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Correspondence

Isolated arterial calcifications of the lower extremities: A clue for *NT5E* mutation

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Dear Editor

A forty-two year old man was referred to the Dept. of Vascular Medicine because of severe intermittent claudication of both legs, that started at the age of 27. He never smoked, used alcohol in moderation and didn't use illicit drugs. His medication included acetylsalicylic acid and rosuvastatin. His family history was unremarkable, notably with no atherosclerotic disease and no consanguinity. His medical history revealed pain in several small joints of the hands, knees, and ankles since 1994, without rheumatological diagnosis.

Physical examination showed absent pulsations of the dorsal pedal arteries on both feet. Laboratory examination revealed normal serum concentration of total cholesterol (3.2 mmol/l), creatinine (71 μmol/l), glucose (5.3 mmol/l), calcium (2.41 mmol/l), inorganic phosphate (0.99 mmol/l), parathyroid hormone (4.3 pmol/l), and 25-OH-vitamin D (56 μmol/l).

A CT angiography of the thorax and abdomen was performed in 2007 revealing extensive arterial lower limb calcifications starting from the femoral artery (Fig. 1). Remarkably, the aorta and coronary

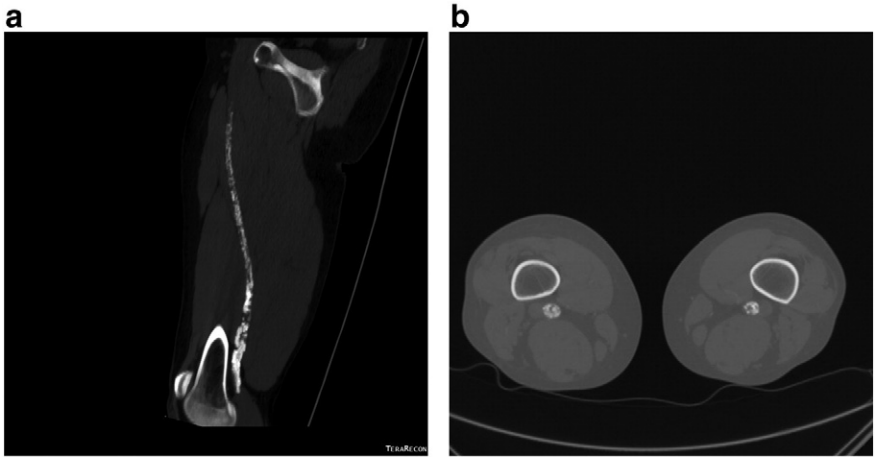

Fig. 1. Curved MPR (a) and transversal slide (b) of the upper leg demonstrating extensive calcifications in the wall of the superficial femoral artery.

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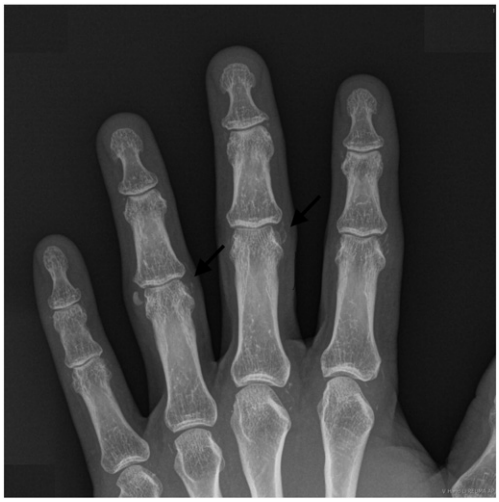

Fig. 2. X-ray of the hands demonstrating small periarticular calcifications (see arrows).

circulation were free from calcifications. Conventional X-ray of his hands revealed extensive pericapsular calcifications of the small hand joints (Fig. 2).

Based on the combination of extensive isolated lower limb calcifications and small joint calcification, the patient was subsequently referred to the Dept. of Human Genetics and a genetic screening of the *NT5E* gene was proposed after informed consent.

Sanger sequencing of the 9 exons and exon–intron boundaries of the *NT5E* gene (NM_002526.3) was performed as described elsewhere [1], and a novel homozygous splice-site variation of intron 3 was identified (c.751 + 2 T>C). This variation was predicted to abolish splicing by four bioinformatics tools (MAxEntScan, NNSPLICE, GeneSplicer, and Human Splicing Finder). After skin biopsy and fibroblast culture, RNA was extracted and RTPCR, gel electrophoresis and Sanger sequencing revealed total in-frame skipping of exon 3 (Fig. 3), with no minor transcripts, compared to control cDNA of an unaffected individual.

The clinical picture of arterial calcification of the lower extremities and joint calcifications has been described as early as 1914 and an inherited nature of this disease was suggested in 1954 (CALcification of Joints and Arteries; CALJA; #211800) [2]. In our patient, the typical distribution of the vascular calcifications in combination with the small joint calcification, suggested a recessive defect in the *NT5E* gene, as previously described in three families with CALJA [1]. *NT5E* encodes CD73, which is a membrane-bound ecto-5′-nucleotidase catalyzing the conversion of AMP to adenosine [3]. It is hypothesized that adenosine acts as a natural break on tissue calcification by inhibiting tissue-nonspecific alkaline phosphatase which degrades pyrophosphate, a potent tissue calcification inhibitor. Recently, the phenotype was extended to upper limb arterial calcifications, raising the question of phenotypic spectrum in CALJA [4]. Bisphosphonates, which are pyrophosphate analogs and potent inhibitors of tissue calcification, might prove beneficial in patients with *NT5E* mutations, which is currently under investigation (ClinicalTrails.gov Identifier: NCT01585402).

Conflict of interest

None.

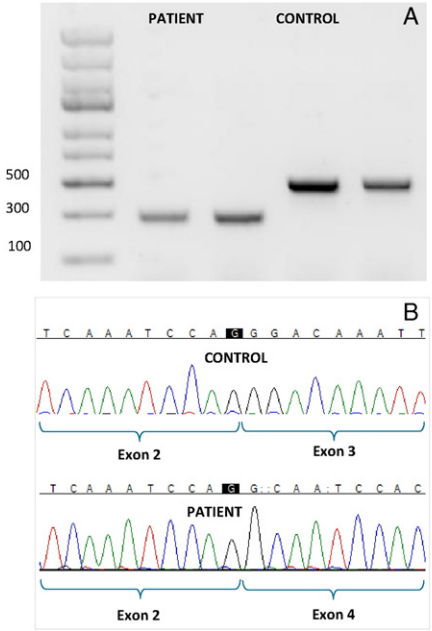

Fig. 3. Gel electrophoresis (a) and Sanger electropherogram (b) of the *NT5E* cDNA exons 2 to 4 from the patient's fibroblast culture compared to an unaffected control. The 300 bp band (3a) corresponds to complete homozygous skipping of exon 3 (3b).

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