

Lysosomal Pathology and Osteopetrosis

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Osteopetrosis is a generic term applied to several clinical disorders of varying severity associated with pathologic high bone mass resulting in obliteration of bone marrow which causes pancytopenia and extramedullary hematopoiesis in the spleen and liver, narrowing of cranial foramina leading to loss of cranial nerve function (sight, hearing), paradoxical osseous fragility, and other manifestations.^{1,2} It is due to abnormalities in osteoclast formation or function due to loss of function mutations in at least 10 genes that may be transmitted by autosomal recessive or dominant inheritance patterns. Bone resorption takes place in subosteoclast resorptive pits or lacunae into which are secreted acid (H^+ as hydrochloric acid) that solubilizes the mineral phase of bone and metalloproteases (cathepsin B) that dissolve the protein matrix of bone. The chloride channel in osteoclast lysosomes, which secretes H^+ into the subosteoclast resorptive pit, is encoded by *CLCN7* (OMIM 602727, chromosome 16p13), a protein that is expressed in many tissues including brain (Figure). Inactivating mutations of *CLCN7* result not only in severe to moderate osteopetrosis (depending on the site of the mutation) but also in lysosomal storage, retinal atrophy, and neurodegeneration (OMIM 611490). The *CLCN7* lysosomal chloride channel is primarily a chloride-proton (H^+) exchanger—ie, H^+ exits the lysosome through *CLCN7* as Cl^- enters and accumulates within this organelle. In the osteoclast's ruffled border, the *CLCN7* channel exchanges Cl^- for H^+ in the resorptive pit while a second channel

driven by the conversion of ADP to ATP (H^+ - transporting ATPase) secretes H^+ into the resorptive lacuna.³

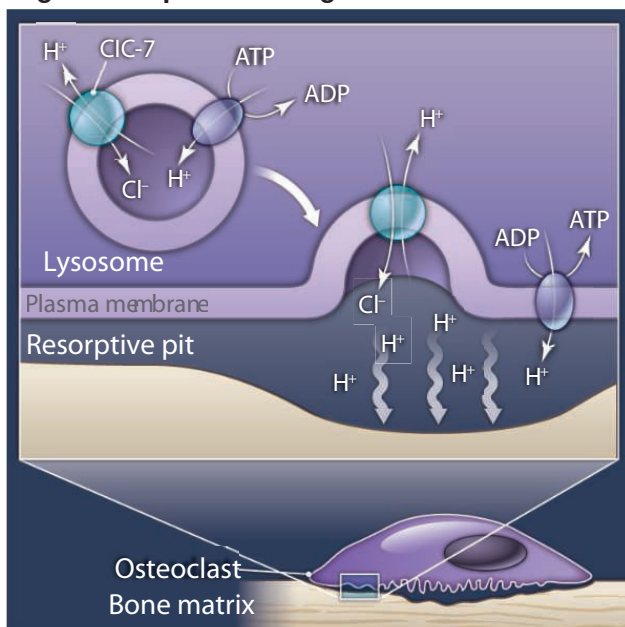
In order to determine whether the H^+ - Cl^- exchange function of *CLCN7* was essential or whether *CLCN7* might function simply as a passive Cl^- conductor, Weinert and co-workers⁴ generated mice in which the H^+ - Cl^- exchange function was abolished leaving the residual protein to function as an uncoupled Cl^- conductor. They did so by mutating glutamate (E) to alanine (A) in codon 245 of *Clcn7*, a site essential for H^+ transport by *Clcn7*. Mice homozygous for uncoupled *Clcn7* (*Clcn7^{unc/unc}*) developed osteopetrosis and associated neural and retinal abnormalities similar to mice with complete loss of *Clcn7* (*Clcn7^{-/-}*) but of somewhat less severity. Thus, *Clcn7^{unc/unc}* mice were retarded in growth and died at or before 5 weeks of postpartum age as did *Clcn7^{-/-}* mice. However, compared to *Clcn7^{-/-}* mice there was a more developed ruffled border, the volume of subosteoclast resorptive pits was larger, and bone mass was less in *Clcn7^{unc/unc}* animals. Although initially phenotypically normal, heterozygous mice (*Clcn7^{unc/+}*) developed slowly progressive hippocampal neurodegeneration at 5 months of age. The investigators concluded that both the conductance of Cl^- and the exchange of H^+ and Cl^- are essential for normal lysosomal function not only in osteoclasts but in other tissues as well.

The present data are important because they further unravel the pathophysiology of loss of *CLCN7*. Such data may ultimately permit more physiologically appropriate therapy of neonates and children with inactivating mutations of *CLCN7*. *OSTM1* (OMIM 607649, chromosome 6q21) and *CLCN7* form a molecular complex that is localized to endosomes, lysosomes, and to the ruffled membrane that caps the subosteoclast resorptive pit, a complex that stabilizes *CLCN7*. In humans, loss of function mutations in *OSTM1* produce a clinical picture that is similar to that of loss of *CLCN7*.

In man, inactivating mutations of *CLCN5* (OMIM 300008, chromosome Xp11.22) are associated with Dent's disease 1 (OMIM 300009)—X-linked hypercalciuric, hyperphosphaturic nephrolithiasis with microglobulinuria, a phenotype that is mirrored in the *Clcn5^{-/-}* knock-out mouse. In the same issue of *Science*, Novarino and Weinert et al⁵ reported the effects of separating renal H^+ - Cl^- exchange from Cl^- conductance by substituting glutamate for alanine in codon 211 (E211A) in *Clcn5* in mice. In *Clcn5^{unc/unc}* mice, clinical and pathophysiological findings were similar to those in *Clcn5^{-/-}* animals indicating the critical importance of H^+ - Cl^- exchange for normal renal tubular function.

Editor's Comment: Osteopetrosis is a rare human genetic disorder due to markedly decreased bone resorption. In the past, the only gene whose inactivation was known to be responsible for human osteopetrosis⁶ was that encoding carbonic anhydrase type II. Now it is known that osteopetrosis may be due to abnormalities in osteoclast formation or function due to loss of function mutations in at least 10 genes that may be transmitted by autosomal recessive or dominant

Figure. Coupled exchange.



Transporters that import chloride ions in exchange for the export of protons control the function of intracellular vesicles in mammalian cells.

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inheritance patterns as reviewed by Allen Root above. Sclerosing bone disorders are usually due to mutations in genes required for osteoclast function that can be subdivided according to their clinical presentation, the primarily affected cell type, and the cellular pathways.⁷ Clinical aspects of osteopetrosis and the consequences for our understanding of bone biology are discussed by de Vernejoul and Kornak.

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References

1. Stark Z, Savarirayan R. Osteopetrosis. Orphanet J Rare Dis. 2009;4:5.
2. Villa A, Guerrini MM, Cassani B, et al. Infantile malignant, autosomal recessive osteopetrosis: The rich and the poor. Calcif Tissue Int. 2009;84:1-12.
3. Smith AJ, Schwappach B. Think vesicular chloride. Science. 2010;328:364-5.
4. Weinert S, Jabs S, Supanchart C, et al. Lysosomal pathology and osteopetrosis upon loss of H⁺-driven lysosomal Cl⁻ accumulation. Science. 2010;328:1401-3.
5. Novarino G, Weinert S, Rickheit G, Jentsch TJ. Endosomal chloride-proton exchange rather than chloride conductance is crucial for renal endocytosis. Science. 2010;328:1398-401.
6. de Vernejoul MC, Bénichou O. Human osteopetrosis and other sclerosing disorders: recent genetic developments. Calcif Tissue Int. 2001;69:1-6.
7. de Vernejoul MC, Kornak U. Heritable sclerosing bone disorders: presentation and new molecular mechanisms. Ann N Y Acad Sci. 2010;1192:269-77.