

REVIEW

GENETICS IN ENDOCRINOLOGY

Autosomal dominant osteopetrosis revisited: lessons from recent studies

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Abstract

Systematic studies of autosomal dominant osteopetrosis (ADO) were followed by the identification of underlying mutations giving unique possibilities to perform translational studies. What was previously designated ADO1 turned out to be a high bone mass phenotype caused by a missense mutation in the first propeller of *LRP5*, a region of importance for binding inhibitory proteins. Thereby, ADO1 cannot be regarded as a classical form of osteopetrosis but must now be considered a disease of *LRP5* activation. ADO (Albers-Schönberg disease, or previously ADO2) is characterized by increased number of osteoclasts and a defect in the chloride transport system (ClC-7) of importance for acidification of the resorption lacuna (a form of Chloride Channel 7 Deficiency Osteopetrosis). *Ex vivo* studies of osteoclasts from ADO have shown that cells do form normally but have reduced resorption capacity and an expanded life span. Bone formation seems normal despite decreased osteoclast function. Uncoupling of formation from resorption makes ADO of interest for new strategies for treatment of osteoporosis. Recent studies have integrated bone metabolism in whole-body energy homeostasis. Patients with ADO may have decreased insulin levels indicating importance beyond bone metabolism. There seems to be a paradigm shift in the treatment of osteoporosis. Targeting ClC-7 might introduce a new principle of dual action. Drugs affecting ClC-7 could be antiresorptive, still allowing ongoing bone formation. Inversely, drugs affecting the inhibitory site of *LRP5* might stimulate bone formation and inhibit resorption. Thereby, these studies have highlighted several intriguing treatment possibilities, employing novel modes of action, which could provide benefits to the treatment of osteoporosis.

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Introduction

The concept of osteopetrosis was introduced in the 1920s to describe patients with radio-opaque bones and universal osteosclerosis. The disease corresponded to marble bone disease (1) first described in 1904 by the German radiologist Albers-Schönberg (2). He described a man with multiple fractures and a radiographic osteosclerosis characterized by increased cortical thickness and reduced marrow space. It became clear that osteopetrosis could be seen in families, and based on mode of inheritance, Johnston *et al.* (1968) (3) suggested two different forms: a benign form designated autosomal dominant osteopetrosis (ADO) and a malignant form seen in childhood and inherited in an autosomal recessive manner, termed autosomal recessive osteopetrosis (ARO). However, other forms were described during the following years, and moreover, it became clear that the clinical presentation of each of the

heritable forms varied. Intermediate forms with relatively mild course but a recessive mode of inheritance were identified. In one of these, the patients presented basal ganglia calcifications and renal tubular acidosis, and this syndrome was subsequently found to be caused by carbonic anhydrase deficiency (4, 5, 6, 7). With these observations, malfunctioning bone resorption was introduced as a common pathogenic factor in osteopetrosis, and at least in the intermediate forms, this was demonstrated in the beginning of the 1980s to be due to an acidification defect across the osteoclast cell membrane (5), see section on Genetic studies identifying *CLCN7*.

Osteopetrosis was described as a heterogeneous group of diseases related to defective bone resorption (3). However, also within ADO, a systematic search for the disease revealed radiographic heterogeneity (8). Based on several families with ADO from the county of Funen, Denmark, in the mid-1980s, we described two distinct

radiological forms based on plain radiographs (8). Further studies characterizing ADO at the clinical, biochemical, histological, and biomechanical levels revealed that these types corresponded to two distinct disorders, which we designated as ADO type 1 (ADO1) and ADO2 (9, 10, 11, 12, 13). These early studies were reviewed in 1989 (14). ADO was defined as a condition with diffuse osteosclerosis, primarily involving the axial skeleton, but with symmetrical effects on the long bones and with little or no modeling defects (14). Based on bone biopsies, it was hypothesized that the pathogenesis of these two forms involved defective bone resorption, directly or indirectly.

During the recent 20 years, investigations of osteopetrosis have contributed significantly to our insight into bone biology. After the millennium, it turned out that ADO1 was caused by an activating mutation in *LRP5* (15) and thus cannot be recognized as a classical form of osteopetrosis but rather should be regarded as a *LRP5*-activating bone disease, or high bone mass (HBM) phenotype. Also, the disease most probably described by Albers-Schönberg (2) turned out to be a form of Chloride Channel 7 Deficiency Osteopetrosis (16). However, it should be noted that a mutation in *CLCN7* has not been demonstrated in up to 30% of patients presenting with a clinical phenotype of ADO, indicating further heterogeneity (17, 18).

The aim of the present review is to give an overview of clinical lessons learned by studies on large and homogenous populations of patients with ADO and HBM. This review will discuss genetic studies leading to the identification of the mutated genes in the original cohorts, of instrumental importance for separating the syndromes into an intrinsic bone-resorptive defect (Chloride Channel 7 Deficiency Osteopetrosis (ADO)) and a *LRP5* activation disease related to the bone formative site. Moreover, we will describe subsequent *in vitro* investigations on bone-resorptive cells in ADO, increasing the understanding of bone biology and the coupling principle. Also, we will critically review recent data on metabolic consequences of disturbed bone resorption in ADO. Finally, perspectives from these studies regarding treatment of other metabolic bone diseases are outlined. For this purpose, literature was searched through PubMed for the key words autosomal dominant (or benign) osteopetrosis, osteoclasts, bone resorption, HBM, and coupling.

Summary of recent systematic clinical studies

Clinical symptoms, bone mass, and bone structure

Two large cohorts of well-characterized patients with ADO have been described in detail after the millennium (19, 20). Moreover, Frost *et al.* (21) recently updated the

HBM_{T2531} cohort. Bone mass in adults based on dual-energy X-ray absorptiometry (DXA) is almost increased to the same level in patients with the bone-resorptive defect (ADO) as in patients with *LRP5* activation bone disease. Thus, ADO patients and our HBM_{T2531} cohort had increased bone mineral density (BMD) at all skeletal sites measured. Z-scores ranged from +2 to +12 at the lumbar spine, which is mostly comprised of trabecular bone, and the whole-body compartment, consisting primarily of cortical bone, Fig. 1, also illustrating studies by high-resolution peripheral quantitative computed tomography (CT) (20, 21, 22).

Penetrance is high in ADO; however, several asymptomatic carriers have been described (14, 20). Waguespack *et al.* (20) described a total of 32 obligate carriers and presented data on BMD in six. In these, BMD Z-scores were modestly elevated with values ranging from 0 to +4 (Fig. 1). The authors had the possibility to reevaluate six patients and one carrier after more than 30 years since the original description (3, 20). In all patients, clinical symptoms progressed, whereas the carrier remained asymptomatic (20). The progression of symptoms is in alignment with progression of the universal osteosclerosis, as indicated by cross-sectional studies using DXA and histomorphometry (11, 12, 20, 21, 23).

Patients with ADO have typical and prevalent symptoms (2, 3, 10, 19, 20, 24). The typical findings are increased fracture frequency, delayed healing, and osteomyelitis, especially in the jaw (2, 10, 19, 20). The latter is of special interest in relation to the current discussion of osteonecrosis of the jaw (ONJ) associated with bisphosphonates and denosumab, i.e. drugs that decrease the number and activity of osteoclasts (25, 26, 27, 28, 29), as will be discussed in more detail below.

With the prevalent and often serious symptoms, the term benign osteopetrosis could be questioned (19, 20). As an example of this, Waguespack *et al.* (20) found early-onset vision loss in one-fifth of the patients and signs of bone marrow failure in 3%. Thus, their cohort had clearly more serious symptoms than we found in our original studies (14). However, the findings are prone to selection bias, as patients with symptoms are found by clinical work-up, whereas patients with sparse symptoms and carriers are mostly found by family studies (14, 19, 20).

Osteonecrosis in the jaw

ONJ occurs in cancer patients with bone metastasis treated with monthly dosages of i.v. administered bisphosphonates or denosumab (with a frequency of 1.3 and 1.8% respectively) (30) and in rare cases (two cases per 100 000 patient years) of patients with osteoporosis treated with oral bisphosphonates (31). ONJ has also been reported in patients with osteoporosis treated with denosumab every 6 months (32). The risk of ONJ in patients with osteoporosis treated with

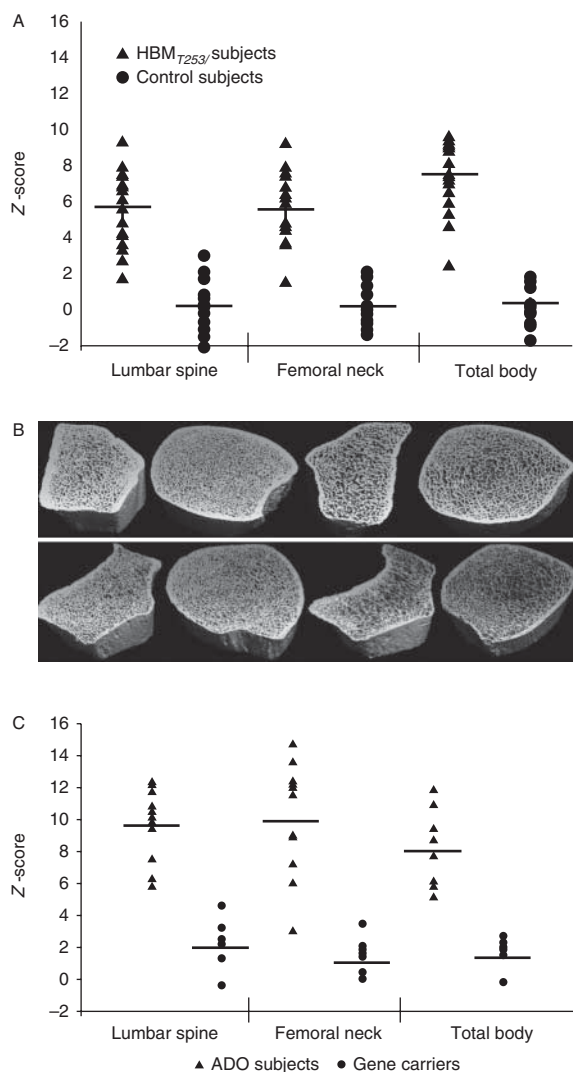


Figure 1 Bone mass by DXA and high-resolution pQCT in autosomal dominant osteopetrosis and the HBM phenotype. (A) Individual BMD Z-scores in 19 patients with HBM_{T253I} compared with controls. The figure is based on data and reproduced from Frost M, Andersen T, Gossiel F, Hansen S, Bollerslev J, Van Hul W, Eastell R, Kassem M & Brixen K. Levels of serotonin, sclerostin, bone turnover markers as well as bone density and microarchitecture in patients with high bone mass (HBM) phenotype due to a mutation in *Lrp5*. *Journal of Bone and Mineral Research* **26** 1721–1728, 2011, with permission. (B) Upper panel: high-resolution pQCT of radius and tibia in a case with HBM_{T253I} (left two pictures) and a control subject (right two images); reproduced from Frost M, Andersen T, Gossiel F, Hansen S, Bollerslev J, Van Hul W, Eastell R, Kassem M & Brixen K. Levels of serotonin, sclerostin, bone turnover markers as well as bone density and microarchitecture in patients with HBM phenotype due to a mutation in *Lrp5*. *Journal of Bone and Mineral Research* **26** 1721–1728, 2011, with permission. Lower panel: high-resolution pQCT of radius and tibia in a case with ADO (left two pictures) and a control subject (right two images). (C) Individual BMD Z-scores from with ADO and obligate carriers. The figure is reproduced from Waguespack SG, Hui SL, Dimeglio LA & Econs MJ. Autosomal dominant osteopetrosis: clinical severity and natural history of 94 subjects with a chloride channel 7 gene mutation. *Journal of Clinical Endocrinology and Metabolism* **92** 771–778, 2007, with permission.

bisphosphonates increases following tooth extractions and with the duration of therapy. The pathogenesis of ONJ related to bisphosphonates and denosumab is currently unknown. Infection, osteocyte death, soft tissue toxicity, interference with angiogenesis, concomitant therapy, and decreased bone turnover have been suggested as causative factors (33). Occurrence of ONJ following two completely different drugs such as bisphosphonates and denosumab sharing only the common effect of decreasing bone turnover (and number of osteoclasts) as well as case reports indicating that bisphosphonate-related ONJ may respond well to therapy with teriparatide suggests that low bone turnover is essential in the pathogenesis of this condition. In ADO, however, inflammation and not necrosis is the usual feature. Patients with ADO also have other dental abnormalities such as delayed tooth eruption, enamel hypoplasia, and dental caries (34) that may predispose to infection of the jaw. Also, non-jaw osteomyelitis is seen in ADO (19) but not following bisphosphonate therapy. Bisphosphonates are taken up not only by osteoclasts but also by macrophages and enhance apoptosis in these cells. The role of macrophages in ONJ and osteomyelitis in patients with ADO, however, is currently unknown.

Bone remodeling

Histomorphometric analyses of bone biopsies from adult patients with ADO are limited. Early studies at the trabecular (11) and cortical envelope (23) indicated virtually normal bone remodeling and an endosteal resorption defect. However, the studies were limited by few individuals investigated and the immense analytical variability of histomorphometry with the risk of a type 2 error (35). The data were in accordance with syndromes of defective bone resorption and normal, or at least not increased, bone formation. In normal subjects, administration of triiodothyronine (T_3) activates bone remodeling (36). In agreement with the relative benign character of ADO, bone turnover can be stimulated by T_3 (37, 38, 39), but the response is blunted for bone resorption as well as for formation markers (38, 39, 40).

ADO is osteoclast rich

Detailed ultrastructural investigations of bone biopsies from ADO patients revealed that osteoclasts were markedly increased in size and number (almost threefold) compared with controls (41) and of the typical multinucleated type. Moreover, biochemical markers of osteoclast number, TRACP and CKBB, were markedly increased in ADO (13, 16, 42, 43, 44, 45). Based on these studies, ADO was described as an osteoclast-rich form, to be discussed in detail below.

Genetic studies identifying *CLCN7*

Positional cloning was used to identify the disease-causing genes in the original populations. This involved a two-step procedure first aiming to localize the affected gene to a chromosomal region and subsequently perform mutation analysis from the delineated region (46). For the first step, we performed genetic linkage analysis in extended families (47).

Identification of the role of the *CLCN7* gene in ADO

In the context of epidemiological and clinical studies in the county of Funen, Denmark, an extended family was ascertained with a large number of family members affected with the classical radiographic appearance of ADO (9). Genome-wide linkage studies revealed a gene localization on chromosome 16p13.3 based on the analysis of six extended families, including the original Danish pedigree (48). By far, the most interesting candidate gene within the delineated region was the *CLCN7* gene encoding a chloride channel. Kornak *et al.* (49) showed that loss of the ClC-7 chloride channel in mice results in severe osteopetrosis. Furthermore, they reported one patient with ARO due to two compound heterozygous mutations in this gene. We identified heterozygous *CLCN7* mutations in all ADO families used for the linkage study as well as in six additional ADO patients (50). The ClC-7 chloride channel has 12 transmembrane domains, which turned out to function as a slowly voltage-gated $2\text{Cl}(-)/1\text{H}(+)$ -exchanger (51). Currently, 25 different mutations have been documented and found in all regions of the gene but with a cluster around amino acids 313–318 and in the intracellular part before the carboxyterminal end of the protein (Fig. 2). Most mutations are missense mutations causing an amino acid substitution with two exceptions. One results in a deletion of one amino acid

(ΔL688), but it can be assumed that this might not disturb the topology of the chloride channel (50). The other is a deletion of two nucleotides (2423delAG) found in two families and causing a frameshift mutation. However, this mutation is located very close to the carboxyterminal part of the protein (50). A possible effect of missense mutations on the functioning of the encoded protein can be complete loss of function. However, for the *CLCN7* mutations, this seems to be unlikely. First, severe ARO has been described in patients with loss of function mutations (49), but the parents were clinically and radiographically normal despite having a heterozygous loss of function mutation. Secondly, ClC-7 is known to function in a dimeric state. Together, this indicates that for the ClC-7 protein, there is no effect of haplo-insufficiency in humans while amino acid substitutions might have a dominant negative effect. Several studies have investigated the direct effects of the mutations. Analyses of human osteoclasts showed that the G215R mutation, which is the most common, reduces acid secretion into lysosomes (52, 53). Further studies using over-expression systems indicated that this was related to ER retention of the mutated protein, a finding also observed for other mutations, such as G240R and R526W (51, 54). Another group of mutations was shown to alter the gating kinetics, thereby rendering the transporter inactive (51). However, the full understanding of the relationship between the mutations and osteoclast functionality is generally unclear, and it is further clouded by the presence of unaffected carriers of the mutations, a phenotype that is manifested even in isolated osteoclasts (55).

Genotype–phenotype correlation

Many of the mutations identified are private to one family but some have been reported in several families. So far, no clear-cut genotype–phenotype correlation has been established (56). This is not unexpected taking into account the high intra-familial clinical variability ranging from asymptomatic to severely affected. This also implies a reduced disease penetrance, which has been estimated to be between 66 and 94% (3, 19, 20, 57, 58). A clear explanation for the reduced penetrance and the intra-familial variability is not available. In theory, single-nucleotide polymorphisms in the promoter region might influence the ratio between the mutated and the wild-type copy of the protein. Interestingly, a coding polymorphism, V418M, has been reported to be associated with the severity of the ADO presentation (59, 60). Furthermore, a 50 bp variable number of tandem repeat (VNTR) polymorphism with a copy number between 2 and 9 is present in intron 8 of the human *CLCN7* gene. This repeat is associated with BMD in the general population, but also with the severity of ADO (61). The mechanism by which this repeat influences the function of ClC-7 is

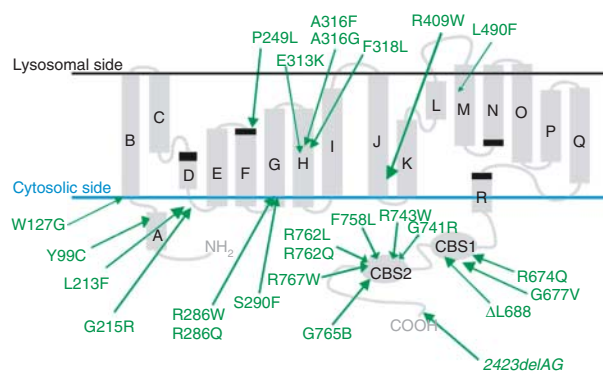


Figure 2 Topology of the chloride channel 7 (ClC-7) protein. The lysosomal side is indicated with a black line, while the cytosolic side is indicated with a blue line. Known mutations in patients with autosomal dominant osteopetrosis type 2 are indicated. The original Danish pedigree harbored the G215R mutation (50).

unclear, but an effect on exon–intron splicing cannot be excluded. The V418M and the VNTR polymorphism are to some degree in linkage disequilibrium (62). Therefore, the real causal variant remains to be demonstrated.

In addition to this, it is clear that ADO is genetically heterogeneous as no *CLCN7* mutation could be found in up to 30% of patients presenting with a clinical phenotype of ADO (17, 18). No clinical or radiological difference between those with or without a *CLCN7* mutation has been reported.

Analysis of osteoclasts from ADO patients

Osteoclast-rich forms of osteopetrosis and in particular ADO have been studied in detail *in vitro*. Histomorphometric analyses of ADO and osteoclast-rich ARO patients showed increased numbers of very large osteoclasts *in vivo* (17, 41). *In vitro* analyses showed that osteoclastogenesis was normal, both with respect to time frame and numbers of osteoclasts, and with respect to morphology (17, 52, 53, 63, 64). The underlying reason for the lack of bone resorption by these osteoclasts was shown to be reduced acid secretion into the resorption lacunae (52, 53, 63, 64, 65), an effect also seen in *CLC-7*-deficient mice (49).

Further studies of the ADO osteoclasts indicated that the reason for the increased number of osteoclasts *in vivo* is related to increased survival of the osteoclasts. The osteoclasts have an attenuated capacity to resorb the calcified bone matrix. This mechanism, at least partially, seems to have an auto-regulatory effect of calcium directly on osteoclast survival (63, 66). However, other studies have also shown that release of transforming growth factor β from the bone matrix caused induction of osteoclast apoptosis (67) and thus also participates in this auto-regulatory control of osteoclast activity. In addition to increased survival due to decreased release of pro-apoptotic factors from the mineral, increases in parathyroid hormone could contribute to the increased number of osteoclasts (68). However, this increase in survival is also seen *in vitro* in pure cultures of osteoclasts where acid secretion is reduced due to mutations or blocked pharmacologically hence showing at least some PTH-independent osteoclast survival effects (63, 66, 69).

In addition to shedding light on the regulation of the life span of osteoclasts, another highly interesting aspect of bone remodeling has been studied extensively based on the pathophysiology of patients with ADO, namely the coupling of bone resorption to bone formation. In the ADO patients, as well as other osteoclast-rich forms of osteopetrosis, it has been shown that bone formation is ongoing, despite the absence of bone resorption (11, 17, 41, 42), a phenomenon that is also found in rodent models of osteoclast-rich osteopetrosis (70, 71), and in animal

models treated with inhibitors of osteoclastic acid secretion (63, 72, 73, 74).

In accordance with these studies, a series of *in vitro* studies of osteoclasts have shown that independent of their resorptive activity, osteoclasts produce anabolic signals for osteoblasts (69, 75, 76, 77), thus explaining the origin of the ongoing bone formation in the ADO/osteoclast-rich osteopetrosis patients, see Fig. 3. Furthermore, these studies showed that only mature osteoclasts produce the anabolic signal (76) and hence illustrated the specificity of this phenomenon, while also providing some evidence as to why bone formation appears very low or even missing in the absence of osteoclasts, as seen in the RANK-deficient patients (78). However, controversies still exist, as demonstrated recently where inhibition of bone resorption *in vitro* with the V-ATPase inhibitor bafilomycin blunted release of anabolic factors from the bone matrix (79). Thus, there are still discussions related to the origin of the anabolic molecules initiating and driving bone formation as a consequence of bone resorption by osteoclasts.

With respect to molecular candidates for the bone anabolic factors derived from the osteoclasts, there are several. They include well-known bone-stimulating

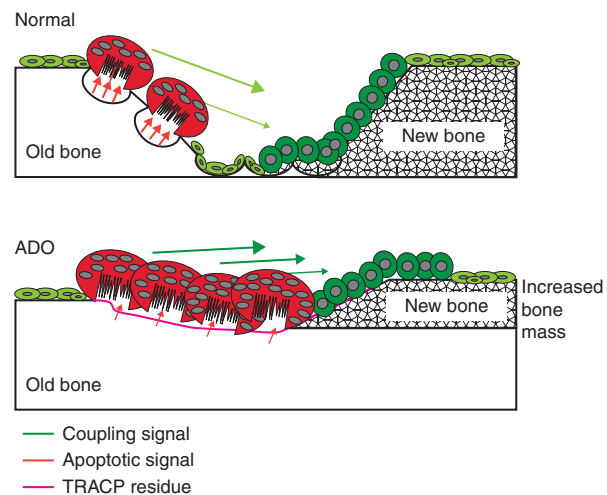


Figure 3 Schematic illustration of the interplay between bone lining cells, osteoclasts, and osteoblasts under normal circumstances (upper panel) and with autosomal dominant osteopetrosis (ADO; lower panel). Upper panel: bone resorption releases apoptotic factors ensuring termination of resorption and the osteoclasts secrete anabolic molecules for the osteoblasts ensuring that bone formation follows bone resorption and thereby completing the bone remodeling cycle. Lower panel: in ADO, resorption per osteoclast is greatly reduced, and this has been shown to lower the release of apoptotic factors from the bone matrix, resulting in larger numbers of large and multinuclear osteoclasts. These osteoclasts are still anabolically active, and they secrete factors to the osteoblasts ensuring rather normal bone formation, which when combined with defective bone formation is the reason for the increased bone mass in these patients. An interesting phenomenon, namely deposition of TRACP residue along the resorbed surface, has been shown in these patients and corresponding animal models; however, the functional consequence of this has not been shown yet. The figure was inspired by Segovia-Silvestre *et al.* (213).

molecules, such as IGF1, BMP6, and Wnt10b (76, 79, 80). Furthermore, there are also molecules that are still being explored in detail, such as the ephrinB2–ephB4 interaction and sphingosine-1-phosphate (76, 77). However, what remains to be demonstrated is clear-cut *in vivo* proof that these are indeed coupling factors. Conversely, showing this is immensely complex when considering the ubiquitous nature of the molecules, and the very likely possibility that it is a combination of molecules that serves this function *in vivo*.

In addition to osteoclast-produced and resorption-derived coupling factors for osteoblasts, there is also some evidence that the bone surface in the resorption pits is of utmost importance for bone formation (81). In this context, it is of interest that the resorbed surface area in the ADO patients is very high, likely as a consequence of the increased numbers of osteoclasts, which initiate, but fail, to perform a complete process of resorption (41), thus leaving the bone surface 'scratched' as opposed to completely resorbed. Along the same line, data from *in vitro* experiments show that allowing osteoclasts to resorb bone facilitates bone formation by osteoblasts in the resorbed areas (82, 83). Furthermore, studies indicate that osteoclasts deposit TRACP on the bone surface leading to recruitment of osteoblasts (84), a finding that correlates with the high levels of TRACP found on the resorbed bone surfaces in ADO patients and other acid secretion-deficient systems (41, 85, 86) (Fig. 3).

In summary, *in vitro* studies of ADO osteoclasts have highlighted relevant and important aspects not only related to bone resorption but also for the coupling principle and thereby for bone formation. Thus, it has been clearly demonstrated that the chloride–proton antiporter ClC-7 is a highly relevant potential pharmacological target for treatment of osteoporosis.

Treatment of and potential therapies identified from studies of ADO

Treatment of ADO

The pivotal studies by Walker (87) in the early seventies for the first time demonstrated that osteoclast-rich forms of osteopetrosis could be cured by parabiosis, also demonstrating that osteoclasts derive from hematopoietic stem cell. Thereby, the scientific basis was established for experimental treatment of the severe clinical (malignant) forms of ARO with bone marrow transplantation in children (88, 89) in the early 1980s. This is now an established treatment modality (90, 91). However, no specific pharmacological therapy for ADO is currently available. Treatment of osteopetrosis including ADO is mainly supportive. Multidisciplinary management is required in order to manage skeletal related complications such as osteoarthritis, fractures, and osteomyelitis, as well as compression of cranial

nerves including most notably the optic nerve (14, 19, 20, 24, 45, 47). Osteopetrosis may cause bone marrow failure and seizures due to hypocalcaemia, but these complications are infrequent in late-onset ADO.

Insight into the pathophysiology of the different forms of osteopetrosis may be important for the design of therapy for these diseases. A number of drugs have been shown to increase osteoclast activity including calcitriol, which ameliorates ARO (92). Furthermore, treatment of ARO with interferon γ 1b increases bone resorption, causing a reduction in trabecular bone area and an increase in bone marrow space (93). While treatment of ARO with RANKL recently was shown to improve the bone phenotype in mice and may prove effective in humans (94), specific pharmaceutical treatment of ADO has not been investigated systematically.

Patients with ADO have a single-allele dominant negative mutation of *CLCN7*. Consequently, it has been suggested that siRNA therapy could transform the phenotype by silencing the affected allele. Indeed, preliminary results have shown that siRNA rescued the phenotype of human osteoclasts transfected with mutant ClC-7 constructs (95). Another possible approach is suggested by the demonstration that osteoclasts cultured from unaffected gene carriers function normally while osteoclasts from affected patients resorbed much less bone *in vitro* (55, 60). These studies strongly suggest that modifying pathways might exist. Potentially, these pathways could be drug targets. Age, sex, and the specific *CLCN7* mutation do not seem to be important in this respect and osteoclastic response to RANKL did not differ between carriers and affected patients either. Finally, it is unknown whether reduction in osteoclast number by treatment with, e.g. bisphosphonates or denosumab, could be beneficial.

Insight into the pathophysiology of ADO is also important for the design of anti-osteoporosis therapy. A number of limitations in this respect should be recognized. First, ADO exists throughout life and thus still show effects that are also related to bone development (modeling) as opposed to bone remodeling (70). The studies have provided proof-of-concept that the chloride–proton antiporter ClC-7 is an attractive target as well.

ClC-7 inhibitors

Studies of ADO patients have demonstrated the potential mode of action of a ClC-7 inhibitor, and the most intriguing finding is the apparent uncoupling between bone resorption and bone formation, a finding that clearly indicates that bone resorption can be inhibited without detrimental effects on bone formation (81, 96, 97).

Studies in the aged ovariectomized rat model using these inhibitors have shown that bone resorption is lowered, while osteoclast numbers and bone formation

are maintained (63, 73), thereby mimicking the phenotype of the ADO patients and *Clc-7*-deficient mice (11, 41, 85, 86). Treatment with these inhibitors also resulted in increased BMD and bone strength (63, 73), thereby underlining the potential of these molecules. The contrast to the fracture pattern in ADO patients (10) might be related to the difference between an inherited condition with early effects during development and bone modeling. As osteopetrosis patients, including ADO patients, show poor bone quality and increased numbers of fractures (14, 18, 98), it should be considered whether molecules mimicking these phenotypes would also result in bone fragility. However, a recent study in which osteopetrosis was induced in adult mice (>3 months of age) showed that the bone brittleness observed in osteopetrosis is a developmental phenomenon during growth and bone modeling caused by the presence of calcified cartilage (70) and thus not a phenomenon likely to occur in adult osteoporotic patients with normal childhood bone development.

In addition, the patients and mice with complete loss of function or expression of *Clc-7* are known to show primary neurological issues (49, 91, 99); however, analyses of the ADO patients, which, based on gene doses, have 25% residual *Clc-7* activity remaining, have not shown any primary neurological phenomena (14, 20, 91), indicating that the neurological problems only arise when *Clc-7* is completely inactive or absent, and this level of inhibition of *Clc-7* is not expected with small-molecule inhibitors.

In relation to the specificity of the target, the A3 subunit of the osteoclastic proton pump, which is functionally close to identical to *Clc-7*, in addition to only having a function in the osteoclasts, has also been studied in detail (100). However, as the A3 subunit is a structural component docked in the membrane and as the enzymatic subunit of the V-ATPase complex has a broad tissue distribution, this has complicated development of small-molecule inhibitors of this target significantly (100).

Hence, the acid secretion process in the osteoclasts is a highly attractive target from a pharmacological point-of-view. However, it is also a difficult target, and more research is clearly needed before a final conclusion can be reached, although the phenotype observed in ADO with decreased resorption and normal or increased bone formation in adults is very promising.

Interestingly, inhibition of cathepsin K, the acid-activated protease mediating type I collagen cleavage in the resorption lacunae, has been explored extensively as a pharmacological target, including publication of data from phase II trials (101, 102, 103, 104). Initially, inhibition of cathepsin K was thought to lead to inhibition of resorption, increases in osteoclast numbers, and no secondary inhibition of formation, as seen in the ADO patients, due to the very close relationship between these processes and the bone

phenotype observed in cathepsin K-deficient mice (105). However, studies on monkeys, the clinical trials, and the phenotype observed in the cathepsin K-deficient pycnodysostosis patients have demonstrated that inhibition/ablation of cathepsin K in humans is more complex, as bone formation is reduced secondary to the inhibition of resorption. Hence, the final data from the phase III study of the cathepsin inhibitor odanacatib is awaited with interest. In summary, inhibitors of *Clc-7* are attractive as candidates for treatment of postmenopausal osteoporosis, inflammatory osteolysis, and other bone metabolic disorders.

Identification of the role of the *LRP5* gene in bone

Two Danish families with HBM were instrumental in localizing the disease-causing gene by linkage analysis on chromosome 11q12–13 (106). Within the delineated region, two genes of interest were localized: *TCIRG1* encoding for the $\alpha 3$ subunit of the proton pump V-ATPase and *LRP5* encoding for the LDL receptor-related protein 5. Mutations in *TCIRG1* were found to cause ARO (107) but could not be demonstrated in our cohort. On the other hand, loss-of-function mutations in *LRP5* were identified to be causative for autosomal recessive osteoporosis-pseudoglioma syndrome (OPPG), characterized by congenital blindness and severe juvenile-onset osteoporosis (108). Heterozygous carriers of these mutations showed an increased incidence of osteoporotic fractures, indicating a dominant negative effect on bone mass (108). At the same time, Little *et al.* (110) and subsequently Boyden *et al.* (109) reported the identical missense mutation, G171V, in two kindred diagnosed with the so-called HBM phenotype. Patients from the first family were asymptomatic but showed radiographic very dense bones, especially involving the cortices of the long bones, as well as an increased thickness of the skull. In the second family, some additional features including a wide and deep mandible and a torus palatinus were described (55). In both families, the patients seem to be protected against fractures. Analysis of the *LRP5* gene in patients from the two Danish families indicated a heterozygous missense (T253I) mutation in both (15). In addition, a more extended set of patients was screened for *LRP5* mutations. All these patients, despite different diagnoses such as endosteal hyperostosis, Van Buchem disease, or autosomal dominant osteosclerosis, showed a similar radiographic and clinical picture (111, 112). In several of these families, we found a missense mutation in *LRP5* (15). As shown in Fig. 4, all these mutations cluster within the first propeller domain of the *LRP5* protein. This has also been the case for all mutations reported later on (113, 114, 115). The clinical variability observed in patients with these *LRP5* mutations cannot be correlated with the specific mutation identified.

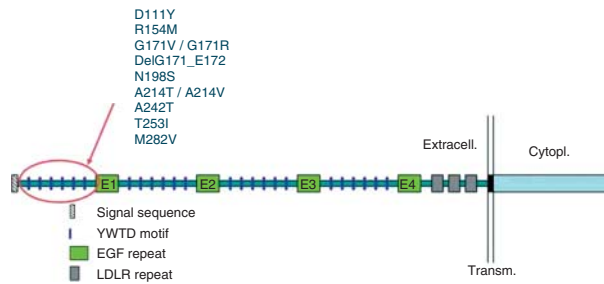


Figure 4 Gain-of-function mutations in the gene encoding the LDL receptor-related protein 5 (LRP5). All identified mutations in the high bone mass phenotypes cluster in the first β -propeller of the extracellular domain, thereby affecting the binding of inhibitory proteins as DKK1 and sclerostin. The consequence of these mutations is an increased signaling of the canonical Wnt pathway. The original Danish pedigrees harbor the T253I mutation (15).

Moreover, intra-familial variability supports the idea that other genetic modifiers as well as environmental factors influence the expression and severity of the phenotype.

Minor changes in the *LRP5* gene, i.e. polymorphisms in *LRP5*, have been associated with slightly lower bone mass and an increased risk of fracture (116, 117). This suggests that the pathway may be a drug target to achieve a regulated response on bone homeostasis.

As discussed in detail below, the *LRP5* protein plays a role as a co-receptor for extracellular Wnt proteins to induce the canonical Wnt signaling and this signaling pathway is regulated by inhibitors including DKK1 and sclerostin (118). Structural analysis of the *LRP5* protein revealed that all amino acids involved in any of the HBM are clustered at an open binding pocket near the surface of the first β -propeller of *LRP5* (119). The mutations do not have any effect on the functioning of the protein but rather disrupt the ligand binding of the extracellular inhibitors DKK1 and sclerostin (120, 121, 122), in which a short binding motif was found by structure analysis (123). The latter protein was identified by positional cloning for two other sclerosing bone disorders, Van Buchem disease and sclerosteosis (124, 125, 126, 127). The radiographic picture in these patients is very reminiscent to HBM. However, the phenotype is more severe (128, 129), with pronounced enlargement of the mandible and extreme thickening of the skull causing cranial nerve encroachments resulting in facial nerve palsy, optic atrophy, and hearing loss. Thus, the similarities between the dominant phenotypes (HBM) and the recessive phenotypes (Sclerosteosis and Van Buchem disease) can be explained by a shared increased Wnt signaling, but by different mechanisms. In the latter conditions, the patients are lacking an inhibitor (sclerostin) while in the former ones the inhibitor can no longer bind its mutated receptor. However, a recent study indicated that this might be a somewhat simplified model. Niziolek *et al.* (130) made knock-in mouse models for two HBM

mutations and compared them to a *SOST* knockout model. All models showed an increased thickness of the skull, but clear differences were noticed at the appendicular skeleton. The A214V and *SOST* mutants were identical with increased bone formation periosteally, while in the G171V model, bone was added preferentially at the endocortical envelope (130), as suggested (14) and demonstrated by histomorphometry in the HBM_{T253I} cohort (11, 23). This indicates, at least for the G171V mutant, that additional mechanisms not associated with sclerostin are involved.

The involvement of the canonical Wnt signaling pathway in bone metabolism

The most important implication of the *LRP5* mutations revealed by positional cloning efforts in skeletal disorders is that they linked, for the first time, the canonical Wnt/ β -catenin signaling pathway to bone biology. This is the best-known pathway triggered by extracellular Wnt molecules. In the absence of Wnts, intracellular β -catenin is bound to a protein complex including axin1/2, APC, casein kinase 1, glycogen synthase kinase 3 β (GSK3 β), as well as WTX (AMER1) and subsequently degraded in the proteasome (131). However, upon the presence of Wnt molecules, these will bind to the extracellular frizzled receptor and a co-receptor (Lrp5/Lrp6). This results in destabilization of the destruction complex and release of β -catenin, leading to intracellular accumulation of β -catenin that can translocate to the nucleus and, after binding to Tcf/Lef transcription factors, induce the expression of target genes (131).

Over the last years, plenty of evidence became available supporting a role of canonical Wnt signaling in bone homeostasis from molecular genetic studies on skeletal diseases. In addition to the already mentioned mutations in the co-receptor *LRP5* and the Wnt inhibitor sclerostin, mutations were found in *WTX*, a member of the destruction complex of β -catenin, causing osteopathia striata (132, 133, 134). Furthermore, a loss-of-function mutation in *LRP6* results in a condition with osteoporosis, coronary artery diseases, and metabolic syndrome (135). Finally, patients diagnosed with Williams–Beuren syndrome have a low BMD, and this condition is associated with a deletion of *FZD9*, another Wnt co-receptor (136). Both *in vitro* and *in vivo* studies have been performed gaining insight into the underlying mechanisms involving both the processes of bone formation and bone resorption.

Evidence for a role of canonical Wnt signaling in bone formation

Canonical Wnt signaling is involved in the bone formation process at different levels. This pathway is a key regulator of the differentiation of mesenchymal stem

cells toward chondrocytes, osteoblasts, or adipocytes. The Wnt/ β -catenin signaling pathway has been shown to inhibit the adipogenic differentiation potential, thus altering the fate of cells from adipocytes to osteoblasts (137, 138, 139, 140). This is induced by suppressing the expression of the adipogenic transcription factors peroxisome proliferator-activated receptor γ (*PPAR γ* (*PPARG*)) and CCAAT/enhancer-binding protein α (*C/EBP α* (*CEBPA*)) (141). Next, an extended set of mouse models was generated to evaluate the role of LRP5 and β -catenin in osteoblastogenesis and bone formation. *Lrp5* knockout mice showed a decreased bone formation rate mainly due to reduced osteoblast proliferation (142). In order to model the human HBM phenotype, Babij *et al.* (143) generated a *Lrp5* G171V transgenic mouse using the 3.6 *Col1A1* promoter and were able to also show a decrease in the apoptosis of osteoblasts and osteocytes, which might also contribute to the phenotype. Mouse models in which β -catenin was conditionally deleted indicated an essential role in osteoblast differentiation as absence of β -catenin in embryonic mesenchymal progenitors abolished the generation of mature osteoblasts (144) and had an effect on the balance between osteo- and chondroblastogenesis (145).

In different studies, the role of Wnt/ β -catenin signaling in bone formation was linked to the responsiveness to mechanical loading on bone, which seemed to be increased in the *LRP5* (G171V) mutant models and reduced in the *LRP5* knockout models (146). Furthermore, deletion of one copy of the β -catenin gene in osteocytes was linked to reduced new bone formation upon mechanical loading (147). Thus, a model has been suggested combining a direct β -catenin-dependent but LRP5-independent osteocytic effect with an LRP5-dependent feedback loop to explain the responsiveness to mechanical loading (148).

Evidence for a role of canonical Wnt signaling in bone resorption

Mouse studies have provided strong indications that at least β -catenin influences the process of bone resorption. The generation of conditional knockout mouse models for β -catenin supports that regulation of bone resorption is mediated by osteoblasts and osteocytes. Deletion of β -catenin in a later stage of osteoblast differentiation results in a severe bone loss (149). An increased number of osteoclasts were present, and subsequently, it was demonstrated that canonical Wnt signaling within differentiated osteoblasts induces the expression and secretion of osteoprotegerin (OPG), an important inhibitor of osteoclastogenesis (137). Thus, by stabilizing β -catenin, the OPG:RANKL ratio increased followed by decreased osteoclastogenesis and defective tooth eruption (137, 150), which are classical signs of osteopetrosis in murine models (151, 152, 153, 154). Along the same lines, deletion of β -catenin in

osteocytes resulted in decreased BMD due to an increased number and activity of osteoclasts (155). This effect was associated with a decreased level of OPG, thus increasing the RANKL:OPG ratio and consequently stimulating osteoclastogenesis.

Because β -catenin is also involved in many Wnt-independent processes, this might suggest, but does not confirm, a direct role for Wnts in osteoclast differentiation. However, Ruan *et al.* (156) recently reported an increased osteoclast differentiation of osteoclast precursors lacking functional LRP5 and LRP6, indicating that canonical Wnt signaling indeed suppresses osteoclast differentiation. Furthermore, the administration of anti-sclerostin antibody, which is assumed to increase canonical Wnt signaling, also results in a decreased osteoclastogenesis and reduction in bone resorption in rats (157).

Early based investigations of our HBM_{T253I} cohort identified low bone resorption biochemically (68) reflecting reduced osteoclast profiles by immunohistochemistry and electron microscopy (41). Although we confirmed the biochemical findings recently (21, 40), these findings have never been established in other HBM cohorts, as systematic bone metabolic studies have not been published.

Treatment of and potential therapies identified from studies of HBM

Treatment of HBM

Owing to the benign nature of the HBM disorders, treatment has so far been mainly supportive, as for ADO. As the pathogenic defect is on the formative side of bone remodeling, bone marrow transplantation is not an option (151, 158, 159). Two considerations are of importance in relation to treatment of HBM: i) serious side effects are unacceptable given the relatively benign nature of the disease and ii) bone formation is increased and accompanied with some alteration in bone resorption (118, 160).

With respect to reversing the phenotype, there appears to be some possibilities as both glucocorticoids and glitazones are characterized by reducing bone formation and increasing bone resorption (161, 162). However, these drugs are associated with unacceptable side effects with high dosages and long-term treatment. Moreover, it would be more appealing to treat directly by targeting the regulation of the Wnt signaling.

Sclerostin and stimulators of sclerostin secretion such as calcitonin (163) could potentially be used for the treatment of HBM, but calcitonin is also a potent inhibitor of bone resorption. Mechanical unloading (i.e. bed rest) is followed by increased resorption and decreased formation, controlled at least to some extent by increased sclerostin levels and thereby inhibition of the overactive Wnt cascade (164). It is at present

unknown whether manipulating sclerostin levels will have unintended effects on the neuromuscular response as seen in humans in relation to unloading (165). Moreover, some of the HBM mutations appear to prevent sclerostin by itself from binding to LRP5 (122), possibly explaining the increased level of sclerostin observed in patients with HBM (21).

Alternatively, application of continuous PTH or RANKL could lead to aggressive induction of bone resorption and thereby potential removal of the excess bone (166, 167). However, this would need careful long-term studies and could potentially be complicated by antibody production and difficulties in controlling the RANKL dose.

Taken together, there are several hypothetical approaches to treat HBM. However, so far, they are all limited by side effects and lack clinical validation. For future studies, side effects and long-term consequences of treatment should be balanced against the, in general, benign nature of the disorder.

The Wnt/LRP5 system

With the identification of the highly interesting bone phenotype of increased bone mass and strength in the HBM patients, who have loss-of-inhibition mutations in *LRP5* (15, 109, 140, 160, 168), an intensive search for therapies targeting this molecule was initiated. As the Wnt ligands, as well as the downstream signaling molecules GSK3 β and β -catenin, are rather ubiquitous and have been implicated in cancer progression, these are less attractive targets despite their obvious anabolic potential (169, 170). On the other hand, the identification of the soluble inhibitors of Wnt signaling, such as sclerostin, DKK1-4, WIF, and sFRPs, identified a series of interesting targets for antibody and small-molecule inhibitor therapy (169), and especially sclerostin that appears rather bone specific and DKK1 have been explored extensively (171, 172, 173, 174).

Anti-sclerostin antibodies, which prevent the binding of sclerostin to LRP5 and the co-receptor (175), have been tested in a host of animal models of osteoporosis, and in all these models that resulted in a large increase in BMD, due to a powerful acceleration of modeling-based bone formation, i.e. activation of bone formation at otherwise quiescent surfaces (176). Also, a reduction of bone resorption was demonstrated, and the resulting increase in bone strength at various sites was substantial (173, 174). AMG-785, a sclerostin MAB, is currently tested in an extensive clinical program. In a phase-1 trial, a large increase in the bone formation marker P1NP and a decrease in the bone resorption marker CTX-I, led to significant increases in BMD at both the lumbar spine and the hip, confirming the pre-clinical results (177).

Anti-DKK antibodies have also been explored, although not to the level of the anti-sclerostin antibodies. Recent studies indicated that these antibodies induce

bone formation, reduce bone resorption, and thereby lead to an increase in bone volume in rodent and monkey models (171, 172, 177). Thus, anti-DKK may have a potential as treatment of osteoporosis.

While there is no doubt about the anabolic potential of these molecules, a question is the potential effect of inducing bone formation at surfaces that are normally quiescent or slowly remodeled. A site in question is the subchondral plate in the joints, which has been highlighted by a series of studies indicating that loss of the Wnt inhibitors is a key player in the development of osteoarthritis (178, 179); however, whether this will occur with the therapeutic strategies and thereby become a potential serious side effect remains to be elucidated. In addition, nerve compression is frequently observed in osteopetrotic/osteosclerotic phenotypes (14, 18, 24), and in the case of the mutations related to the Wnt/LRP5 system, this is a consequence of the high bone volume.

In summary, the antibodies against soluble Wnt inhibitors are highly promising in terms of the bone response, but they will need to be carefully monitored in clinical development for osteoporosis due to the potential consequences of inducing bone formation systemically.

Metabolic aspects in relation to a homeostatic model involving bone

Interaction between bone and glucose metabolism

Recent investigations have linked bone metabolism and whole-body glucose homeostasis, and studies indicate that the ADO and HBM phenotypes may include changes in glucose metabolism.

Lee *et al.* (180) showed that bone cells interact with glucose metabolism through the osteoblast-specific protein, uncarboxylated osteocalcin. Mice expressing lower levels of uncarboxylated osteocalcin were hyperglycemic, hypoinsulinemic, had reduced insulin sensitivity in peripheral tissue, lower-cell mass, and increased fat mass (180). Subsequently, Ferron *et al.* (65) demonstrated that insulin signaling in osteoblasts increased osteoclast activity and thereby the level of uncarboxylated osteocalcin, thus affecting glucose homeostasis. Fulzele *et al.* reported that insulin promotes osteoblast development and osteocalcin expression and that bone formation and the number of osteoblasts were reduced in mice without insulin receptors in bone (181, 182). With age, these mice developed adiposity and insulin resistance (181).

Circumstantial evidence of interaction between bone and whole-body metabolism in humans has recently become available. Thus, osteocalcin was inversely associated with plasma glucose, insulin levels, and serum triglycerides in elderly men and women as well as elderly men with high cardiovascular risk

(183, 184, 185, 186, 187, 188). Also, osteocalcin was lower in patients with T2DM (189, 190, 191) and gestational diabetes (192). Moreover, in active acromegaly, a condition with profound insulin resistance, osteocalcin levels were the major determinant of insulin resistance and β -cell function, both *in vivo* and *in vitro* (193). In animals, the potent inhibitor of bone resorption, alendronate, changed the levels of both uncarboxylated osteocalcin and glucose metabolism. In accordance with these results, treatment of humans with alendronate reduced whereas PTH 1–84 (a bone-forming agent) increased circulating levels of uncarboxylated osteocalcin. These changes were associated with fat mass and other markers of metabolism including adiponectin (194). In contrast to these findings, teriparatide (PTH (1–34)) had no impact on glucose homeostasis (195). Furthermore, it has recently been reported that treatment with antiresorptive drugs including alendronate, zoledronic acid, and denosumab has no clinically important effect on fasting glucose levels (196). However, these findings need to be studied further in trials designed to fully clarify to what extent bone resorption has an effect on glucose homeostasis.

Glucose and fat metabolism in relation to ADO and HBM

LRP5 is expressed in several tissues including bone and pancreas (197, 198). Mutations in *LRP5* have been shown to influence the differentiation of human mesenchymal stem cells into osteoblasts or adipocytes (140). Moreover, polymorphisms in *LRP5* have been associated with osteoporosis and osteoporotic fractures, as well as obesity and metabolic syndrome (116, 117, 199, 200, 201). Glucose-induced insulin secretion is impaired in mice deficient in *Lrp5* (202). Recently, Palsgaard *et al.* (203) reported that *LRP5* promotes insulin signaling in pre-adipocytes, suggesting that modulation of *LRP5* could promote insulin sensitivity in type 2 diabetes. Furthermore, patients with OPPG due to a loss of function mutation in *LRP5* are more frequently glucose intolerant or diabetic, possibly due to β -cell dysfunction (204). However, glucose homeostasis remains to be investigated in detail in patients with HBM.

Compared with age- and sex-matched controls, fat mass and BMI were higher in our patients with HBM_{T253I} (21). Additionally, patients with clinical, not genetically verified, HBM had higher BMI compared with their relatives (205). Thus, these observations suggest that the clinical phenotype of HBM and increased bone mass may also involve fat mass and glucose metabolism.

Individuals with impaired bone resorption due to ADO appeared to have lower levels of uncarboxylated osteocalcin and decreased levels of insulin after food intake, strengthening the notion that bone metabolism

at least in part regulates glucose homeostasis (65). However, as the study comprised few individuals with ADO, further studies on glucose metabolism in ADO are needed for confirmation, in as much as ADO seems not to entail a higher risk of diabetes, based on the literature.

Taken together, these studies suggest that bone metabolism and whole-body glucose and fat homeostasis are integrated. Monogenetic bone disorders characterized by abnormal bone formation or resorption may prove useful as a platform for further investigations.

Serotonin in relation to HBM

Rather than acting directly on osteoblast differentiation, *LRP5* may regulate bone formation through changes in the expression of tryptophan hydroxylase I, an enzyme that influences the serotonin synthesis in the gut (206), and pharmacological inhibition of gut-derived serotonin may increase bone mass (207). In accordance with the animal study, a subsequent study demonstrated that the level of serotonin was lower in patients with HBM (207). We reported lower levels of serotonin in platelet-poor plasma as well as serum serotonin measured in samples collected at two different time points in our patients with HBM_{T253I} (21, 208), but the level of serotonin was not associated with bone mass or structure. Furthermore, a recent study did not find any effect of gut-derived serotonin on bone metabolism (209). Instead, the study indicated that *LRP5* regulates bone mass through osteocytes and, possibly, late-stage osteoblasts. Taken together, the *LRP5*–HBM has provided support of a potential association between serotonin levels and bone mass; however, further studies including other genotypes are clearly needed for confirmation.

Conclusions and perspectives

Updated definition of clinical osteopetrosis

Previously, we have described ADO in two forms (ADO1 and ADO2) (14). However, it turned out that the former was caused by an activating mutation in *LRP5* (*LRP5* activation bone disease) defined as an HBM phenotype (15), leaving ADO to the disease related to chloride channel 7 deficiency. An updated definition taking into account recent advantages in pathophysiological understanding should describe osteoclast-rich and osteoclast-poor forms (17, 18). Thus, in a modern sense, osteopetrosis is an inherited group of generalized bone disorders characterized by increased bone mass in all compartments due to osteoclast failure and impaired bone resorption. This definition focuses on the resorptive side of bone remodeling, however, and recognizes direct (for example, ADO due to *ClC-7* defects within the osteoclast) and indirect regulatory defects. Examples of

the latter are defects in the receptor for RANKL at the resorptive, osteoclastic site (RANK) (78), and mutations in RANKL by itself on the osteoblastic site (210). This definition acknowledges regulatory (autocrine/paracrine/endocrine) pathways within bone remodeling and also points to the fact that bone formation is ongoing in osteopetrosis. For ADO, the defective osteoclasts seem to regulate bone formation (75, 81, 96). This updated definition is also in alignment with the original description of the naturally occurring murine forms of osteopetrosis, depicting osteoclast-poor (ex the *tl*-rat, (151, 158, 159)) and -rich forms (ex the *ia*-rat, (211, 212)).

Potential therapeutic aspects

From a therapeutic perspective, the studies of ADO have highlighted several new targets, all of which employ novel modes of action and could thereby provide benefits to the field of osteoporosis. The possibility to have a pure anabolic response or an anti-resorptive response without the secondary reduction in bone formation associated with presently available treatments is enticing (97).

Based on the studies of the HBM phenotype, an array of new drugs for treatment of osteoporosis is in development and several of these target the canonical Wnt signaling pathway. The antibodies against soluble Wnt inhibitors are promising in terms of the bone response, but they will need to be carefully monitored in clinical development due to the potential serious side effects.

Whole-body energy homeostasis

The role of bone as an integrated part of whole-body energy homeostasis is controversial. Both ADO and HBM have contributed directly to this discussion. Detailed metabolic studies hypothesized a positive feed-forward loop integrating bone remodeling in glucose and insulin homeostasis, exemplified with ADO as the human counterpart (65). Insulin via the insulin receptor on osteoblasts stimulates bone resorption and thereby the release of uncarboxylated osteocalcin from the bone matrix, thus in turn stimulating insulin secretion. Indeed, ADO patients had decreased uncarboxylated osteocalcin (reduced bone resorption) and reduced insulin levels (65). The extensive, however, diverging studies performed in various models have clearly challenged previous concepts in this field. However, only very few patient samples have been presented and the clinical importance remains unclear.

Declaration of interest

K Henriksen has economical interest in development of CLC-7 inhibitors. The other authors have nothing to declare.

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