morphologically distinct areas. However, in this case report, as in several others,^{6–8} all components of the adrenal tumour appeared benign. Composite pheochromocytomas and neuroblastic tumours are simply explained by their shared embryological origin from the neural crest. In composite corticomedullary tumours, it is difficult to understand how a single clone of cells could give rise to a tumour with elements of two distinct embryological origins. Collision tumour is a possibility, and it has been previously hypothesised that hormones or other factors acting in a paracrine manner could stimulate growth of both adrenal cortical and medullary-type cells simultaneously.⁶ Recent animal research has noted apparently bipotential progenitor cells in rat kidneys, which may give rise to cells in both the adrenal cortex and medulla.¹ Whether a common progenitor may play a role in humans is currently unknown. It has also been postulated by some authors that adrenal neoplasms in children may arise from a cell more akin to the fetal adrenal cortex than the adult adrenal cortex;⁴ it is feasible this precursor cell may have a different biological potential to that giving rise to tumours in adults. Ultimately, the pathophysiological origin of this tumour is currently undetermined and requires further study.

An additional notable feature of this case was the extensive degenerate change. These findings (hyalinisation, calcification, ossification and lipomatous metaplasia) have been previously reported in a non-functional adrenal cortical neoplasm in an adult,¹¹ but not in children. The main clinical significance of this finding is in the potential for misdiagnosis on imaging, as the presence of fat on computed tomography scan has been previously suggested as an exclusionary factor for a diagnosis of adrenal cortical adenoma.¹¹ Indeed in this case, the imaging differentials provided were of myelolipoma or teratoma.

Determination of the prognosis of this tumour also raised several issues. There is an inherent difficulty in predicting the behaviour of endocrine neoplasms in general, which is further confounded by the relative paucity of such neoplasms occurring children.⁴ Several scoring systems exist for predicting malignant behaviour in adrenal cortical neoplasms in adults, however these do not apply well to the paediatric setting. When adult scoring systems are used, the majority of paediatric adrenal neoplasms will be classified as carcinomas, but despite this will follow a benign clinical course.^{3–5} Important independent prognostic criteria for adrenal cortical neoplasms in children as described in the literature have included size (>200 g), local invasion beyond the adrenal gland, increases mitoses (>15 per 20 high power fields) and necrosis,^{3,4} though no single feature alone can accurately predict behaviour. This case did not display any of these concerning features, though prediction of outcome is complicated by the unique nature of the tumour. In the literature, the reported behaviour of corticomedullary composite tumours has been predominantly benign, though as previously noted there are very few cases described.^{6–8} Taking these factors into account, the outcome for this patient was predicted to be excellent. The patient is currently well at 6 months following primary resection.

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1. Steenblock C, Rubin de Celis MF, Androutsellis-Theotokis A, et al. Adrenal cortical and chromaffin stem cells: is there a common progeny related to stress adaptation? *Mol Cell Endocrinol* 2017; 441: 156–63.
2. Hartley AL, Birch JM, Marsden HB, Reid H, Harris M, Blair V. Adrenal cortical tumours: epidemiological and familial aspects. *Arch Dis Child* 1987; 62: 683–9.
3. Wieneke JA, Thompson LD, Heffess CS. Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. *Am J Surg Pathol* 2003; 27: 867–81.
4. Dehner LP, Hill DA. Adrenal cortical neoplasms in children: why so many carcinomas and yet so many survivors? *Pediatr Dev Pathol* 2009; 12: 284–91.
5. Lloyd RV, Osamura RY, Klöppel GN, Rosai J. *WHO Classification of Tumours of Endocrine Organs*. 4th ed. Lyon: IARC, 2017.
6. Wieneke JA, Thompson LD, Heffess CS. Corticomedullary mixed tumor of the adrenal gland. *Ann Diagn Pathol* 2001; 5: 304–8.
7. Michalopoulos N, Pazaitou-Panayiotou K, Boudina M, Papavramidis T, Karayannopoulou G, Papavramidis S. Mixed corticomedullary adrenal carcinoma. *Surg Today* 2013; 43: 1232–9.
8. Lau SK, Chu PG, Weiss LM. Mixed cortical adenoma and composite pheochromocytoma-ganglioneuroma: an unusual corticomedullary tumor of the adrenal gland. *Ann Diagn Pathol* 2011; 15: 185–9.
9. Kao CS, Grignon DJ, Ulbright TM, Idrees MT. A case report of adrenocortical carcinosarcoma with oncocytic and primitive neuroectodermal-like features. *Hum Pathol* 2013; 44: 1947–55.
10. Bielle F, Fréneaux P, Jeanne-Pasquier C, et al. PHOX2B immunolabeling: a novel tool for the diagnosis of undifferentiated neuroblastomas among childhood small round blue-cell tumors. *Am J Surg Pathol* 2012; 36: 1141–9.
11. Masugi Y, Kameyama K, Aiba M, et al. Non-functional adrenocortical adenoma with extensive degeneration. *Pathol Int* 2003; 53: 241–5.

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McArdle disease presenting as abnormal liver function: biochemical, anatomical and genetic characterisation in the first genetically confirmed Chinese family with a novel splicing variant

Sir,

Glycogen storage disease V; GSD5 (MIM # 232600), also known as McArdle disease, is an autosomal recessive disorder of skeletal muscle carbohydrate metabolism due to the deficiency of the muscle specific glycogen phosphorylase, coded by the *PYGM* gene (MIM * 608455) for the breakdown of glycogen in skeletal muscles.¹ It is one of the most common glycogen storage disorders in Caucasian populations, with an estimated prevalence of 1 in 100,000 in the United States, or even up to 1 in 42,355 using variant frequency from exome sequencing. However, information on this condition in East Asian populations is relatively limited, with sparse reports on East Asian patients.² Here we report for the first time genetically confirmed McArdle disease in a Chinese family.

Fig. 1 Ischaemic forearm test results showing flat lactate response with elevation of ammonia.

The proband was a 25-year-old Chinese male with unremarkable past health. He first presented with asymptomatic elevation of parenchymal liver enzymes alanine aminotransferase (ALT) 71 U/L and aspartate aminotransferase (AST) 81 U/L (reference intervals not available) noted during a health check. He was referred for further work up for deranged liver function. Initial blood tests showed elevated ALT 81 U/L (reference interval <61 U/L) and AST 183 U/L (5–34 U/L) but unremarkable alkaline phosphatase (ALP) 46 U/L (53–128 U/L), gamma glutamyltransferase (GGT) 23 U/L (12–64 U/L) and total bilirubin 9 μmol/L (reference interval 3–21 μmol/L). Investigations of deranged liver function, viral serology (HBsAg, anti-HCV), autoimmune serology (anti-nuclear, anti-smooth muscle and anti-mitochondria antibodies), iron profile with saturation% and ferritin, alpha-fetoprotein and ultrasonography of the abdomen revealed no abnormalities. Of note, his creatine kinase (CK) was grossly elevated 27,282 U/L (65–270 U/L),

as well as his lactate dehydrogenase (LDH) 1042 U/L (125–220 U/L), but his urine myoglobin was negative. He denied recent vigorous exercise, intramuscular injection and trauma. Subsequently, he presented 3 months after initial presentation with dark coloured urine associated with low back pain after heavy lifting. He also recalled similar previous episodes of passing reddish urine after exercise. His CK on admission was 146,104 U/L with a positive urine myoglobin, elevated ALT (262 U/L) and AST (576 U/L), and normal plasma creatinine. He was treated with intravenous 0.9% sodium chloride solution and his CK decreased to 4988 U/L before he was discharged.

Further investigations for rhabdomyolysis were performed. Nerve conduction study and electromyography were normal. A normal dried blood spot for amino acids, free carnitine and acylcarnitines excluded fatty acid oxidation defects. His acid alpha glucosidase activity was normal. Ischaemic forearm exercise test was performed and showed flat lactate response with normal rise of ammonia (Fig. 1). Muscle biopsy of his left vastus lateralis under light microscopy showed a few scattered atrophic fibres with no evident vacuole in the sarcoplasm. Periodic acid–Schiff staining showed generalised increase in staining intensity in the fibres. Further electron microscopic examination showed accumulation of glycogen granules in the sarcoplasm among the nuclei, mitochondria and myofibrils, but not within lysosomes or enveloped by membranes. Some mitochondria possessed type I paracrystalline inclusions within the matrix compartment (Fig. 2).

With consistent clinical, biochemical and biopsy findings, a provisional diagnosis of McArdle disease was made. Confirmatory genetic analysis on *PYGM* gene (Refseq accession numbers: NG_013018.1/NM_005609.3/NP_005600.1) was performed on genomic DNA isolated from white blood cells with polymerase chain reaction (PCR) and bidirectional Sanger sequencing. The patient

Fig. 2 (A) Electron micrograph showing granular glycogen particles within the sarcoplasm adjacent to myofibrils. The particles are not bound by a membrane. (B) Electron micrograph showing mitochondria with paracrystalline inclusions in the matrix (black arrow).

was found to be apparently homozygous for c.1970-1G>A (dbSNP rs1057516598), which is a 3' acceptor splice site variant involving intron 16 out of a total of 20 coding exons of *PYGM*. It was not reported in Gnome Aggregation Database. *In silico* algorithms such as MaxEntScan, SpliceSiteFinder-like and NNSPLICE predicted that this variant may abolish the 3' acceptor splice site. It was previously reported in ClinVar (RCV000410171.1) as a likely pathogenic variant, but only limited information and evidence of this pathogenicity is available. We performed complementary DNA (cDNA) sequencing with RNA extracted from white blood cells, and cDNA prepared with reverse transcription using random hexamers. We confirmed the skipping of exon 17 using forward primers at the boundary of exon 14 and 15 and reverse primer at the boundary of exon 19 and 20. Subsequent PCR with another set of primers flanking the known exon 17 deletion c.1969+214_2177+369del showed a single product, and Sanger sequencing did not show any break point or heterozygous variant. Considering it being a null variant involving a canonical splice site (PVS1), absent from controls (PM2) and supported pathogenicity by multiple lines of computational evidence (PP3), this variant is classified as pathogenic according to the American College of Medical Genetics and Genomics guideline. Further family cascade screening identified that the patient's younger brother, who also demonstrated features of exercise induced myoglobinuria, was also apparently homozygous for c.1970-1G>A. Their asymptomatic parents refused genetic testing due to the lack of perceived benefits.

The diagnosis of McArdle disease in this Chinese family was confirmed by biochemical, anatomical and molecular genetic testing. In the literature, no genetically confirmed McArdle disease has been reported in the Chinese population, suggesting it is much rarer than in Caucasians. Compounding with its tendency to be misdiagnosed as being lazy or unfit, growing pains, or rheumatic disorders,³ it is important that the clinicians be aware of this glycogen storage disorder which would prevent further unnecessary diagnostic testing, potential complications of recurrent rhabdomyolysis and/or stigmatisation. This patient presented with elevated liver enzymes, which with a closer inspection showed a reversed De Ritis ratio, i.e., AST activity being higher than that of ALT. Due to the more rapid clearance of AST, the serum ALT activity is usually higher than that of AST in health.⁴ In this patient, his De Ritis ratio was elevated both on initial presentation with abnormal liver function (ratio = 2.3) and subsequently with rhabdomyolysis (ratio = 2.2). In a patient with reversed De Ritis ratio but no history or clinical feature suggestive of a hepatic pathology, alternative explanations of the elevated liver enzymes should be considered. For suspected McArdle disease, classically an ischaemic forearm exercise test would be performed, as in this case, to evaluate the lactate and ammonia response during anaerobic exercise. However, ischaemia was associated with myalgia, cramps, or even more severe complications such as myoglobinuria and acute renal failure; therefore, a non-ischaemic version of the forearm exercise test was proposed which showed good sensitivity and specificity to replace the ischaemic version.⁵ The mitochondrial paracrystalline inclusions together with glycogen accumulation in the muscle biopsy in this patient were previously described in patients with McArdle disease who underwent investigation

for myopathy due to use of corticosteroid⁶ and statin.⁷ However, as this patient was not taking any statin or corticosteroid, such electron microscopy findings provided evidence on the direct relationship between the pathology of myophosphorylase deficiency and the mitochondrial ultrastructural derangement, without the confounding effects of drugs. Exon 17 of *PYGM*, which is considered to involve the catalytic site of the enzyme, is one of the hotspots for pathogenic variants.² Gross deletions of genomic DNA directly involving exon 17 (c.1969+214_2177+369del,⁸ c.1970_2177del⁹ and a 1.1 kb deletion¹⁰) have been reported, together with a splice site variant c.2380-1G>A in intron 19 which was associated with skipping of exon 17 at cDNA level.⁹ It is interesting that in the cDNA analysis by Bruno *et al.*⁹ they also identified a cDNA transcript with exon 17 skipping in two patients, one with an apparently homozygous c.2380-1G>A variant, while the other one with no identified genomic DNA variant, suggesting that this transcript may be due to an unidentified variant in genomic DNA. In our patients, without parental genotype, we could not confirm the homozygosity of the rare splice site variant c.1970-1G>A. The parents were not known to be consanguineous, but they did originate from the same city in China, so this possibility could not be excluded. We could not detect the presence of the known exon 17 deletions (c.1969+214_2177+369del and c.1970_2177del), but the possibility remains that the actual genotype in our patients would involve another unrevealed gross deletion of *PYGM* on genomic DNA, as often found in patients with apparent homozygosity,¹¹ or an unidentified splicing variant giving rise to the same aberrant cDNA transcript.

In summary, we report the first genetically confirmed McArdle disease in a Chinese family. The biochemical finding of elevated liver enzymes should be evaluated carefully in conjunction with other clinical features and serum enzymes such as ductal enzymes, e.g., ALP or GGT, or muscle enzyme, e.g., CK. Further specific provocative testing, microscopic characterisation and molecular genetics would help confirm the diagnosis and optimise management in the patient.

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1. Taylor RL, Davis M, Turner E, *et al.* Clinical utility gene card for McArdle disease. *Eur J Hum Genet* 2018; 26: 758–64.
2. Nogales-Gadea G, Brull A, Santalla A, *et al.* McArdle Disease: update of reported mutations and polymorphisms in the *PYGM* gene. *Hum Mutat* 2015; 36: 669–78.
3. Scalco RS, Morrow JM, Booth S, Chatfield S, Godfrey R, Quinlivan R. Misdiagnosis is an important factor for diagnostic delay in McArdle disease. *Neuromuscul Disord* 2017; 27: 852–5.

4. Botros M, Sikaris KA. The de Ritis ratio: the test of time. *Clin Biochem Rev* 2013; 34: 117–30.
5. Hogrel JY, van den Bogaart F, Ledoux I, *et al*. Diagnostic power of the non-ischæmic forearm exercise test in detecting glycogenosis type V. *Eur J Neurol* 2015; 22: 933–40.
6. Mastaglia FL, McCollum JP, Larson PF, Hudgson P. Steroid myopathy complicating McArdle's disease. *J Neurol Neurosurg Psychiatry* 1970; 33: 111–20.
7. Baker SK, Vladutiu GD, Peltier WL, Isackson PJ, Tamopolsky MA. Metabolic myopathies discovered during investigations of statin myopathy. *Can J Neurol Sci* 2008; 35: 94–7.
8. García-Consuegra I, Rubio JC, Nogales-Gadea G, *et al*. Novel mutations in patients with McArdle disease by analysis of skeletal muscle mRNA. *J Med Genet* 2009; 46: 198–202.
9. Bruno C, Cassandrini D, Martinuzzi A, *et al*. McArdle disease: the mutation spectrum of PYGM in a large Italian cohort. *Hum Mutat* 2006; 27: 718.
10. Wu L, Brady L, Shoffner J, Tamopolsky MA. Next-generation sequencing to diagnose muscular dystrophy, rhabdomyolysis, and hyperCKemia. *Can J Neurol Sci* 2018; 45: 262–8.
11. Landsverk ML, Douglas GV, Tang S, *et al*. Diagnostic approaches to apparent homozygosity. *Genet Med* 2012; 14: 877–82.

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EBV positive fibrin/chronic inflammation associated diffuse large B-cell lymphoma: an incidental finding associated with a breast implant

Sir,

We report a rare case of Epstein–Barr virus positive (EBV+) fibrin/chronic inflammation associated large B cell lymphoma (FA-DLBCL) occurring in association with a textured breast implant.

A 70-year-old well woman presented for removal of a right-sided breast implant after a 4 month history of minor pain and capsular contracture. The Allergan macrotextured silicone implant had been *in situ* for 9 years, initially placed as part of a breast reconstruction following mastectomy treatment alone for a primary breast carcinoma. *En bloc* surgical removal of the intact implant and capsulectomy was performed without any prior biopsy or fine needle aspiration procedure. During specimen grossing, yellow-cream fluid was present between the internal layer of the fibrous capsule and the surface of the implant (Fig. 1A). The capsule was slightly thickened without discrete masses. Systemic work-up showed no other sites of disease including a normal lactate dehydrogenase (LDH) level. There was no history of prior lymphoma or systemic immunosuppression. A diagnosis of EBV+ FA-DLBCL was rendered after incorporating the clinical and radiological findings. Surgical removal of the implant alone was performed. There is no evidence of disease progression, recurrence or systemic lymphoma after one year of close clinical follow-up and interval PET imaging.

Microscopic examination of the capsule showed clusters and aggregates of atypical large pleomorphic cells, localised to the space between the breast capsule and implant. The cells were mostly enmeshed within fibrin on the inner aspect of the capsule and also very focally within the fibrous capsule (Fig. 1B,C). There was no angiocentric growth, vascular wall invasion or destruction.

Fig. 1 (A) Thickened yellow fluid present in the space between the fibrous capsule and surface of the implant. (B,C) Aggregates of pleomorphic large cells within the fibrin and fibrous capsule.

The atypical cells expressed CD45, CD79a (Fig. 2A), CD20, PAX5, CD30, BCL6, MUM1, PD-L1 (SP142 clone) and were negative for CD10, pan-T-cell markers (CD3, CD2, CD4, CD5, CD7, CD8), cytotoxic markers (TIA1, Perforin, Granzyme B), ALK, CD68, carcinoma markers (AE 1/AE3, Cam 5.2, EMA) and for HHV-8 latent nuclear antigen-1 (Fig. 2B,D). Only small numbers of CD3/CD8 co-expressing reactive small T cells were present in the background. Ki-67 was confined to the pleomorphic large cells. The majority of cells harboured EBV, demonstrated by *in situ* hybridisation (Fig. 2E). LMP1 and EBNA2 were not available in our laboratory for latency typing. Immunoglobulin heavy chain rearrangement by polymerase chain reaction (PCR) detected a monoclonal population. There was no evidence of *MYC* gene rearrangement, copy number variation or amplification. Next generation sequencing was attempted but did not yield a result due to the nature of the DNA.

FA-DLBCL is a provisional clinicopathological entity in the 2017 World Health Organization (WHO) Classification,¹