Challenging arterial calcification disease associated with rare NT5E gene mutation

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Challenging arterial calcification disease associated with rare *NT5E* gene mutation

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DESCRIPTION

A 47-year-old women has been suffering from intermittent claudication since she was 33 years. The symptoms have slowly worsened during the following years and the patient was unable to walk more than 200 meters without leg pain (Leriche – Fontain IIb) at admission. She had an history of hydroxyapatite crystal calcification disease diagnosed at the age of 19 years, after the onset of bilateral symmetric hand arthritis and Raynaud phenomenon.

On physical examination peripheral pulses were weak at palpation and ankle-brachial index (ABI) measurement was markedly decreased (0.59 on the right and 0.38 on the left leg). Echo-colour-Doppler ultrasounds of the lower limbs documented severely calcified arteries (**Figure 1A, B**), while no calcification of the carotid walls was found. The patient underwent coronary CT scan, abdominal and thoracic aorta CT scan and lower limbs artery CT scan and X-ray to accurately assess the systemic calcium deposit and to determine the global cardiovascular risk. The images revealed severe calcific arterial disease limited to femoral and popliteal arteries bilaterally with developed collateral neovascularization (**Figure 1C, D**); coronary arteries and the whole aorta were free from vascular wall calcification. Hands X-ray displayed pericapsular calcification of the finger joints. A wide panel of laboratory examination, including serum calcium and phosphate, vitamin-D, parathyroid hormone, anti-nuclear antibodies (ANA), anti-extractable nuclear antigen antibodies (ENA) were unremarkable.

We performed Sanger sequencing the coding exons of the *NT5E* gene (Table) and identified the homozygous c.1608dupA – p.Val537SerfsTer7 variant, consistent with the diagnosis of "Arterial Calcification due to Deficiency of CD73" also known as "CAlcification of Joints and Arteries" (CALJA) syndrome (MIM211800). The variant is predicted loss-of-function, described in another case with the disease [1] and reported in ClinVar database (VCV000029689.1). Segregation analysis showed both parents were heterozygotes (Figure 2).

Arterial Calcification due to Deficiency of CD73 is a rare syndrome defined by arterial calcification which targets vessels below the diaphragm, notably the large lower extremity arteries, and spares coronary circulation. Mutations in *NT5E* gene, encoding for the plasma membrane enzyme CD73 which hydrolyses AMP to adenosine and inorganic phosphate (Pi), were identified in adults from three different families affected with the disease. [1] From this first description only four other families have been described worldwide. [2-5]

In vitro assays demonstrated that CD73-deficient fibroblasts reduced extracellular adenosine levels and enhanced the activity of tissue non-specific alkaline phosphatase (TNAP), which generates procalcifying extracellular Pi. CALJA is an adult-onset disease, with the first symptoms occurring during the second decade of life. The pathogenetic calcification progresses for years, possibly leading to neoangiogenic vascular remodelling and development of small collateral vessels. This fact could explain the marked decrease of ABI index with a mild functional impairment of our patient.

An approved therapy for patients affected by Arterial Calcification due to Deficiency of CD73 has not yet

been available, however an ongoing clinical trial is testing the effectiveness and the safety of Etidronate as a standard treatment for this syndrome.

Table. PCR primers used for Sanger sequencing of NT5E gene

Name	*Sequence*
NT5E-1F	*5'-cctagctgctcgcccctactc*
NT5E-1R	*5'-actctgccatccgctgcttttc*
NT5E-2F	*5'-tgtctcatagagcttagtcagttttga*
NT5E-2R	*5'-ataatgccaagctgtgatttagggc*
NT5E-3F	*5'-ttaaggtgtttaacctttgcatgta*
NT5E-3R	*5'-agttacaaaggcaaaagagacacag*
NT5E-4F	*5'-agccatgtatgtacaagggctgac*
NT5E-4R	*5'-ggagcaaaatatccagccatctaa*
NT5E-5F	*5'-ccagaatttagcccagtgtgagat*
NT5E-5R	*5'-tcgcatccttccttcctcttcc*
NT5E-6F	*5'-gatcctaaggaagaagagccaga*
NT5E-6R	*5'-ggcaagaaccataacagaaaagga*
NT5E-7F	*5'-attttctcaagtctattttccttct*
NT5E-7R	*5'-aggaaatgccatgagacttggga*
NT5E-8F	*5'-gaaatctccctttggatctggtg*
NT5E-8R	*5'-cttggcccaattttgttgttctt*
NT5E-9F	*5'-acaaaggactaccttactgttgattga*
NT5E-9R	*5'-agcctgtaaagatggtttttgctg*

Note: PCR conditions were identical for all amplimers. PCR was performed using the KAPA DNA PCR kit (Roche Diagnostics, Risch-Rotkreuz, Switzerland) under the specified conditions and 50 ng of genomic DNA. We performed a touch-down PCR using the following protocol: 95°C 3min; 14 cycles 95°C15 sec, 63°C 15sec, 72°C 3sec; 29 cycles 95°C15 sec, 63°C-0.5°C/cycle 15sec, 72°C 3sec. Final extension 72°C 1 min. PCR products were purified using ExoSAP, cycle sequenced and run on a 3130XL ABI Prism apparatus (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA).

LEARNING POINTS/TAKE HOME MESSAGES

- *Arterial Calcification due to Deficiency of CD73 is a rare genetic syndrome secondary to NT5E gene mutation characterized by arterial calcification which selectively targets vessels below the diaphragm together with periarticular calcification.*
- *A complete imaging evaluation with Duplex-ultrasounds, CT-scan and X-ray is of utmost importance to identify the typical arterial and articular calcification pattern.*
- *Arterial Calcification due to Deficiency of CD73 treatment is still a challenge with bisphosphonates as the only pharmacological therapy under evaluation by clinical trials.*

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FIGURES

Figure 1. Imaging panel showing wide calcification of the common femoral artery with signs of partial demodulated blood flow on echocolorDoppler scan (panel A) with distal evidence of post-stenotic flow (panel B); X-ray (panel C) and CT scan (panel D) of both legs displaying the typical pattern of massive arterial calcification with several collateral vessels.

Figure 2. Family pedigree and electropherograms of the pathogenic *NT5E* gene variant in exon 9.

PATIENT'S PERSPECTIVE

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Figure 1

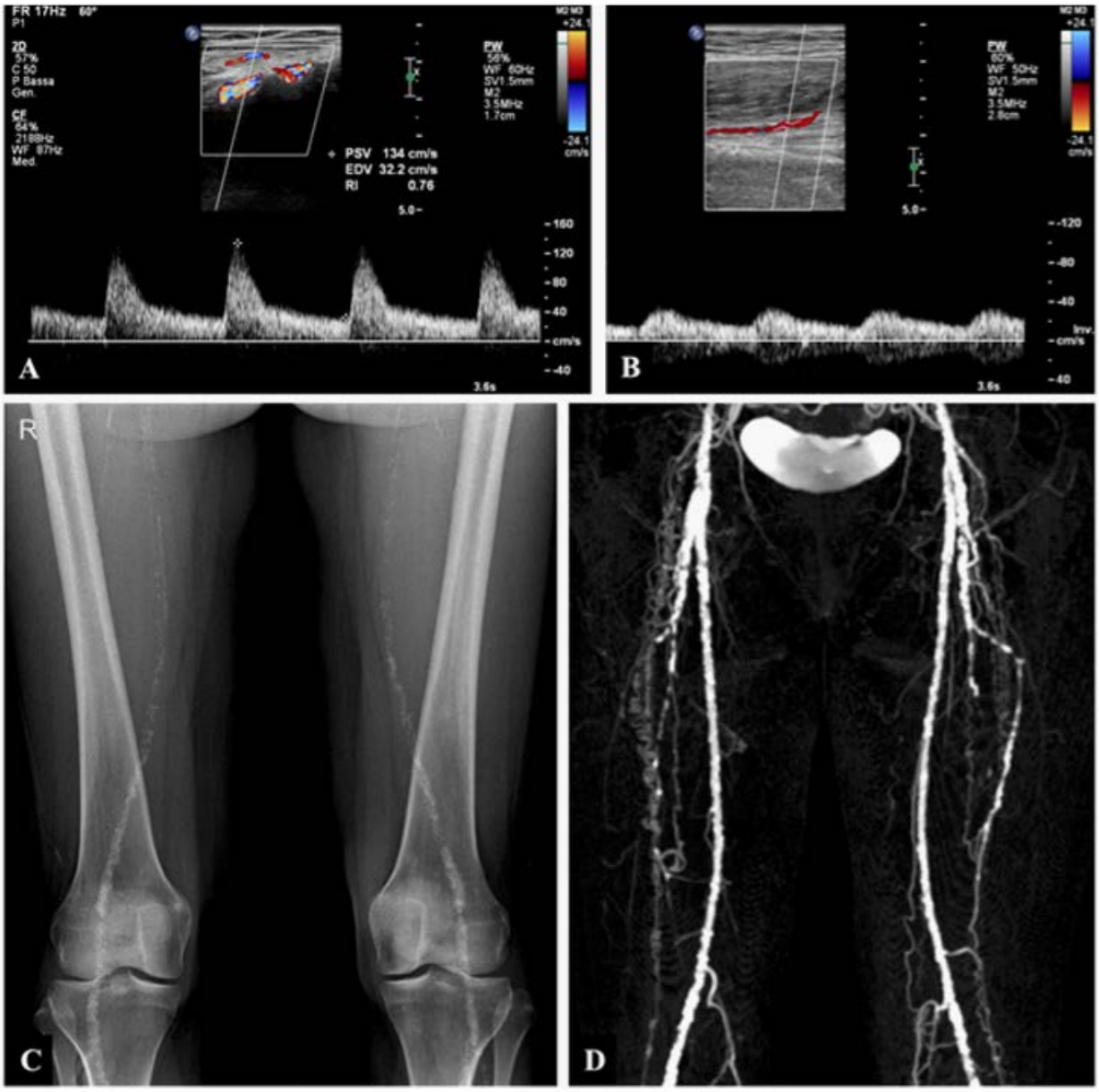
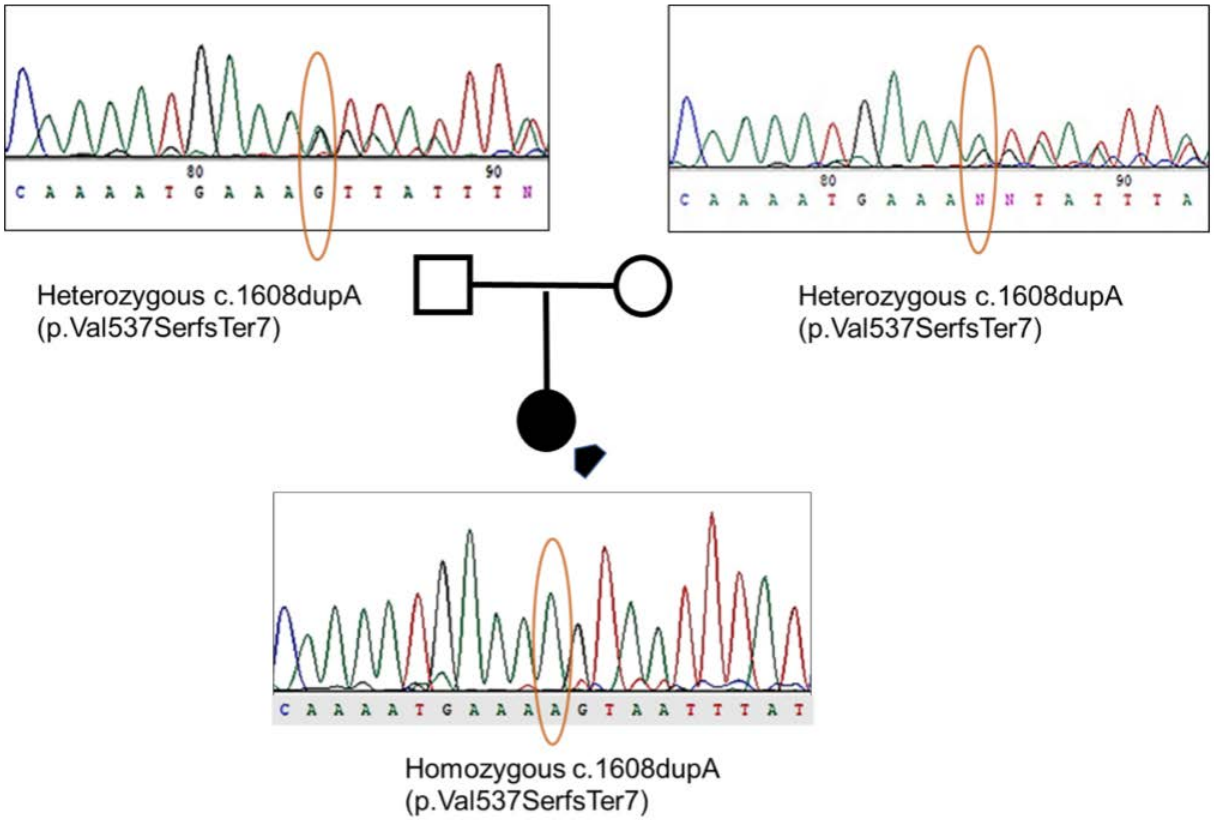


Figure 2


Heterozygous c.1608dupA
(p.Val537SerfsTer7)
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