the patient and could also lead to decisions on the appropriate treatment. Furthermore, novel treatments may evolve from the future identification of other osteopetrosis genes. This might soon become true for osteoclast-poor patients with RANKL administration. Also, research is ongoing for direct treatment strategies based on the nature of a specific mutation in a patient. Modified U1 small nuclear nRNAs are considered to correct specific splicing defects in the *TCIRG1* gene (26). Alternative strategies are also being considered for ADOII. As these patients are heterozygous with a dominant-negative effect of the mutated CLCN7 protein, one could try to specifically knock down the mutated copy, thus reversing the pathogenic mechanism. Finally, Frattini *et al.* (28) were able to rescue murine malignant osteopetrosis by HSCT *in utero*. The future will tell whether any of these

potential strategies will be of value for the treatment of at least a subset of osteopetrosis cases.

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