

Images in...

Table 1 PCR primers used for Sanger sequencing of *NT5E* gene

NT5E-1F	5'-cctagctgctgccctactc
NT5E-1R	5'-actctgccatcgctgtcttc
NT5E-2F	5'-tgtctcatagagcttagctgtttga
NT5E-2R	5'-ataatgccaagctgtgatttagggc
NT5E-3F	5'-ttaaaggtttaaccttgcagtga
NT5E-3R	5'-agttacaaggcaaaagacacag
NT5E-4F	5'-agccatgtatgtacaagggtgac
NT5E-4R	5'-ggagcaaaatccagccatctaa
NT5E-5F	5'-ccagaatttagccagtgtagat
NT5E-5R	5'-tcgcatcctctctctctcc
NT5E-6F	5'-gatcctaaggaagaagaccaga
NT5E-6R	5'-ggcaagaaccataacagaaagga
NT5E-7F	5'-atcttcaagctatctctctct
NT5E-7R	5'-aggaaatgccatgagacttgga
NT5E-8F	5'-gaaatcccttggatctggtg
NT5E-8R	5'-cttgcccaattttgttctt
NT5E-9F	5'-acaaggactactctgttgattga
NT5E-9R	5'-agcctgtaaaagatggtttgtg

PCR conditions were identical for all amplicons. 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 3 min; 14 cycles 95°C 15 s, 63°C 15 s, 72°C 3 s; 29 cycles 95°C 15 s, 63°C–0.5°C/cycle 15 s, 72°C 3 s. 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, Massachusetts, USA).

Learning points

- ▶ Arterial calcification due to deficiency of CD73 is a rare genetic syndrome secondary to *NT5E* gene mutation characterised 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.

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.

Acknowledgements We would like to thank the patient for participating in this study.

Contributors GA, MM and AA performed and interpreted clinical evaluation and drafted the manuscript. AB performed and interpreted genetic analyses. All authors revised the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs

Mauro Massucci <http://orcid.org/0000-0003-0895-440X>

Angelo Adamo <http://orcid.org/0000-0001-7668-8938>

Alfredo Brusco <http://orcid.org/0000-0002-8318-7231>

REFERENCES

- 1 St Hilaire C, Ziegler SG, Markello TC, *et al*. NT5E mutations and arterial calcifications. *N Engl J Med* 2011;364:432–42.
- 2 Ichikawa N, Taniguchi A, Kaneko H, *et al*. Arterial calcification due to deficiency of CD73 (ACDC) as one of rheumatic diseases associated with Periarticular calcification. *J Clin Rheumatol* 2015;21:216–20.
- 3 Zhang Z, He J-W, Fu W-Z, *et al*. Calcification of joints and arteries: second report with novel NT5E mutations and expansion of the phenotype. *J Hum Genet* 2015;60:561–4.
- 4 de Nijs T, Albuissin J, Ockeloen CW, *et al*. Isolated arterial calcifications of the lower extremities: a clue for NT5E mutation. *Int J Cardiol* 2016;212:248–50.
- 5 Yoshioka K, Kuroda S, Takahashi K, *et al*. Calcification of joints and arteries with novel NT5E mutations with involvement of upper extremity arteries. *Vasc Med* 2017;22:541–3.

Copyright 2020 BMJ Publishing Group. All rights reserved. For permission to reuse any of this content visit <https://www.bmj.com/company/products-services/rights-and-licensing/permissions/>
BMJ Case Report Fellows may re-use this article for personal use and teaching without any further permission.

Become a Fellow of BMJ Case Reports today and you can:

- ▶ Submit as many cases as you like
- ▶ Enjoy fast sympathetic peer review and rapid publication of accepted articles
- ▶ Access all the published articles
- ▶ Re-use any of the published material for personal use and teaching without further permission

Customer Service

If you have any further queries about your subscription, please contact our customer services team on +44 (0) 207111 1105 or via email at support@bmj.com.

Visit casereports.bmj.com for more articles like this and to become a Fellow