C5b/9 (membrane attack complex) staining was negative in capillary endothelium and muscle fibres, excluding dermatomyositis. Further tests on serum for anti-U1RNP and anti-NXP2 antibodies were negative, and also did not support the diagnosis of mixed connective tissue disease or dermatomyositis.

Electron microscopy showed a monophasic process characterised by multifocal myonecrosis with early regenerating fibres, endomysial and perimysial lymphocytes and perivascular lymphocytes with invasion of the vessel walls and associated reactive endothelial changes (Fig. 1C). There were no tubulofilamentous inclusions or rimmed vacuoles to suggest a diagnosis of IBM.

Immune-related adverse events (irAEs) are being increasingly described for immune checkpoint inhibitors,[1] however myositis as a potentially life-threatening neurological irAE to nivolumab monotherapy is rare.[2] In the case described, we consider the findings of widespread perivascular T-lymphocytes and monophasic immune-mediated myositis to be part of the same process, presumptively caused by activated CTLs targeting muscle fibres and associated vasculature. The CD8+ effector lymphocytes were accompanied by a significant CD4+ T-cell population, including numerous presumptive Tregs. This has not previously been described in case reports of nivolumab-induced myositis,[3,4] despite the morphological inflammatory changes conforming to the polymyositis-like findings described in these case reports.[3] Alternative causes of immune-mediated myositis and connective tissue disorder overlap syndromes were considered as differential diagnoses. The light microscopy findings, immunophenotype of the inflammatory changes and additional investigation results, including negative myositis and other autoimmune auto-antibodies, excluded conventional immune-mediated myositides, such as dermatomyositis or IBM, and connective tissue disorders. The relatively monophasic nature of the histopathological changes also argued against malignancy-associated polymyositis. Due to the temporal relationship of onset of symptoms after nivolumab administration, lack of a known co-morbid myopathology and histological, biochemical and immunological features supporting T-lymphocyte mediated myositis, we believe that our findings are consistent with nivolumab-induced myositis.

With the increase in the number of approved indications for immune checkpoint inhibitors, clinicians need to be aware of rare and potentially life-threatening irAEs.

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Urinary bladder stone due to adenine phosphoribosyltransferase deficiency: first genetically confirmed case in a Chinese patient

Sir,

Urinary stone disease is uncommon in prepubertal children. Its presence, if recurrent or with no apparent cause, is a strong indication for further investigations of a possible underlying inborn error of metabolism. Amongst these possible inborn errors, adenine phosphoribosyltransferase (APRT) deficiency (OMIM #614723) is an autosomal recessive inborn error of adenine metabolism, which has been reported globally in Japan, France and Iceland.[1] The deficiency of APRT activity is characterised by excessive production of 2,8-dihydroxyadenine (DHA) (Fig. 1). This water-insoluble compound is cleared in the kidneys, where it may further precipitate as crystals leading to urolithiasis or crystalline nephropathy, while some patients may be asymptomatic.[1]

It is important to diagnose APRT deficiency early because it is readily treatable with allopurinol, and if left undiagnosed patients may potentially develop devastating consequences such as recurrent urinary stone disease, end-stage renal failure requiring renal replacement therapy,[2] or even recurrent crystalline nephropathy in renal allograft.[3] Early diagnosis and treatment may improve renal function and achieve normal growth and development in children with APRT deficiency.[4]

Here we report the first Chinese family with genetically confirmed APRT deficiency. The proband was a 30-month-old Chinese boy born full term to non-consanguineous parents. His perinatal, developmental and family histories were unremarkable. He presented with recurrent urinary tract infection one week after initial presentation. There was no haematuria and no noted stone passage. Physical examination showed a round suprapubic mass which was also noted on plain radiograph (Fig. 2A), and confirmed by ultrasonography to be a bladder stone (Fig. 2B). The kidneys otherwise were normal in size and no renal mass lesion was detected. Initial blood tests showed elevated white blood cell count 18.8 $\times$ 10^9/L, predominantly neutrophils. Biochemical tests of the blood and urine, including blood gas, plasma calcium, phosphate, amino acids, and urine calcium, oxalate, cystine, organic acids and pH, were unremarkable (Supplementary Table 1, Appendix A). Urine microbiological studies showed white blood cell >100 per µL, and culture grew *Morganella morganii*. The patient was subsequently put on intravenous piperacillin and tazobactam, and arranged for surgical stone removal. Intraoperative cystoscopy showed a single

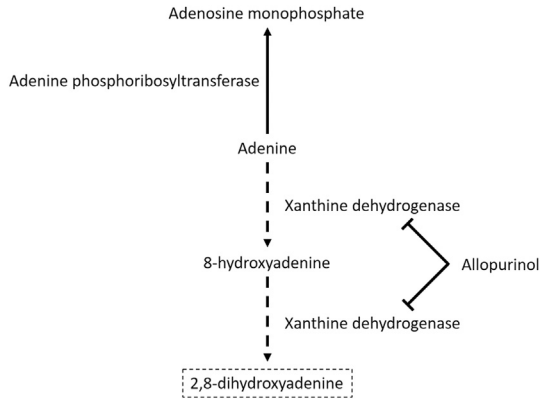

Fig. 1 Metabolic pathway of adenine and the formation of 2,8-DHA in the alternative pathway in APRT deficiency. Note the role of allopurinol in inhibiting the formation of the insoluble 2,8-DHA crystals and prevention of stone formation.

bladder stone with normal bilateral ureteric openings and no bladder trabeculation. The stone was extracted by open midline cystolithotomy. The post-operative course was uneventful, and the patient was discharged after a week. Post-operative ultrasonography of the urinary system showed no other stone in the urinary system.

The stone specimen was submitted for chemical analysis using the Frontier Fourier Transform Infra-red (FTIR) spectrometer coupled with the Universal Attenuated Total Reflection (ATR) Sampling Accessory (PerkinElmer, USA). A piece of brown, oval-shaped stone with rough surface and hard consistency, measuring 26×23×10 mm, was washed, dried, pulverised into powder form, and then applied to the ATR Sampling Accessory. An infra-red spectrum of the powdered stone sample was obtained and matched to 2,8-DHA in the NICODOM IR Kidney Stones Library (Nicodom, Czech Republic) (Fig. 3A). Microscopy of fresh urine smear showed roundish brown crystals with dark outline, with occasional vague central spicules. Under polarised light, yellowish crystals with a central Maltese cross pattern were observed, morphologically compatible with 2,8-DHA (Fig. 3B,C).

Further analysis for urine 2,8-DHA and adenine was performed at the Chemical Pathology Laboratory, Princess Margaret Hospital, Hong Kong. A liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) method (in-house developed, unpublished) for purines and pyrimidines was performed using a dilute-and-shoot approach. A brief description of the method is included as follows, courtesy of Mr Chi-Kong Lai, Scientific Officer at the Chemical Pathology Laboratory, Princess Margaret Hospital, Hong Kong. Filtered urine sample was diluted with 5% acetonitrile in 0.1% formic acid and then injected into Zorbax Eclipse Amino Acid Analysis reversed-phase column (4.6×150 mm, 5 μm) (Agilent, USA) in ACQUITY UPLC system (Waters Corporation, USA). Gradient elution was performed with eluent A (0.05% formic acid in deionised water) and eluent B (0.05% formic acid in acetonitrile) at a flow rate of 0.5 mL/min and an elution gradient as follows: 0−9 min 96% A, 4% B; 9−11 min 65% A, 35% B; 11−15 min 96% A, 4% B. Following polarity switching electrospray ionisation, MS/MS analysis was performed in Xevo TQ-S (Waters Corporation, USA) triple quadrupole mass spectrometer in multiple-reaction monitoring (MRM) mode with product-ion confirmation. With this assay, hyperexcretion of adenine and 2,8-DHA was demonstrated in the patient's urine (Fig. 3D,E).

To confirm the biochemical diagnosis of APRT deficiency, Sanger sequencing was performed for the proband, his parents and elder sister. In brief, genomic DNA was extracted with QIAamp DNA Blood Mini Kit (Qiagen, Netherlands) from blood samples collected in ethylenediaminetetraacetic acid (EDTA) tubes (Vacuette; Greiner Bio One, Austria), then amplified by polymerase chain reaction using AmpliTaqGold polymerase (Applied Biosystems, USA) with primers targeting all of the coding exons and 40 nucleotides of the flanking introns of the *APRT* gene (OMIM*102600; Refseq NG_008013.1/NM_000485.2) using the QIAgility system (Qiagen, Netherlands) and sequenced in both directions with capillary electrophoresis by the 3500 Genetic Analyzer (Applied Biosystems). Two heterozygous variants c.200G>A p.(Arg67Gln) and c.461_462del p.(Val154Glufs*9) were detected in the proband and compound heterozygosity was confirmed with parental genotyping. The first variant c.200G>A (rs762509151) is a missense mutation of a highly conserved amino acid which interacts with the adenine base.[5] It was absent in East Asian control subjects in Genome Aggregation Database, and was previously reported in a case of APRT deficiency in a Japanese patient.[6] The second variant c.461_462del was a novel frameshift variant which was absent from control subjects in

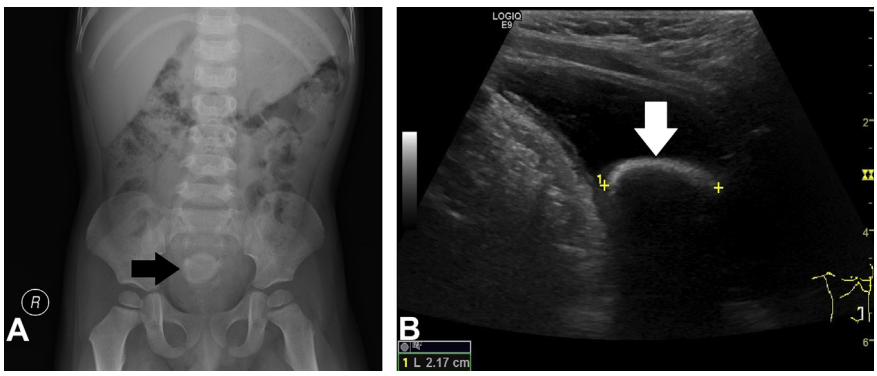

Fig. 2 (A) Plain radiograph of the abdomen showing a suprapubic radio-opaque mass (solid black arrow) measuring 2.2×2.4 cm. (B) Ultrasonography of the urinary system showed a hyperechoic stone (solid white arrow) measuring 2.2 cm longitudinally in the urinary bladder.

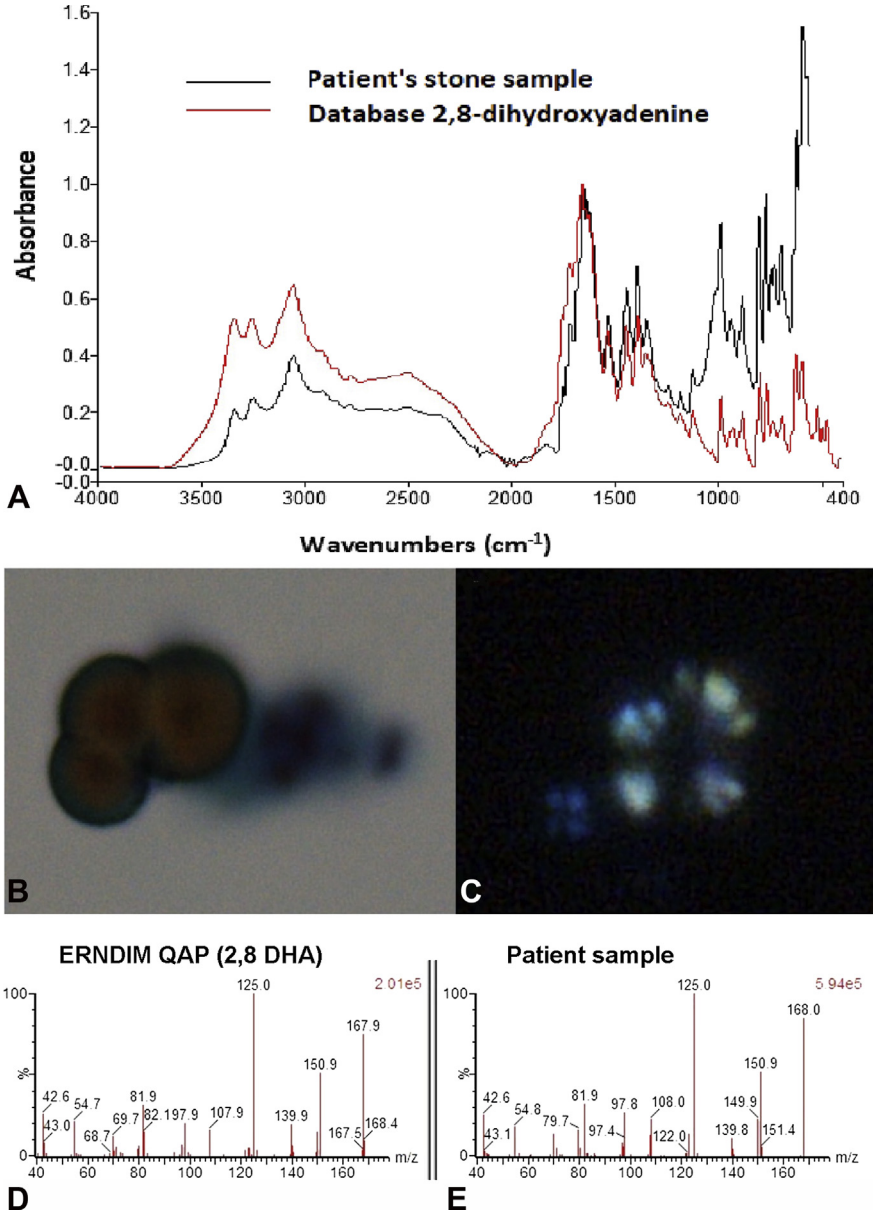

Fig. 3 (A) The ATR-FTIR stone analysis spectrum of the patient's sample (in black), compared to its best-match spectrum of 2,8-DHA (in red) in the NICODOM IR Kidney Stones spectra library. (B) Urine smear showed brownish round crystals with dark outline, with occasional vague central spicules. (C) Under polarised light, yellowish crystals with a central Maltese cross pattern were observed. Morphologically these crystals were compatible with 2,8-DHA crystals. (D,E) Mass spectrum of the product ions from the patient's urine sample (E) compared to a proficiency urine sample from of ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) Quality Assurance Programme containing known 2,8-DHA (D), confirming urinary hyperexcretion of 2,8-DHA in the patient.

Genome Aggregation Database. This results in the insertion of nine amino acids at residue 154, out of 180 amino acids of the normal protein, followed by premature termination. The C-terminal of this protein, especially the residue Leu159 which is important for the specificity of the enzyme towards adenine,[5] is predicted to be lost as a result. The variant c.461_462del was hence classified as pathogenic.[7] The above biochemical and genetic findings confirmed the diagnosis of APRT deficiency in the proband. His elder sister was subsequently also found to have the same genotype as the proband, and demonstrated significant 2,8-DHA crystalluria. Allopurinol treatment was started for both of them, and significant reduction of 2,8-DHA crystal count in the urine was observed.

Laboratory diagnosis of APRT deficiency requires (1) identification of 2,8-DHA stones; (2) polarised light microscopy for 2,8-DHA crystalluria; (3) APRT activity assay; (4) urinary metabolite assay for adenine and 2,8-DHA; (5) renal biopsy showing 2,8-DHA crystal deposition; or (6) genetic analysis of *APRT* gene.[1] For routine clinical laboratories where specialised investigations such as enzyme activity assay or urine metabolite assay are not available, stone analysis and polarised light microscopy of urine can be considered as reasonable screening tests for the investigation of urolithiasis, especially in children. APRT deficiency potentially may have been under-diagnosed before introduction of ATR-FTIR for urinary stone analysis. Traditional wet chemistry urinary stone analysis was inadequate as it

misclassified 2,8-DHA as uric acid.[2] Also, patients may have no sizable urinary stones, as in the proband's sister, therefore urine microscopy for crystalluria can be complementary to stone analysis in case detection. The proband's elder sister remained asymptomatic and had no urinary stone at the age of six. Compared to his sister, the earlier presentation in the proband might be due to environmental factors such as fluid and dietary purine intake,[8] or precipitating factors such as concomitant urinary tract infection. Although studies on APRT deficiency in adults suggested no gender differences,[2] in children there were significantly more males than females (2.5 males: 1 female),[4] which may provide indirect evidence that males present earlier, consistent with our findings in this family. The variability in the age and presentation of APRT deficiency is also complicated by the observation that 2,8-DHA stones can be radio-opaque, while they were typically described as radiolucent.[8] In our patient, this phenomenon was potentially associated with intercurrent urinary tract infection. The radio-opaque component, although unidentified in this study, might also explain the imperfect matching in the infra-red spectrum.

Genetic testing of *APRT* gene is usually a confirmatory test and is usually offered only to patients with compatible investigation results. However, nowadays with next-generation sequencing it is also possible to diagnose APRT deficiency with a renal disease or urolithiasis panel without the need for the above biochemical investigations. Nevertheless this approach requires significantly higher cost and technical expertise.[9] Given the genetic similarities between the Han Chinese and the Japanese populations, it might be unexpected that APRT deficiency, which is common in Japan, has been rarely reported in Chinese. The common *APRT* mutation in Japanese p.Met136Thr was not found in 231 Chinese subjects from Taiwan,[10] supporting possible genetic heterogeneity in the two populations. However, our family carried a known variant c.200G>A which was reported in Japanese, so the exact degree of genetic heterogeneity is yet to be determined. There was a previous case report of APRT deficiency in a 17-month-old Chinese boy presenting with severe acute kidney injury secondary to obstructive uropathy from multiple renal calculi.[11] However, no genetic study was reported as the diagnosis was only made by infrared spectrometric stone analysis and red blood cell enzyme assay. Genetic characterisation would be helpful for better understanding of the genetic basis of APRT deficiency in the Chinese population and molecular testing strategy. Another heterozygous p.Ala116Thr variant was reported in a Chinese male who presented with gout at the age of 18,[12] with APRT enzyme activity compatible only with a carrier phenotype. Therefore, the two siblings we reported here would be the first Chinese family with genetically confirmed APRT deficiency.

In summary, to our best knowledge, this is the first Chinese family of APRT deficiency presenting with urolithiasis, and confirmed with urinary stone analysis, urine metabolite screening, urine microscopy and genetic testing. Clinicians and pathologists should be aware of the appropriate laboratory investigations required for making a proper diagnosis of this pan-ethnic disease, as early treatment with allopurinol can prevent kidney damage and its related morbidities.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2019.02.008.

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