Radiology Case Reports 15 (2020) 1883–1886

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/radcr

Case Report

Morphology and chemical identity of periarticular and vascular calcification in a patient with the rare genetic disease of arterial calcification due to deficiency of CD73 (ACDC)☆,☆☆

Deepak R. Lakshmipathy, BS[a], Cornelia D. Cudrici, MD[a], Frederick Dyda, PhD[b], Wenqian Xu, PhD[c], Elisa A. Ferrante, PhD[a], David T. Nguyen, BS[a], Katherine M. Carney, BSN[a], Shirley Rollison, RT (R) (CT)[a], Marcus Y. Chen, MD[a], Leon J. Nesti, MD, PhD[d], Manfred Boehm, MD[a], Alessandra Brofferio, MD[a], Han Wen, PhD[a,]*

[a] *National Heart, Lung, and Blood Institute, National Institutes of Health; 9000 Rockville Pike, Bethesda, MD 20892 USA*

[b] *Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health; 9000 Rockville Pike, Bethesda, MD 20892 USA*

[c] *X-ray Science Division, Advanced Photon Source, Argonne National Laboratory; 9700 S. Cass Ave, Lemont, IL 60439 USA*

[d] *Clinical and Experimental Orthopedics, Walter Reed National Military Medical Center, Bethesda, MD USA*

ARTICLE INFO

Article history:
Received 9 July 2020
Revised 20 July 2020
Accepted 20 July 2020

ABSTRACT

A 54-year old female patient with the genetic disease of arterial calcification due to deficiency of CD73 was studied under the Undiagnosed Disease Program of the National Institutes of Health. She presented with symptoms of claudication in her 40s and later developed arthritic symptoms, ectopic calcification in her left hand and severe arterial calcifications of the lower extremities. Since little was known about the composition of the calcifications in arterial calcification due to deficiency of CD73, we investigated their chemical identity and microscopic morphology in this patient with imaging and x-ray diffraction analysis.

☆ Acknowledgments: We would like to thank Mr. Eric Bennett (National Heart, Lung, and Blood Institute, National Institutes of Health; 9000 Rockville Pike, Bethesda, MD 20892) for assistance with operation of the x-ray micro tomosynthesis scanner. This research was funded by the Division of Intramural Research, National Heart, Lung and Blood Institute, and the Intramural Program of the National Institute of Diabetes and Digestive and Kidney Diseases. This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

☆☆ Competeing Interests: None.

* Corresponding author.
E-mail address: han.wen@nih.gov (H. Wen).

https://doi.org/10.1016/j.radcr.2020.07.056

We found that, microscopically, the bulk calcifications consisted of fragments of either solid or porous internal structure. Both periarticular and arterial calcifications were primarily hydroxyapatite crystals of the same crystalline anisotropy, but different crystalline grain sizes. This was consistent with the presence of hydroxyapatite crystals along with birefringent calcium pyrophosphate dihydrate crystals in the synovial fluid of the patients by polarized light microscopy. The result suggests that tissue calcification in both locations follow a similar biochemical mechanism caused by an increase in extracellular tissue-nonspecific alkaline phosphatase activity.

Background on ACDC

Arterial calcification due to deficiency of CD73 (ACDC) is a rare, hereditary autosomal recessive disease that has been identified in less than twenty people worldwide. The cause of the disease is recently identified as a mutation in the 5′-nucleotidase Ecto (*NT5E*) gene that encodes the CD73 enzyme.[1-3] Inactivation of CD73 results in decreased extracellular adenosine and inorganic phosphate, and increased tissue-nonspecific alkaline phosphatase (TNAP) activity, inducing the formation of calcifications in the hands and lower extremities [2]. Left untreated, this buildup can lead to severe peripheral obstructive arterial disease and arthritis [1].

Little is known about the chemical identity and microscopic morphology of the calcium deposits in ACDC. While isolated crystals in the synovial fluid are traditionally characterized with polarization microscopy [4], the dense calcifications in the hands and lower extremities require the use of x-ray technologies capable of penetrating thick samples.

Case report

A 54-year-old female patient presented with symptoms of claudication in her 40s and later developed arthritic symptoms. She had significant calcium deposits in her lower-extremity arteries and hand and foot joint capsules, pain and cramping, poor circulation and reduced mobility. The diagnosis of ACDC was confirmed by genomic DNA analysis from a punch-biopsy skin specimen. Figure 1A is a radiography of the left hand prior to the surgical removal of a radially based calcified nodule adjacent to the metacarpophalangeal joint of the index finger. The patient also received an ultra-high-resolution lower extremity CT scan of 16 cm length (Figs. 1B and C) on a Cannon Aquilion Genesis One CT scanner (Tustin, CA). Previously, the patient had undergone a femoral endarterectomy procedure on the same vessel, from which vascular tissue samples were preserved.

The hand radiograph revealed a dense, 11 mm-long calcified nodule adjacent to the metacarpophalangeal joint of the left index finger (Fig. 1A). The lower-extremity CT showed dense and contiguous arterial calcification with cross-sectional sizes up to 30 mm (Fig. 1B and C). The arterial calcification appears to be dense aggregates of heterogeneous fragments.

Fresh tissue samples from the periarticular lesion in the left hand were scanned with an x-ray micro tomosynthesis scanner in a time window of 15 minutes prior to pathology tests. A preserved vascular tissue sample from the previous endarterectomy procedure was also scanned with micro tomosynthesis.

Microscopically, calcification in the periarticular lesion appeared to be aggregates of heterogeneous particles up to 500 μm in size (Fig. 2A). Calcification in the vascular tissue from the femoral artery had an interconnected porous internal structure (Fig. 2B).

Tissue samples were preserved in formalin for x-ray diffraction analysis. The analysis concluded that the calcification in both the periarticular and vascular tissue samples were primarily hydroxyapatite crystals (Figs. 3A and B). Both possessed the same crystalline anisotropy, where the grains were elongated along the crystallographic c-axis. The average crystalline grain size was larger in the periarticular sample (25 nm) than the vascular arterial sample (14 nm).

Conclusion

Bulk calcium deposits in the periarticular and vascular tissues of the patient were identified as primarily hydroxyapatite crystals. The calcification at these 2 locations shared similar structures at the nano scale, but differing appearance at the microscopic scale. The finding is consistent with the presence of hydroxyapatite along with calcium pyrophosphate dihydrate crystals in her synovial fluid, as identified by polarized light microscopy. [4] The hydroxyapatite identity of the calcium deposits can be explained by the originally proposed metabolic pathway of ACDC, where severe calcification is considered to be caused by an increase in extracellular TNAP activity. [1-3] It is currently accepted that hydroxyapatite crystal formation is induced by low pyrophosphate levels, a potent inhibitor of mineralization, as a result of high local extracellular TNAP activity and increased local levels of inorganic phosphate. [3].

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the in-

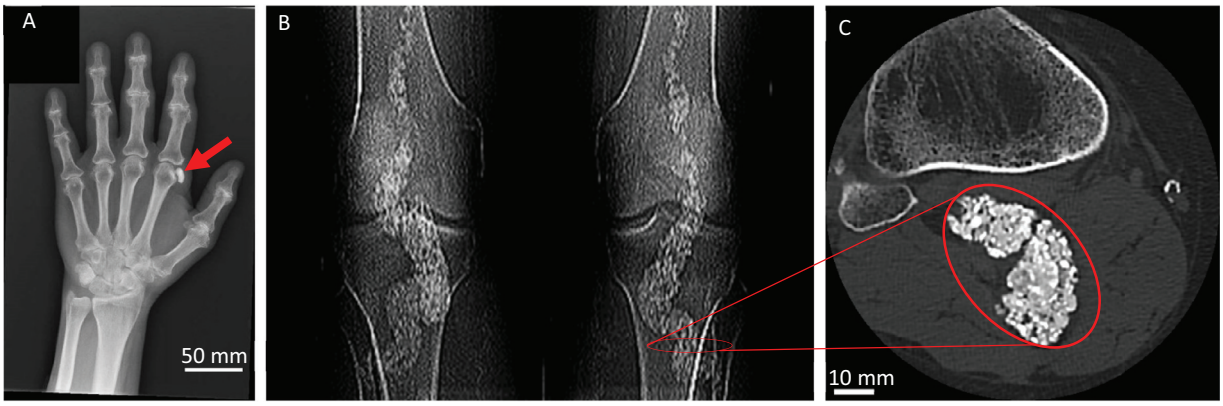

Fig. 1 – (A) A radiograph of the patient's left hand shows a solid calcification of 11 mm length near the metacarpophalangeal joint of the index finger (red arrow). (B) An AP planar projection image shows extensive, contiguous calcification within the patient's femoral arteries. (C) A CT cross-section below the patella of the right knee provides a detailed view of the calcium deposits (red oval), which appeared to be a compacted mass of heterogeneous fragments. (Color version of figure is available online.)

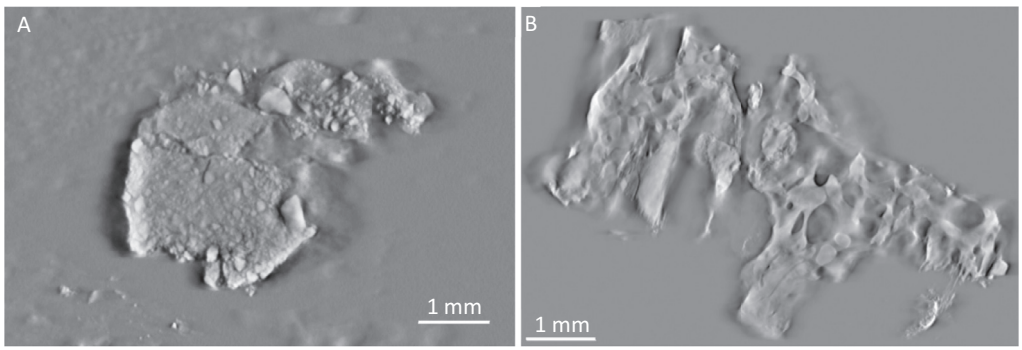

Fig. 2 – X-ray micro tomosynthesis scans of tissue samples. (A) A cross-sectional image of a fresh tissue sample that was part of the surgically removed periarticular lesion in the left hand. Bulk calcification appears as aggregates of heterogeneous particles up to 500 μm in size. (B) A cross-sectional image of a preserved vascular tissue sample from a femoral endarterectomy procedure. Bulk calcification has an interconnected porous internal structure.

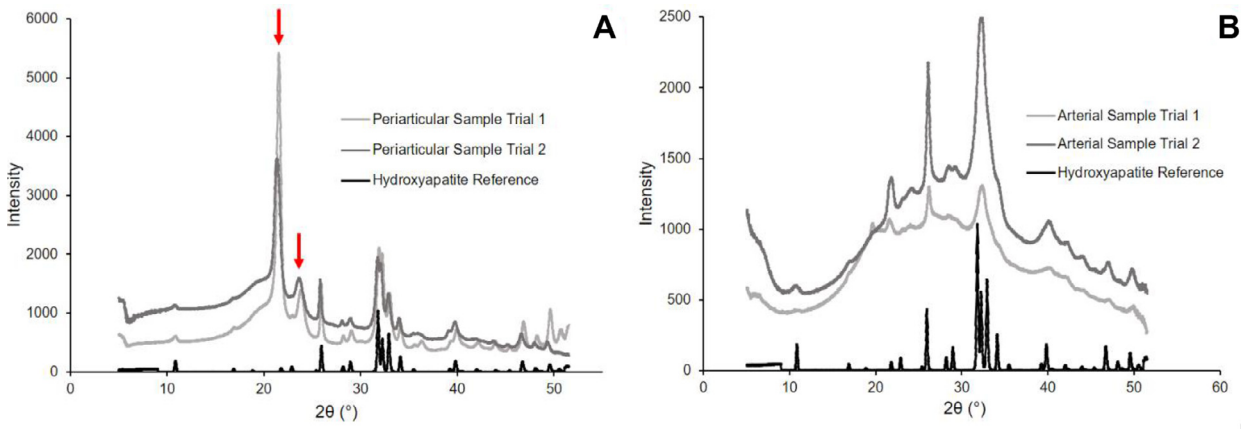

Fig. 3 – (A) and (B) are x-ray diffraction radial patterns of the periarticular and arterial samples, respectively. The hydroxyapatite reference pattern for chemical fingerprinting is also shown. Red arrows point to background peaks from the sample holder. (Color version of figure is available online.)

stitutional and/or national research committee and with the Helsinki Declaration of 1975 for experiments involving humans and its later revisions or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

REFERENCES

[1] Hilaire CST, Ziegler SG, Markello TC, Brusco A, Groden C, Gill F, et al. NT5E mutations and arterial calcifications. N Engl J Med 2011;364:432–42.

[2] Zhang Z, He JW, Fu WZ, Zhang CQ, Zhang ZL. Calcification of joints and arteries: second report with novel NT5E mutations and expansion of the phenotype. J Hum Genet 2015;60(10):561–4.

[3] Jin H, St Hilaire C, Huang Y, Yang D, Dmitrieva NI, Negro A, et al. Increased activity of TNAP compensates for reduced adenosine production and promotes ectopic calcification in the genetic disease ACDC. Sci Signal 2016;9:ra121.

[4] Cudrici et al., Multifocal calcific periarthritis with distinctive clinical and radiological features in patients with CD73 deficiency, manuscript in preparation.