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REVIEW ARTICLE

A positive correlation between osteopetrosis and genetic disorder of *CA II, TCIRG1, SNX10, CLCN7* genes and importance of CRISPR (Cas 9) in gene therapy: A review

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ABSTRACT

Osteopetrosis can be defined as a group of uncommon skeletal disorders that are genetically and clinically heterogeneous, often stemming from family history. In osteopetrosis, abnormal osteoclast function disrupts bone homeostasis. Thus, patients have unusually high bone density as their primary symptom, which can lead to easy bone fractures. As a result, the disorderly, excessively dense bone that is brittle continues to grow unregulated. Depending on the inheritance pattern, three distinct types of osteopetrosis have been identified: X-linked osteopetrosis, autosomal recessive osteopetrosis (ARO), and autosomal dominant osteopetrosis (ADO). One of the primary causes of osteopetrosis is the carbonic anhydrase II gene. Carbonic acid is produced from carbon dioxide and water by the enzyme, which is then utilized to produce the acidic surroundings necessary for the breakdown of bone minerals and osteoclast activity. Therefore, autosomal recessive osteopetrosis occurs when genetic alterations bring on a CAII deficit, while CLCN7, TCIRG1, and SNX10 are genes most frequently impacted by osteopetrosis. SNX10 and TCIRG1 have been demonstrated to engage with protons pumped by vacuolar-type H(+)-ATPase (V-ATPase) at the osteoclast contact, respectively, while CLCN7 works in concert with TCIRG1 to transport hydrogen ions outside of the cell. Therefore, defects of these genes cause nonfunctional osteoclasts to induce ineffective bone resorption and, thus, osteopetrosis. Finding genetic mutations in osteopetrosis patients can have significant predictions and therapeutic consequences. In this review, we hope to raise the consciousness of osteopetrosis in medicinal society and encourage more research into potential mutations in CA II, TCIRG1, SNX10, and CLCN7 that may be linked to elevated osteoblast activity and decreased osteoclast activity in patients with osteopetrosis and highlight the importance of the CRISPR technology in gene therapy in the future.

Keywords: Osteopetrosis, Genetic disorder, *CA II*, *TCIRG1, SNX10, CLCN7, CRISPR*

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INTRODUCTION

Osteopetrosis can be defined as one of the rare heritable skeletal disorders in which the bones become denser (Emadian *et al*., 2014). The condition is caused by prolonged calcification of primary chondroids and bones, as well as malfunction of osteoclasts (Hosseinzadeh *et al*., 2024). The main elaborations of osteopetrosis are unusual fractures with a typical slow fracture amalgamation (Cohen-Solal *et al*., 2023).

Like other uncommon illnesses, osteopetrosis is seen differently depending on whether it runs in the family or is a first-time occurrence (Del Fattore *et al*., 2008; Thomas *et al*., 2009). When other family members are impacted, a common understanding of the disease's etiology, key outcomes, and associated consequences helps them accept the diagnosis and enhances patient monitoring. Therefore, Osteopetrosis may not be diagnosed for months or even years without a family history (Cohen-Solal *et al*., 2023).

Diagnostic suspicion is typically raised by suggestive radiographic findings along with a clinical pattern of fractures (Calder *et al*., 2022), as shown in Figure 1A. Once osteopetrosis has been diagnosed radiographically, we advise genetic testing since it can differentiate between different variants of the disease that have different implications (Wu *et al*., 2017; Gong *et al*., 2023) and helps with treatment choices and it provides a potential avenue for prenatal diagnosis in impacted families (Arunachalam *et al*., 2024).

Multiple genetic mutations that produce defective osteoclasts were linked to osteopetrosis pathogenesis with varying inheritance patterns (Moore *et al*., 2017). Consequently, based on the pattern of inheritance, three distinct kinds of osteopetrosis were identified: autosomal dominant osteopetrosis (ADO), X-linked osteopetrosis, and

autosomal recessive osteopetrosis (ARO) (Teti and Econs, 2017).

Current medical treatments for osteopetrosis are mostly palliative approaches aimed at relieving symptoms (Maurizi, 2022), Surgical alternatives benefit fractures of the proximal femoral (Hiyama *et al*., 2020), as seen in image 1 (B, C). Gene therapy with analogous hematopoietic stem and ancestor cells offers a potentially beneficial therapeutic option for this multi-systemic illness (Moscatelli *et al*., 2021). To improve the healing process of major non-unions and bone defects, the engineering of bone tissue is critical (Carlier *et al*., 2014). An effective method for examining the biological processes taking place at the tissue, subcellular, and cellular levels through bone healing from fractures is in silico modeling analysis (Lafuente-Gracia *et al*., 2021). By predicting the environment inside scaffolds made of biomaterial and the kinship between inducement and bone tissue renewal, in silico approaches can help guide the requirements of bone tissue engineering studies (Zhao *et al*., 2018).

Symptoms and Signs

Osteoporosis is distinguished by unusual osteoclast differentiation, and unbalance of bone turnover, and results in abnormalities such as deformities, abnormalities in the teeth, and defective mineral homeostasis. It also leads to structural fragility (Penna *et al*., 2019). Clinical personifications of osteopetrosis often involve fractures in bones (Jodeh *et al*., 2024). Increased bone density is the defining characteristic of typical osteopetrosis (Ishaque et al., 2022). A problem in the bone marrow system or neural systems may result from macrocephaly (Bhati and Goyal, 2017), altered remodeling and modeling system, changed craniofacial morphology, increased bone surface area, and bone encroachment on bone marrow space (Mwakalinga *et al*., 2023).

Genes Associated with Osteopetrosis

Almost all osteopetrosis cases indicated result from defects in gene products that are involved in regulating intracellular as well as extracellular pH of osteoclasts (Gaytán-Morales *et al*., 2021; Yu *et al*., 2014). Moreover, mutations in no less than ten genes were identified as causative in different osteopetrosis case types in humans (Stark & Savarirayan, 2009; Yu *et al*., 2014), as illustrated in Figure 2.

CA II Gene

The chromosome 8q22 contains the CA II gene, is 20 kb long, and has 7 exons (Awad *et al*., 2002; Suliman *et al*., 2010; Alhuzaim *et al*., 2015). The cytoplasmic enzyme CAII, which is mostly expressed in bone, brain, distal renal tubules, and erythrocytes, is encoded by the CAII gene. It accelerates the changeover of carbonic acid (H2CO3) to bicarbonate (HCO3-) and hydrogen ions (H+), which controls intracellular pH. CAII contributes to the resorption of bone, osteoclast differentiation, and kidney acid-base physiology (Borthwick *et al*., 2003; Leite *et al*., 2023). The genetic condition known as carbonic anhydrase II (CAII) enzyme deficiency, which is brought about by mutations in the CA2 gene, manifests as symptoms such as brain calcification, renal tubular acidosis, and osteopetrosis (Shaik *et al*., 2020). Most cases of osteopetrosis result from changes in genes encoding enzymes convoluted in the process of acidic disintegration in the space between the osteoclasts and the bone matrix. Since a major cause of osteopetrosis is a lack of carbonic anhydrase II, activators of this enzyme have many uses in the treatment of osteopetrosis (Alkhayal *et al*., 2023). Mutational investigation revealed two new mutations in the CAII gene: Both Asian and Chinese families have a nonsense mutation in exon 4 (c.T381C p.Y127X); one family (Chinese population) has a splice mutation at the splice donor site of intron 3 (c.350+2T>C, IVS3+2T>C), exon 3 skipping in the patient's mRNA due to the splice-site mutation results in an in-frame deletion and a new premature stop codon. It was anticipated that these alterations would cause CAII to stop functioning (Pang *et al*., 2015).

TCIRG1 Gene

TCIRG1 (T cell immune regulator 1) biallelic defects are the most common ARO cause (Yu et al., 2014), which can be defined as one of the heterogeneous skeletal diseases known as "osteoclast-rich osteopetrosis, encoding osteoclast specific 116 kD sub-unit of the vacuolar pump (Makaryan et al., 2022), representing a crucial cusp in understanding genetic and molecular heterogeneity of ARO. According to a genetic study, TCIRG-1 makes up roughly 58.6% of ARO, which typically presents shortly after birth (Capo *et al*., 2022). Its location is on chromosome 11q13.2 (Chen *et al*., 2023). The A3 subunit of VPP-1, expressed on the ruffled osteoclast membrane border, is encoded by this gene (Chu *et al*., 2021). Roughly 50% of ARO1 instances are represented by alterations on *TCIRG1*(Pangrazio *et al*., 2012). The two functional domains that make up the A3 subunit are membrane-spanning VO, which accomplishes proton pump activation and ATP hydrolysis, and the hydrosoluble V1 (Chu *et al*., 2021), which carries out ATP hydrolysis and operates extracellular membrane acidification. On osteoclasts, the A3 isoform is highly expressed (Frattini *et al*., 2000; Chávez-Güitrón *et*

al., 2018). *TCIRG1*-deficient ARO cohort exhibits a high degree of genetic heterogeneity, as evidenced by no less than 120 distinct mutations in the gene that were identified to date (Pangrazio *et al*., 2012). These mutations include nonsense mutations, small insertions/deletions, missense mutations, splicing defects, and large genomic deletions (Palagano *et al*., 2018; Nadyrshina and Khusainova, 2023). According to Pangrazio *et al*., (2012), almost 50% of ARO patients have the disease due to 41 new mutations in the *TCIRG1* gene. The process of designing drugs to treat osteoporosis may benefit from the characterization of mutations in this gene. Five new mutations (c.1020+1_1020+5 dup, c.66delC, c.2236+6T>G, c.2181C>A, and c.692delA) were found in the TCIRG1 gene of Chinese patients with osteopetrosis (Zhang *et al*., 2017). The *TCIRG1* gene polymorphisms c.117 + 83 T > C, c.417 + 11A > G, and c.714-19C > A were found to be associated with autosomal recessive osteopetrosis, according to Kocak *et al*., 2019.

SNX10 Gene

Six exons make up SNX10, which codes for 224 amino acids, and the PX domain resides in the remnant (Aker *et al*., 2012). Sorting nexin 10 (SNX10) is one of 33 sorting nexin family proteins. According to a blast search alignment of the SNX-10 sequence, it is situated on chromosome 7p15.2 (Xu et al., 2021). SNX-10 gene (OMIM614780), encoding sortin nexin 10, has emerged as a significant role in ARO pathogenesis over the past ten years. According to current estimates, pathogenic variants in SNX10 are accountable for 4-5% of the ARO cases (Huybrechts and Van Hul, 2022). It was demonstrated that SNX10 interacts with V-ATPase, also known as vacuolar-type H(+)-ATPase, to facilitate the pumping of protons to osteoclast bone lacunae. Furthermore, SNX-10 contributes significantly to the preservation of bone homeostasis and is engaged in intracellular vesicular trafficking (Elson *et al*., 2021; Udupa *et al*., 2023). Aker *et al*. (2012) have found that members of three consanguineous Palestinian families carried the first missense variant, c.152G>A; p. Arg51Gln. SNX10 gene has a homozygous stop mutation (c46C > T, p. Arg16X) in an Iraqi patient who was born into a consanguineous family and had a clinical osteopetrosis history, according to a 2013 publication by Mégarbané *et al*. 2013. Figure 3 illustrates how the SNX10 gene mutation was reported in osteopetrosis patients by Xu *et al*., 2014 and Elson *et al*., 2021.

CLCN7 Gene

CLCN7 is found on chromosome 16p13.3 in humans (Zhang *et al.,* 2016), has twenty-five exons, and codes for chloride channel protein 7 (CLC-7) (Rivadeneira and Uitterlinden, 2018), an 803 amino acid protein (Liang *et al*., 2021). An acidic environment is maintained for bone resorption, exchanging ions of chloride against protons, mediated by CLCN7, a protein about the channel of a voltage-gated chloride protein family. In a case when TCIRG1 transports hydrogen ions outside of the cell, CLCN7 functions in concert with it (Frattinni *et al*., 2000, Hoyoux *et al*., 2014, Wang *et al*., 2023). ARO, ADO II, and intermediate ARO (IARO) are a result of the changes in the CLCN-7 gene (Li *et al*., 2019). Most ARO variants (13–16%) result from the biallelic CLCN-7 variants (Klaab *et al*., 2023). A unique variation of the splice acceptor site in CLCN-7 gene variant (c.739-18G > A) has been discovered by genetic testing, according to Won *et al*., 2017; Klaab *et al*., 2023 by interfering with CLCN-7 gene splicing (exons 8 & 9), the newly discovered variant (c739-18G > A) trims all the functional domains of generated protein. Those truncating mutations are known as "loss of function mutations." The function of osteoclast-mediated extracellular acidification is affected by mutations in the CLCN-7 gene, as has been documented by Kornak *et al*., 2001; Okamoto *et al*., 2017. This disrupted disintegration of the bone's inorganic matrix leads to a range of clinical manifestations. P.(Val577Met), one of the mutations. According to Rössler *et al*. (2020), the affected individual had new mutations of the compound heterozygous in the CLCN-7 gene [c.982-1G > C and c.1208G > A (p. Arg403Gln)], which were discovered through whole-exome sequencing (WES) and Sanger sequencing. Subsequent familial segregation revealed that every parent has passed on the mutation.

Gene therapy for Osteopetrosis

Targeted gene therapy has the potential to treat diseases by accurately addressing genetic abnormalities. This approach uses therapeutic genes to target specific cells or tissues (Vickram *et al*., 2024). The process of replacing, deleting, or altering defective genes at precise locations, or adding healthy genes, is known as gene therapy. The genetic materials involved in gene therapy include messenger ribonucleic acid (RNA), deoxyribonucleic acid, microsomal RNA, small interference RNA, and antisense oligonucleotides (Bansal *et al*., 2023). Gene therapy has been thoroughly studied and shows a lot of promise (Kim *et al*., 2016). CRISPR/Cas is the most current of several genome editing techniques developed based on several processes (Robb, 2019). CRISPR-Cas9 technology is a relatively new tool that makes targeting DNA simple and effective (Susani *et al*., 2018). The CRISPR/Cas9 system can the bone and cartilage repair (Wang *et al*., 2022; Li *et al*., 2023). By using clustered regularly

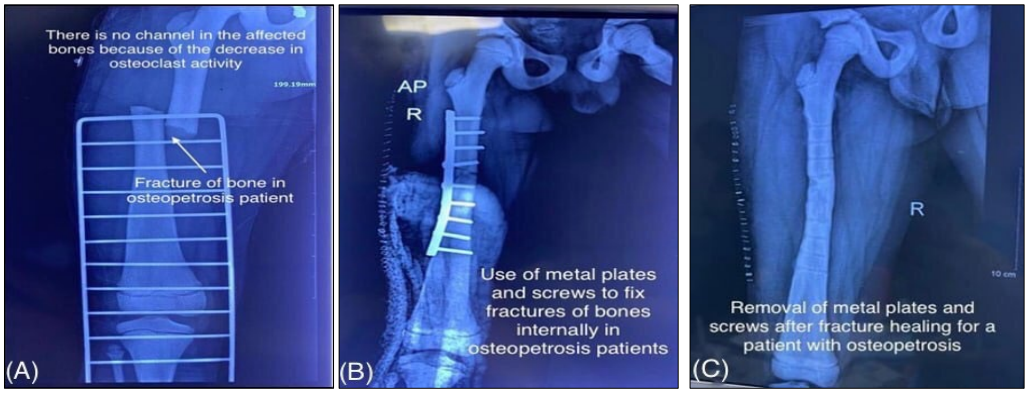

Figure 1. A, B, and C demonstrate using X-rays to diagnose osteopetrosis bone fractures and monitor the patients' surgery operations outcomes.

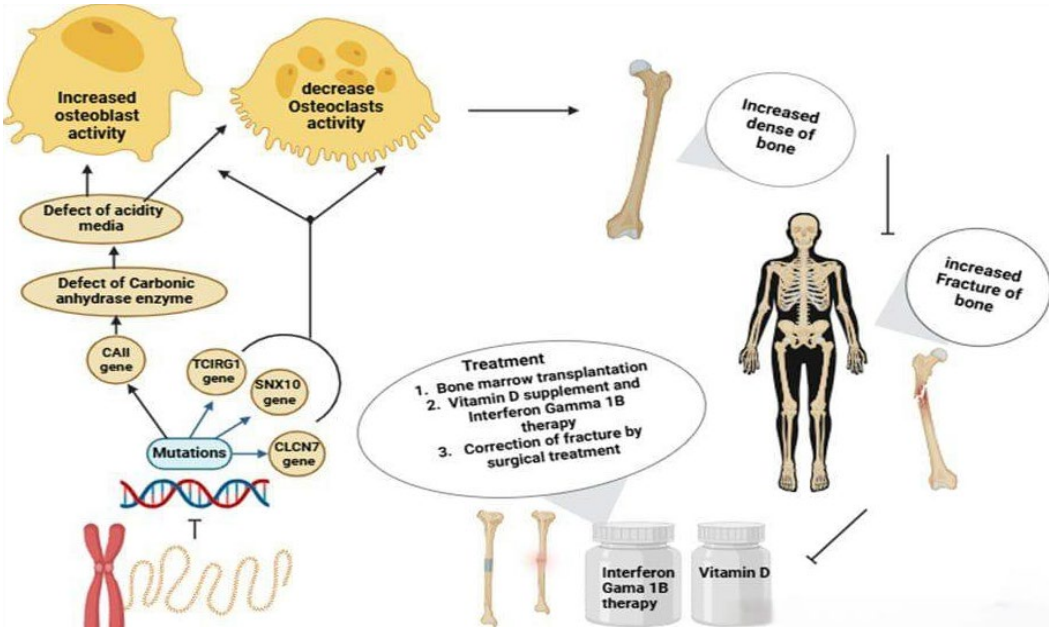

Figure 2. The relationship between genetic mutations in the CAII, TCIRG1, SNX10, and CLCN7 genes and dysfunction of osteoclasts and osteoblasts in patients with osteopetrosis. This picture was manufactured using BioRender (https://biorender.com).

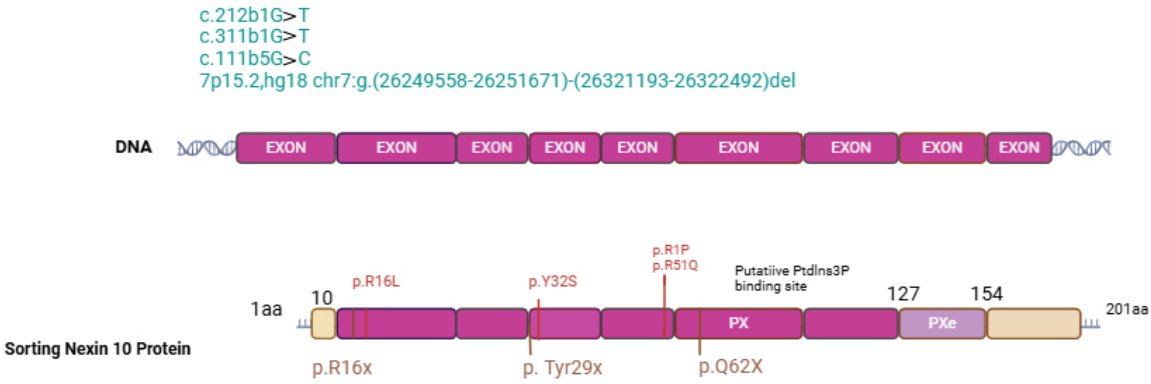

Figure 3. Osteopetrotic mutations in SNX10. Green highlights three exon splicing-related mutations as well as a significant loss in the SNX10 locus. The non-sense mutations highlighted in brown may result in A stop codon that comes early, a very truncated and perhaps protein product that is unstable. Red represents known missense mutations that could impact the SNX-10 protein's stability or its capacity to bind proteins and lipids. There has also been a report that P.Q62X and p.R16L have a compound heterozygous nonsense mutation. Yellow and orange markers, respectively, designate Pxe and PX(https://biorender.com).

interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), Li et al. (2023) were able to correct the point mutation R286W of the *CLCN7* gene in ADO2-iPSCs. O'Brien *et al.* (2015) reported that the homozygous mutation c.630G>A in the *TCIRG1* gene of osteopetrosis patients, producing a hylomorphic allele, CRISPR/Cas9, may enable this defect to be corrected by editing the affected CD34+ cells outside the body and then re-inoculating. Ou *et al*. (2019) revealed a family with osteopetrosis that had a heterozygous mutation in *CLCN7* exon 10 (c.856C>T (p.R286W), CRISPR/Cas9 system was utilized to successfully correct the *CLCN7* mutation in ADO2-iPSCs, restoring the mutated TGG (tryptophan) triplet to the wild-type CGG (arginine). The gene-corrected ADO2-iPSCs (GC-ADO2-iPSCs) displayed iPSC-like morphology and a normal chromosomal pattern. Although there is a lot of therapeutic promise in targeted gene therapy, more studies and technological advancements are required to overcome its drawbacks and provide safe and effective clinical treatments (Vickram *et al*., 2024).

CONCLUSIONS

Osteopetrosis represents a class of hereditary bone diseases brought about by failure of the osteoclast, thus, bones turn excessively thick, fragile, and capable of breaking (fracture) readily. One of the main causes of osteopetrosis and reduced osteoclast and grown osteoblast action is mutations, which alter the expression of genes and the resulting protein. This review shows that osteopetrosis in humans is linked to polymorphisms and mutations in a few genes, including SNX10, TCIRG1, CA II, and CLCN7 in some nations. CRISPR technology can be used to decode genes and alter the DNA of individuals with inherited bone disorders in gene therapy for osteopetrosis sufferers.

RECOMMENDATION

We recommend the use of DNA sequencing technology in the accurate diagnosis along with radiological diagnosis of osteoporosis, thus improving diagnosis and treatment by predicting the gene expression of contributing genes that play an important role in transmembrane transport and regulation of cellular acids, in addition to genetic counselling for families with a family history of osteoporosis, which will help in discovering new genetic and pharmacological therapeutic strategies for this condition. In addition to future studies on other genes contributing to the disease and the possibility of applying gene therapy using CRISPR technology.

ABBREVIATIONS

ARO, autosomal recessive osteopetrosis; ADO, autosomal dominant osteopetrosis; CA II, Carbonic anhydrase II; SNX10, Sorting nexin -10; TCIRG1, T cell immune regulator 1; CLCN7, Chloride channel 7; X-ray, X- radiation; KD, Kilodalton; VPP, Vacuolar proton pump; dup, Duplication; del, Deletion; T, Thymine; C, Cytosin; A, Adenine; G, Guanine; OMIM, Online Mendelian Inheritance in Man; Arg, Arginine; Gln, Glutamine; Q, Glutamin, R, Arginine, L, lysine; Px, Phox-homology; Pxe, Extended phox homology; Intermediate autosomal recessive osteopetrosis, IARO; ADO II, Autosomal dominant osteopetrosis type II; Val, Valine; WES, whole-exome sequencing; CTGT, connective tissue gene testing; siRNA, small interfering RNA; clustered regularly interspaced short palindromic repeats, CRISPR; induced pluripotent stem cells, iPSCs; R, Arginine; W, Tryptophan.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Consent was obtained directly from the clinic of surgeon Dr. Firas Khalid Al-Khalidi for bones, fractures, and joints / Iraq / Maysan and some patients with osteopetrosis visiting the clinic of Dr. Firas Khalid Al-Khalidi for post some pictures of fracture bones with osteopetrosis.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient before the publication of the study.

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AUTHOR CONTRIBUTIONS

Z.Z.G: Writing, editing, reviews. M.A.D and H.D.H: Reviews. F.K.A: Reviews and diagnoses medical cases and enhances the research with some X-rays for patients with osteopetrosis. All the authors contributed to the conduct of this work and the approval of the final version. They have reviewed and given their approval to the manuscript's final draft.

CONFLICT OF INTEREST

None.

REFERENCES

Aker, M., Rouvinski, A., Hashavia, S., Ta-Shma, A., Shaag, A., Zenvirt, S., Israel, S., Weinttraub, M., Taraboulos, A., Shavit, Z., & Elpeleg, O. (2012). An SNX10 mutation causes malignant osteopetrosis in infancy. Journal of Medical Genetics, 49(4), 221-226.

Alhuzaim, O., Almohareb, O., & Sherbeeni, M. (2015). Carbonic anhydrase II deficiency in a Saudi woman. Clinical Medicine Insights: Case Reports, 8, S16897.

Alkhayal, Z., Shinwari, Z., Gaafar, A., & Alaiya, A. (2023). Carbonic anhydrase II activators in osteopetrosis treatment: A review. Current Issues in Molecular Biology, 45(2), 1373-1386.

Arunachalam, A., Aboobacker, F., Sampath, E., Devasia, A., Korula, A., George, B., & Edison, E. (2024). Molecular heterogeneity of osteopetrosis in India: Report of 17 novel variants. Indian Journal of Hematology and Blood Transfusion, 40, 1-10.

Awad, M., Al-Ashwal, A., Sakati, N., Al-Abbad, A., & Bin-Abbas, B. (2002). Long-term follow up of carbonic anhydrase II deficiency syndrome. Saudi Medical Journal, 23(1), 25-29.

Bansal, A., Prakash, R., Agarwal, S., & Advani, U. (2023). Gene therapy and its applications. Journal of Medical Evidence, 4(1), 46-56.

Bhati, P., & Goyal, C. (2017). A rare case of osteopetrosis with unusual feature as microcephaly. Journal of Clinical and Diagnostic Research, 11(8), 01-02.

Borthwick, K., Kandemir, N., Topaloglu, R., Kornak, U., Bakkaloglu, A., Yordam, N., Ozen, S., Mocan, H., Shan, G., Sly, W. S., & Karet, F. E. (2003). A phenocopy of CAII deficiency: a novel genetic explanation for inherited infantile osteopetrosis with distal renal tubular acidosis. Journal of Medical Genetics, 40(2), 115-121.

Calder, D., Arulkumaran, S., & D'Arco, F. (2022). Imaging in osteopetrosis. Bone, 165, 116560.

Carlier, A., Van Oosterwyck, H., & Geris, L. (2014). In silico biology of bone regeneration inside calcium phosphate scaffolds. In Tissue engineering: Computer modeling, biofabrication and cell behavior (pp. 31-48). Dordrecht: Springer Netherlands.

Capo, V., Abinun, M., & Villa, A. (2022). Osteoclast rich osteopetrosis due to defects in the TCIRG1 gene. Bone, 165, 116519.

Chávez-Güitrón, E., Cerón-Torres, T., Sobacchi, C., Ochoa-Ruiz, E., & Villegas-Huesca, S. (2018). Autosomal recessive osteopetrosis type I: description of pathogenic variant of TCIRG1 gene. Boletín Médico del Hospital Infantil de México, 75(4), 255-259.

Chen, Y., Zhou, L., Guan, X., Wen, X., Yu, J., & Dou, Y. (2023). Case report: Gene mutations and clinical characteristics of four patients with osteopetrosis. Frontiers in Pediatrics, 11, 1096770.

Chu, A., Zirngibl, A., & Manolson, F. (2021). The V-ATPase a3 subunit: Structure, function and therapeutic potential of an essential biomolecule in osteoclastic bone resorption. International Journal of Molecular Sciences, 22(13), 6934.

Cohen-Solal, M., Collet, C., Bizot, P., Pavis, C., & Funck-Brentano, T. (2023). Osteopetrosis: the patient point of view and medical challenges. Bone, 167, 116635.

Del Fattore, A., Cappariello, A., & Teti, A. (2008). Genetics, pathogenesis and complications of osteopetrosis. Bone, 42(1), 19-29.

Elson, A., Stein, M., Rabie, G., Barnea-Zohar, M., Winograd-Katz, S., Reuven, N., Shalev, M., Sekeres, J., Kanaan, M., Tuckermann, J., & Geiger, B. (2021). Sorting Nexin 10 as a key regulator of membrane trafficking in bone-resorbing osteoclasts: lessons learned from osteopetrosis. Frontiers in Cell and Developmental Biology, 9, 671210.

Emadian, O., Kariminasab, M., Montazer, F., Azar, S., Saravi, S., & Daneshpoor, M. (2014). Osteopetrosis: A case report and review of the literature. Sudan Journal of Medical Sciences, 9(4), 271-275.

Frattini, A., Orchard, J., Sobacchi, C., Giliani, S., Abinun, M., Mattsson, P., Keeling, D., Andersson, A., Wallbrandt, P., Zecca, L., Notarangelo, L., Vezzoni, P., & Villa, A. (2000). Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis. Nature Genetics, 25(3), 343-346.

Gaytán-Morales, F., Castorena-Villa, I., Mendoza-Camargo, O., Cortés-Flores, C., Gómez-Domíguez, A., Montenegro-Chahar, D., Maldonado-Garcia, P., & Parra-Ortega, I. (2021). Hematopoietic stem cell transplantation in a patient with osteopetrosis and mutation in CLCN7: long-term follow-up. Boletín Médico del Hospital Infantil de México, 78(3), 225-233.

Gong, H. P., Ren, Y., Zha, P. P., Zhang, W. Y., Zhang, J., Zhang, Z. W., & Wang, C. (2023). Clinical and genetic diagnosis of autosomal dominant osteopetrosis type II in a Chinese family: A case report. World Journal of Clinical Cases, 11, 700-708.

Hiyama, S., Takahashi, T., Matsumura, T., & Takeshita, K. (2020). Open reduction and internal fixation using a locking compression plate as treatment for subtrochanteric fracture in two patients with osteopetrosis. Injury, 51(2), 565-569.

Hosseinzadeh, V., Rad, M., Khorashad, R., & Khodashenas, E. (2024). A case report of neonatal osteopetrosis. Revista Colombiana de Reumatología, 31(2), 276-279.

Hoyoux, C., Dresse, F., Forget, P., Piette, C., Rausin, L., Villa, A., Gothot, A., & Florkin, B. (2014). Osteopetrosis mimicking juvenile myelomonocytic leukemia. Pediatrics International, 56(5), 779-782.

Huybrechts, Y., & Van Hul, W. (2022). Osteopetrosis associated with PLEKHM1 and SNX10 genes, both involved in osteoclast vesicular trafficking. Bone, 164, 116520.

Ishaque, A., Farid, E., Nasir, S., Qadar, T., & Jamal, A. (2022). Intermediate autosomal recessive osteopetrosis with an unusual absence of fractures. Ochsner Journal, 22(4), 366-371.

Jodeh, W., Katz, A. J., Hart, M., Warden, J., Niziolek, P., Alam, I., Lng, S., Polgreen, L., Imel, E., & Econs, M. (2024). Autosomal dominant osteopetrosis (ADO) is caused by a missense variant in the TCIRG1 gene. The Journal of Clinical Endocrinology & Metabolism, 109(7), 1726-1732.

Klaab, Z., Al Tuwaijri, A., Umair, M., Aldahmash, B., & Alfadhel, M. (2023). A novel homozygous splice site

variant in the CLCN7 causes osteopetrosis. Journal of King Saud University-Science, 35(1), 102377.

Kim, Y. D., Pofali, P., Park, T. E., Singh, B., Cho, K., Maharjan, S., Dandekar, P., Ratnesh, J., Choi, Y. J., Arote, R., & Cho, C. S. (2016). Gene therapy for bone tissue engineering. Tissue Engineering and Regenerative Medicine, 13, 111-125.

Kornak, U., Kasper, D., Bösl, M. R., Kaiser, E., Schweizer, M., Schulz, A., Friedrich, W., Delling, G., & Jentsch, T. J. (2001). Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell, 104(2), 205-215.

Lafuente-Gracia, L., Borgiani, E., Nasello, G., & Geris, L. (2021). Towards in silico models of the inflammatory response in bone fracture healing. Frontiers in Bioengineering and Biotechnology, 9, 703725.

Leite, L. D. R., Resende, K. K. M., Santos Rosa, L. D., Mazzeu, J. F., de Oliveira, L. C., Scher, M. D. C. S. D., Acevedo, A. C., & Yamaguti, P. M. (2023). Carbonic anhydrase II deficiency syndrome with amelogenesis imperfecta linked to a homozygous CA2 deletion. Intractable & Rare Diseases Research, 12(3), 202-205.

Li, D., Ou, M., Zhang, W., Luo, Q., Cai, W., Mo, C., & Dai, Y. (2023). CRISPR/Cas9-mediated gene correction in osteopetrosis patient-derived iPSCs. Frontiers in Bioscience-Landmark, 28(6), 131.

Li, L., Lv, S. S., Wang, C., Yue, H., & Zhang, Z. L. (2019). Novel CLCN7 mutations cause autosomal dominant osteopetrosis type II and intermediate autosomal recessive osteopetrosis. Molecular Medicine Reports, 19(6), 5030-5038.

Liang, H., Li, N., Yao, R. E., Yu, T., Ding, L., Chen, J., & Wang, J. (2021). Clinical and molecular characterization of five Chinese patients with autosomal recessive osteopetrosis. Molecular Genetics & Genomic Medicine, 9(11), 1815.

Makaryan, V., Kelley, M. L., Bolyard, A. A., Yen, H. J., & Dale, D. C. (2022). Novel TCIRG1 mutations causing congenital neutropenia. Blood, 140(1), 8334-8335.

Maurizi, A. (2022). Experimental therapies for osteopetrosis. Bone, 165, 116567.

Mégarbané, A., Pangrazio, A., Villa, A., Chouery, E., Maarawi, J., Sabbagh, S., Lefranc, G., & Sobacchi, C. (2013). Homozygous stop mutation in the SNX10 gene in a consanguineous Iraqi boy with osteopetrosis and corpus callosum hypoplasia. European Journal of Medical Genetics, 56(1), 32-35.

Moore, J. B., Hoang, T. D., & Shwayhat, A. F. (2017). Case report of clinical vignette: osteopetrosis. Military Medicine, 182(3-4), 1886-1888.

Moscatelli, I., Almarza, E., Schambach, A., Ricks, D., Schulz, A., Herzog, C. D., Henriksen, K., Askmyr, M., Schwartz, J. D., & Richter, J. (2021). Gene therapy for infantile malignant osteopetrosis: review of pre-clinical research and proof-of-concept for phenotypic reversal. Molecular Therapy-Methods & Clinical Development, 20, 389-397.

Mwakalinga, L. K., Temu, R. J., Massawe, H., Ncheye, M., Mahumbuga, Z., Shirima, O. A., Jusabani, M., Shoo, R., & Pallangyo, A. J. (2023). Osteopetrosis case series from Tanzania. Open Access Library Journal, 10(8), 1-13.

Nadyrshina, D. D., & Khusainova, R. I. (2023). Clinical, genetic aspects and molecular pathogenesis of osteopetrosis. Vavilov Journal of Genetics and Breeding, 27(4), 383.

O'Brien, A., Krueger, J., Dupuis, L., Voronov, I., Kannu, P., Cohn, R. D., & Mendoza-Londono, R. (2015). 382. Osteopetrosis: An unusual presentation of a rare disease as a candidate for gene therapy. Molecular Therapy, 23, S152.

Ou, M., Li, C., Tang, D., Xue, W., Xu, Y., Zhu, P., Bo, L., Jiansheng, X., Chen, J., Sui, W., Yin, L., & Dai, Y. (2019). Genotyping, generation and proteomic profiling of the first human autosomal dominant osteopetrosis type II-specific induced pluripotent stem cells. Stem Cell Research & Therapy, 10, 1-17.

Okamoto, N., Kohmoto, T., Naruto, T., Masuda, K., Komori, T., & Imoto, I. (2017). Novel CLCN7 compound heterozygous mutations in intermediate autosomal recessive osteopetrosis. Human Genome Variation, 4(1), 1-4.

Palagano, E., Menale, C., Sobacchi, C., & Villa, A. (2018). Genetics of osteopetrosis. Current Osteoporosis Reports, 16(1), 13-25.

Pang, Q., Qi, X., Jiang, Y., Wang, O., Li, M., Xing, X., Dong, J., & Xia, W. (2015). Two novel CAII mutations causing carbonic anhydrase II deficiency syndrome in two unrelated Chinese families. Metabolic Brain Disease, 30, 989-997.

Pangrazio, A., Caldana, M. E., Iacono, N. L., Mantero, S., Vezzoni, P., Villa, A., & Sobacchi, C. (2012). Autosomal recessive osteopetrosis: report of 41 novel mutations in the TCIRG1 gene and diagnostic implications. Osteoporosis International, 23, 2713-2718.

Pangrazio, A., Fasth, A., Sbardellati, A., Orchard, P. J., Kasow, K. A., Raza, J., Albayrak, C., Albayrak, D., Vanakker, O. M., De Moerloose, B., Vellodi, A., Notarangelo, L. D., Schlack, C., Strauss, G., Kuhl, J. S., Caldana, L., LO Iacono, N., Susani, L., Kornak, U., ... Villa, A., & Sobacchi, C. (2013). SNX10 mutations define a subgroup of human autosomal recessive osteopetrosis with variable clinical severity. Journal of Bone and Mineral Research, 28(5), 1041-1049.

Penna, S., Capo, V., Palagano, E., Sobacchi, C., & Villa, A. (2019). One disease, many genes: implications for the treatment of osteopetroses. Frontiers in Endocrinology, 10, 85.

Rivadeneira, F., & Uitterlinden, A. G. (2018). Osteoporosis genes identified by genome-wide association studies. In Genetics of bone biology and skeletal disease (pp. 377-395). Academic Press.

Robb, G. B. (2019). Genome editing with CRISPR-Cas: an overview. Current Protocols Essential Laboratory Techniques, 19(1), e36.

Rössler, U., Hennig, A. F., Stelzer, N., Bose, S., Kopp, J., Søe, K., Cyganek, L., Zifarelli, G., Ali, S., Hang, M. V., Strassler, E. T., Hahn, G., Pusch, M., Stauber, T., Lzsvak, Z., Gossen, M., Stachelscheid, H., & Kornak, U. (2020). Efficient generation of osteoclasts from human induced pluripotent stem cells and functional investigations of lethal CLCN7-related osteopetrosis. Journal of Bone and Mineral Research, 36(8), 1621-1635.

Shaik, N. A., Bokhari, H. A., Masoodi, T. A., Shetty, P. J., Ajabnoor, G. M., Elango, R., & Banaganapalli, B. (2020). Molecular modelling and dynamics of CA2

missense mutations causative to carbonic anhydrase 2 deficiency syndrome. Journal of Biomolecular Structure and Dynamics, 38(14), 4067-4080.

Stark, Z., & Savarirayan, R. (2009). Osteopetrosis. Orphanet Journal of Rare Diseases, 4, 1-12.

Suliman, O. S., Khedr, M. A., & Al Ruthae, M. S. (2010). Carbonic anhydrase II deficiency syndrome: A report of 18 new Saudi Arabian cases. Journal of Pediatric Neurology, 8(02), 163-174.

Susani, L., Castelli, A., Lizier, M., Lucchini, F., Vezzoni, P., & Paulis, M. (2018). Correction of a recessive genetic defect by CRISPR-Cas9-mediated endogenous repair. The CRISPR Journal, 1(3), 230-238.

Teti, A., & Econs, M. J. (2017). Osteopetroses, emphasizing potential approaches to treatment. Bone, 102, 50-59.

Thomas, A., Francis, L., & James, B. R. (2009). Osteopetrosis. Postgraduate Medical Journal, 85(1003), 250-250.

Udupa, P., Ghosh, D. K., Kausthubham, N., Shah, H., Bartakke, S., Dalal, A., Girisha, K. M., & Bhavani, G. S. (2023). Genome sequencing identifies a large non-coding region deletion of SNX10 causing autosomal recessive osteopetrosis. Journal of Human Genetics, 68(4), 287-290.

Vickram, A. S., Manikandan, S., Richard, T., Lakshmi, S. V., & Chopra, H. (2024). Targeted gene therapy: Promises and challenges in disease management. Journal of Bio-X Research, 7(02), 81-89.

Wang, S. W., Gao, C., Zheng, Y. M., Yi, L., Lu, J. C., Huang, X. Y., Cai, J. B., Zhang, P. F., Cui, Y. H., & Ke, A. W. (2022). Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Molecular Cancer, 21(1), 57.

Wang, X., Wang, Y., Xu, T., Fan, Y., Ding, Y., & Qian, J. (2023). A novel compound heterozygous mutation of the CLCN7 gene is associated with autosomal recessive osteopetrosis. Frontiers in Pediatrics, 11, 978879.

Won, J. Y., Jang, W. Y., Lee, H. R., Park, S. Y., Kim, W. Y., Park, J. H., Kim, Y., & Cho, T. J. (2017). Novel missense loss-of-function mutations of WNT1 in an autosomal recessive osteogenesis imperfecta patient. European Journal of Medical Genetics, 60(8), 411-415.

Wu, C. C., Econs, M. J., Di Meglio, L. A., Insogna, K. L., Levine, M. A., Orchard, P. J., Miller, P. W., Petryk, A., Rush, E. T., Shoback, D. M., Ward, L. M., & Polgreen, L. E. (2017). Diagnosis and management of osteopetrosis: consensus guidelines from the osteopetrosis working group. The Journal of Clinical Endocrinology & Metabolism, 102(9), 3111-3123.

Xu, J., Qiu, H., Zhao, J., & Pavlos, N. J. (2021). The molecular structure and function of sorting nexin 10 in skeletal disorders, cancers, and other pathological conditions. Journal of Cellular Physiology, 236(6), 4207-4215.

Xu, T., Xu, J., Ye, Y., Wang, Q., Shu, X., Pei, D., & Liu, J. (2014). Structure of human SNX10 reveals insights into its role in human autosomal recessive osteopetrosis. Proteins: Structure, Function, and Bioinformatics, 82(12), 3483-3489.

Yu, T., Yu, Y., Wang, J., Yin, L., Zhou, Y., Ying, D., Huang, R., Chen, H., Wu, S., Shen, Y., Fu, Q., & Chen, F. (2014). Identification of TCIRG1 and CLCN7 gene mutations in a patient with autosomal recessive osteopetrosis. Molecular Medicine Reports, 9(4), 1191-1196.

Zhang, X. Y., He, J. W., Fu, W. Z., Wang, C., & Zhang, Z. L. (2017). Novel mutations of TCIRG1 cause a malignant and mild phenotype of autosomal recessive osteopetrosis (ARO) in four Chinese families. Acta Pharmacologica Sinica, 38(11), 1456-1465.

Zhang, Y., Chen, D., Zhang, F., Lv, Q., Tang, L., & Tong, N. (2016). Case report osteopetrosis complicated by schizophrenia results from mutations on Chromosome 16. International Journal of Clinical and Experimental Medicine, 9(9), 18673-18677.

Zhao, F., Mc Garrigle, M. J., Vaughan, T. J., & Mc Namara, L. M. (2018). In silico study of bone tissue regeneration in an idealised porous hydrogel scaffold using a mechano-regulation algorithm. Biomechanics and Modeling in Mechanobiology, 17, 5-18.