RBCK1-related disease: A rare multisystem disorder with polyglucosan storage, autoinflammation, recurrent infections, skeletal, and cardiac myopathy—Four additional patients and a review of the current literature

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Abstract

In this article, we report four new patients, from three kindreds, with pathogenic variants in RBCK1 and a multisystem disorder characterised by widespread polyglucosan storage. We describe the clinical presentation of progressive skeletal and cardiac myopathy, combined immunodeficiencies and auto-inflammation, illustrate in detail the histopathological findings in multiple tissue types, and report muscle MRI findings.

Keywords: cardio; myopathy; auto-inflammation; glycogen; myopathy; polyglucosan; RBCK1

Supplemental Table S1 Laboratory findings in patients 1 and 2. Abnormal results are shown in bold

1 INTRODUCTION

Homozygous or compound heterozygous pathogenic variants in RBCK1 (OMIM #610924) cause a systemic condition characterised by polyglucosan body accumulation. The exact role of RBCK1 in the molecular pathogenesis of underlying polyglucosan formation and/or clearance is still unclear. It is a very rare condition, with current knowledge based on a few case reports. All of the 21 previously reported patients had cardiac and/or skeletal muscle myopathy (Table 1). A proportion of patients may also present with immune deficiency and auto-inflammation, indicating a possible genotype/phenotype correlation. In this article, we report four additional patients, from three kindreds, including two sisters with compound heterozygous mid-domain variants in RBCK1, with a phenotype spanning the entire spectrum of the condition, including skeletal and cardiac myopathy, combined immunodeficiencies and auto-inflammation.
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Note: P1-P4 are described in this publication.

Abbreviations: CM, cardiac muscle; IBD, inflammatory bowel disease; NR, not reported; SM, skeletal muscle; T1DM, type 1 diabetes.
2 CASE SERIES

A summary of the features of patients 1 to 4, as well as comparable data on previously published patients, is presented in Table 1.

2.1 Patient 1 (P1)

P1 first presented in early childhood with delayed motor milestones and poor weight gain due to difficulties feeding. Juvenile arthritis affecting the ankles, knees, hands and wrists, and refractory to treatment, developed age 3 years, with severe episodes requiring frequent hospital admissions. From 4 years of age, she had recurrent episodes of vomiting and diarrhoea. She suffered recurrent infections (gastrointestinal [GI], tonsils, parotid, ears, throat, respiratory) with associated sepsis. Respiratory disease was associated with persistent cervical lymphadenopathy and the development of bronchiectasis. A diagnosis of inflammatory bowel disease (IBD) was made at the age of 16 years. Early onset dilated cardiomyopathy necessitated orthotopic cardiac transplant at the age of 7 years. Proximal leg weakness, progressive in nature and mostly affecting limb girdle and proximal lower limb muscles, limited mobility. Generalised seizures developed age 17 years. Brain MRI at the time showed infarcts in multiple vascular territories with micro-haemorrhages, suggestive of vasculitis. Late dysphagia was reported at 21 years.

She was born (in the United Kingdom) to consanguineous Bangladesh parents. Her mother gave birth to 15 children, of whom 9 died: eight before the age of 5 years due to reported “breathing difficulties,” and one at 17 years due to a respiratory infection. Her sister (P2) with a similar (although not identical) phenotype was diagnosed with an unspecified glycogen storage disease (GSD) based on skeletal muscle biopsy as described below. Following this diagnosis, this patient (P1) was also given the diagnosis of a possible GSD. Four other living siblings (two females, two males) are reported to be well.

At 24 years, she was re-referred to the adult inherited metabolic disease clinic. Physical examination confirmed proximal weakness and wasting. She also had truncal weakness and bilateral ptosis but with a full range of eye movements. She had mild dysarthria and both cough and sneeze were weak. Sensory examination was normal.

Neurophysiology studies showed myopathic features with normal motor and sensory nerve conduction studies. Speech and language assessment showed a moderate to severe oropharyngeal dysphagia.

The combination of cardiomyopathy, skeletal myopathy, immunodeficiency, and therapy-refractory systemic inflammatory diseases raised the suspicion of a possible RBCK1 defect. Genetic mutation analysis by WGS identified a homozygous pathogenic mutation (c.691delC; p.[Gln231Serfs*45]) in the mid-domain of RBCK1.

Age 28 years, she presented with recurrent fever and persistently elevated inflammatory markers without a clear infective focus. CT-PET identified an enlarged mediastinal lymph node which was biopsied and confirmed Hodgkin lymphoma. She was treated with 2 cycles of ABVD chemotherapy regimen, with complete FDG-PET scan response. Chemotherapy was complicated by colitis and bowel obstruction with a stricture of the terminal ileum, which was managed conservatively. However, respiratory function deteriorated and she died of respiratory complications 3 months later.

2.2 Patient 2 (P2)

P2, currently 34 years, was re-referred to the adult inherited metabolic disease services following her sister's (P1) genetic diagnosis. From a young age, she was unable to run due to breathlessness and muscle weakness. Symptoms slowly progressed to include difficulties walking upstairs and moving from a sitting to a standing
position. She also suffered recurrent infections, including ear, respiratory tract, and urinary tract infections. She had a tonsillectomy and adenoidectomy at the age of 11 years.

Cardiomyopathy was diagnosed at the age of 17 years. At the age of 22 years, she had a twin pregnancy. The twins developed twin-to-twin transfusion syndrome and were delivered at 27 weeks gestation by Caesarean section. Both have a severe autistic spectrum disorder. Her cardiac symptoms significantly worsened following delivery and she developed cardiac failure, requiring a cardiac transplant at 4 months post-partum. She reports worsening of recurrent infections following heart transplantation and immunosuppression treatment, with recurrent infections (urinary, respiratory) on a monthly basis. On physical examination performed at 32 years, widespread muscle weakness, worse proximally, was evident.

P2 was given a diagnosis of an “unspecified glycogen storage disorder” at the age of 20 years based on a skeletal muscle (quadriceps) biopsy. Her diagnosis was reviewed following P1’s genetic diagnosis, and she was also confirmed to have the same homozygous pathogenic variant (c.691delC; p.[Gln231Serfs*45]) in the mid-domain of RBCK1.

2.3 Patient 3 (P3)

Patient 3 was initially investigated at the age of 9 years following an episode of severe abdominal pain. Splenomegaly and mildly deranged liver enzymes were noted. An abdominal ultrasound revealed a mildly fatty liver and a liver biopsy showed extensive bridging fibrosis with additional hepatocyte changes including ground glass inclusions.

He was born by normal vaginal delivery at term. At 8 weeks, he presented with four generalised seizures and was found to have splenomegaly and abnormal liver function, attributed to CMV infection at the time. There were no other problems in the neonatal period and his early developmental milestones were all normal.

He was noted to have difficulties running from the age of 3 years. He was referred to the paediatric metabolic team at the age of 10 years. An EMG showed only mild myopathic changes. A muscle biopsy was consistent with a histopathological diagnosis of polyglucosan body myopathy (Figure 1). Sequencing of the GBE gene did not identify any pathogenic variants.
He presented again with episodes of pallor, dizziness, chest pain, and recurrent palpitations at the age of 14 years. An echocardiogram showed thickening of the myocardium suggestive of a storage cardiomyopathy.

By 15 years, he had a minimal one-handed positive Gowers’ sign. He could only walk for 15 minutes at a time and was mainly limited by marked fatigue rather than muscle weakness. A muscle MRI of the upper...
leg (Figure 2), showed increased signal intensity on T1-weighted sequences within vasti, sartorius, adductor magnus and, to a lesser extent, hamstring muscles, with relative sparing of the rectus femoris, adductor longus, and gracilis.

**FIGURE 2** Muscle MRI findings in *RBCK1*-related disease. Muscle MRI from the lower limb, transverse sections, from Patient P3 at 15 years of age, A,B, and from patient P4 at 26 years of age, C,D. T1-weighted images from the thigh in Patient P3, A, show a characteristic pattern with selective involvement of the vasti, sartorius (S), adductor magnus (AM), and hamstring muscles, with relative sparing of the rectus femoris (RF), gracilis (G), and adductor longus (AL). B, Corresponding STIR images suggest the presence of oedema affecting the same preferentially involved muscle groups (white arrows). T1-weighted images in patient P4 indicated, C, a similar but much more severe pattern of selective involvement within the thigh, and, D, diffuse involvement within the lower leg. Patient P4 also had corresponding STIR imaging changes suggestive of oedema (data not shown). St, semitendinosus; VL, vastus lateralis

Weight gain was poor, but he had otherwise remained in good general health, with no recurrent chest or other infections. At aged 15 ½ years his body mass index (BMI) had dropped to 12 kg/m² and he presented with acute ventricular tachycardia which responded to amiodarone. Cardiac function was severely reduced requiring inotropic support. In view of his very low BMI, liver involvement and uncertain prognosis of the underlying condition, cardiac transplantation was not thought appropriate. Palliative care was instituted. His cardiac and liver disease deteriorated and he died 1 month later, aged 16 years.

Genetic testing revealed compound heterozygosity for a paternally inherited c.1522_1526del; p.(Asn508Profs*4) and a maternally inherited c.799C > T; p.(Gln267*) pathogenic variant in the *RBCK1* gene.

### 2.4 Patient 4 (P4)

Patient 4 was the first of two children born to unrelated parents. He was born by vaginal delivery at 42 weeks gestation weighing 2.8 kg. He walked independently at 18 months. At the age of 4 years he was noted to have difficulty climbing stairs. By 9 years he had a clearly waddling gait, difficulty climbing stairs, mild shoulder girdle weakness and significant pelvic girdle weakness. He was also noted to have specific learning difficulties of the dyslexic type with impairment of short-term memory.

An echocardiogram at age 10 years showed mild dilatation of the left ventricle with borderline LVH on ECG but normal QT interval. By 16 years, he had reduced cardiac function with LV dilatation and a fractional shortening of 15.8%.

EMG and nerve conduction studies were normal. Polyglucosan bodies were noted on muscle biopsy (Figure 1).
At age 14, years there was a decline in motor skills with “spasms” in arms and legs, requiring the use of a wheelchair when fatigued. Exercise-induced lactate production (Bruce protocol) was normal. The cardiomyopathy progressed to end-stage cardiac failure and he had an orthotopic cardiac transplantation age 17 years. The explanted heart was grossly enlarged with biventricular dilatation. Microscopy showed myocyte hypertrophy with large perinuclear vacuoles that contained PAS positive diastase resistant material. There were areas of myocyte loss, predominantly sub-endocardial with replacement fibrosis and an infiltrate of inflammatory cells, predominantly lymphocytes (CD45+) and macrophages (CD68+).

Post-transplant he had some generalised seizures, thought to be Cyclosporin induced which settled on its withdrawal. Problems with both long and short-term memory and word recall were noted. An EEG was normal. Brain MRI showed subtle bilateral symmetrical subcortical white matter signal change in the occipital lobes. MRA and cranial Doppler studies were normal.

Aged 20 years, an overnight sleep study documented mild respiratory muscle weakness. Aged 21 years, he developed a generalised non-specific synovial inflammation which was steroid responsive. Immunological testing revealed IgM raised at 7.4 g/L [0.57-2.66], anti-cardiolipin IgG negative, a normal full blood count and rheumatoid factor positive (titre 1/32). Splenomegalgy was noted.

By 26 years, he was wheelchair-bound due to progressive myopathy. A bilateral lower limb MRI showed extensive muscle oedema (Figure 2). He also had small bilateral knee joint effusions. Aged 30 years, he reported several lower respiratory tract infections. Spirometry indicated moderate restrictive respiratory myopathy. Continuing progression of his skeletal myopathy with reduced shoulder girdle strength necessitated use of a motorised wheelchair. Aged 33 years, he presented with acute congestive cardiac failure secondary to severe tricuspid regurgitation of his transplanted heart. He died following valvular heart surgery complicated by progressive multiorgan failure.

Genetic testing by direct Sanger sequencing confirmed compound heterozygosity for (c.817dupC, p.(Leu273Profs*27)) and c.1465delA, p.(Thr489Profs*9) mutations in the RBCK1 gene.

3 LABORATORY INVESTIGATIONS

Laboratory investigations for P1 and P2 are shown in Supplemental Table S1. To investigate possible immunodeficiency, immunoglobulins, IgG subclasses, specific antibody titres, full lymphocyte subsets, B cell phenotype, and T cell proliferation were measured in patients 1 and 2. Results indicate that P1 had a polyclonal hypergammaglobulinemia with positive autoantibody serology but low titres of antibodies against encapsulated bacteria; P2 had essentially normal immunoglobulin levels. P1 had a reduced NK cell count and percentage with borderline B cell numbers. B cell phenotype revealed mild reduction in IgM memory cell percentage, reduced plasmablast percentage and increased CD21-CD38 cells in both sisters. The latter abnormality was more marked in P1, who also demonstrated reduced switched memory B cell percentage. Both also had significant impairment of T cell proliferation, most notably to polyclonal T cell stimulation with Phyto-haemagglutinin.

4 HISTOPATHOLOGICAL FINDINGS

Retrospective analysis of biopsies/explants with additional relevant staining (when material was available) following the genetic diagnosis was performed. These included for P1: four GI (16, 18, 21, 25y), lung (18y) and heart (8y); P2: heart (24y); P3: liver (10y) and quadriceps (11y); P4: quadriceps (9y) and heart (17y) biopsies (Figure 1). Tongue and parotid biopsies for P1 and quadriceps biopsy for P2 could not be traced from the archives, but the original pathology reports were reviewed. There was extensive vacuolar degeneration affecting the majority of the cardiac and skeletal muscle fibres. The vacuoles were present centrally, in the subsarcolemma, often occupied the entire fibre, and were filled with basophilic inclusions that were strongly PAS-positive and alpha-amylose/diastase resistant (Figure 1A-C,E-G,J,K). Fibres containing these inclusions were often depleted of normal PAS staining for glycogen (Figure 1B,F). The inclusions were strongly positive
for myophosphorylase, and largely negative for acid phosphatase. Ultrastructural examination revealed a fibrillar nature of the inclusions unlike normal glycogen, and consistent with polyglucosan (Figure 1I,O). Marked slow fibre predominance and frequent atrophic fibres were noted in all quadriceps biopsies. Extensive interstitial fibrosis, myocyte loss and patchy chronic inflammation were present in the heart. Smaller polyglucosan inclusions were noted in the medial coats of cardiac (Figure 1L) and skeletal muscle arteries and arterioles. Similar inclusions were observed within the lamina propria in all GI and bronchial biopsies with associated chronic inflammation (P1) (Figure 1P-U). Immunophenotyping showed that the polyglucosan inclusions were strongly reactive to ubiquitin and p62 (Figure 1D,M), and partially to desmin (Figure 1H,N).

Liver biopsy in P3 (not illustrated) showed extensive bridging fibrosis and PAS-positive ground glass inclusions in hepatocytes, only slightly resistant to diastase digestion. Ultrastructurally, there was no evidence of polyglucosan storage.

5 DISCUSSION

Here, we report four additional patients with polyglucosan storage myopathy associated with RBCK1 deficiency. Two sisters displayed the full phenotypic spectrum of the condition including skeletal myopathy, cardiomyopathy, immunodeficiency, and auto-inflammation, whereas the other two unrelated patients had a skeletal and cardiac myopathy without any apparent dysfunction of the immune system.

Normal glycogen is PAS-positive, has a particulate ultrastructural morphology (15-20 nm) and contains glucose polymers with α-1,4-glucosidic bonds branched through α-1,6-glucosidic bonds. Abnormal processing of glycogen results in the formation of an amylopectin-like polysaccharide that is less branched, with a fibrillar ultrastructural morphology, contains other proteins, is PAS and Lugol’s iodine-positive, and variably resistant to digestion by alpha-amylase/diastase. It accumulates as corpora amylacea within subpial, subependymal, and perivascular astrocytic processes with ageing, and as polyglucosan within various tissues in a group of inherited disorders of glycogen metabolism. To date, pathogenic pathogenic variants in nine genes with diverse functions have been associated with polyglucosan storage disease with diverse clinical phenotypes. Variants in GYI, GYS1, PRKAG2, and PFKM result mainly in polyglucosan storage in cardiac and/or skeletal muscle, whereas variants in GBE1, RBCK1, EPM2A, EPM2B, and PRDM8 cause more widespread, multisystem polyglucosan storage. GYS1 variants cause equine disease.

RBCK1 is one of the components of the linear ubiquitin chain assembly complex (LUBAC), an E3 ligase complex that adds head-to-tail linear polyubiquitin chains to substrate proteins. LUBAC regulates activation of the canonical NF-κB pathway, which plays a key role in inflammatory and immune responses. Recessive loss-of-expression and loss-of-function variants in the N-terminal part of RBCK1 were first reported in three patients from two unrelated families who presented with a fatal disorder characterised by chronic auto-inflammation, recurrent pyogenic infections and skeletal and cardiac myopathies with polyglucosan storage. Functional studies in patients’ fibroblasts showed compromised NF-κB activation in response to interleukin-1β (IL-1β). By contrast, patients’ monocytes were hyper-responsive to IL-1β. The consequences of RBCK1 and LUBAC deficiencies for IL-1β responses differed between cell types, consistent with the paradox of auto-inflammation and immunodeficiency in these patients. Subsequently, 13 patients from 10 unrelated families were reported with homozygous or compound heterozygous variants in the mid- and C-terminal domains of RBCK1 and presenting with childhood or juvenile-onset progressive skeletal and/or cardiac myopathy with polyglucosan storage without immunodeficiency. It was proposed that RBCK1 deficiency may present either as an immunological disorder or as polyglucosan storage myopathy/cardio(myopathy depending on the variants being present in the N-terminal or the mid- and C-terminal domains of RBCK1, respectively.

In our series, the two female siblings display the full phenotypic spectrum of the condition to include myopathy, cardiomyopathy, immunodeficiency and auto-inflammation. There was clear intrafamilial variability and only one of the siblings (P1) suffered from early onset IBD and inflammatory arthritis. Immunological findings are necessarily confounded by iatrogenic immunosuppression, but nevertheless revealed interesting patterns. Both sisters had a marked increase in the percentage of CD21-CD38 B cells,
known to be elevated in autoimmune diseases, with compensatory decreases in other subsets. In particular, P1 demonstrated reduced switched memory B cell percentage and low baseline titres of antibodies against encapsulated bacteria despite an overall hypergammaglobulinemia: these abnormalities might help to explain recurrent infection in childhood with the development of bronchiectasis. Both sisters also had markedly reduced T cell proliferation, although this may simply reflect medication effects. A recent report also describes two patients from unrelated families with homozygous truncating variants in the mid-domain of RBCK1 presenting with childhood-onset myopathy and cardiomyopathy, polyglucosan storage and signs of auto-inflammation and immunodeficiency. It has been demonstrated that monocytes from patients with LUBAC component deficiencies are constitutively hyper-inflammatory and generate excessive innate cytokine responses upon stimulation, which may underlie the auto-inflammatory phenotype. The mechanism of immune deficiency remains less well understood, but paradoxically impaired NF-κB responses in fibroblasts and B cells have been described. The most common immunological abnormality observed, and consistent with our own cases, has been impaired antibody production and abnormal B cell phenotype. However, it is currently unknown whether this is due to intrinsic B cell dysfunction, a consequence of T cell dysregulation or simply reflects the impact of chronic inflammation. Overall, we believe there is currently no obvious genotype-phenotype correlation that can be demonstrated regarding the presence or absence of immune dysfunction. Figure 3 summarises the location of known pathogenic variants in the RBCK1 gene.

![Figure 3](image)

**Figure 3** Schematic representation of the RBCK1 gene marked with described variants. Variants identified in this study are in bold (novel variants are in red)

This article includes the first report of a pregnancy in a woman with RBCK1-related cardiomyopathy. Her twin sons were born preterm at 27 weeks and have an autism spectrum disorder. It is recognised that preterm infants have a 10-fold higher risk of developing autism spectrum disorders. It is also hypothesized that a link between maternal/foetal inflammation can lead to deregulation of normal brain development. This pregnancy was also complicated by peri-partum progression of symptoms with cardiac transplant required at 4 months post-partum. Normal pregnancy is associated with changes in cardiac and haemodynamic function that are designed to ensure adequate blood supply to the foetus, including increased circulating volume, cardiac output, stroke volume, and decreased peripheral resistance and blood pressure. Although these changes are harmless in a normal pregnant woman, there is increased foetal and maternal morbidity and mortality in women with underlying cardiac disease. Cardiac disease in women with RBCK1-related cardiomyopathy therefore has important implications in terms of management of pregnancy, pain relief, anaesthesia, fluid delivery, surgical intervention and the peri-partum period. Women planning pregnancy require specialist care including pre-conception cardiac risk assessment and surveillance of ventricular function. Combining our data and available information from the published literature, then the mean age at death in patients who underwent a cardiac transplant for cardiomyopathy (N = 8) was 25.3 years (28, 33, and 15 years), while in those with cardiomyopathy who were not transplanted (N = 6), the mean age at death was 17.6 years (17, 16, and 20 years). However, three of our patients presented in this article survived 21 years (deceased at 28 years), 13 years (alive at 35 years), and 16 years (deceased at 33 years) post-transplant for severe cardiac failure—and given that cardiac disease remains the leading cause of early mortality in this condition, we would advocate that all patients continue to be considered for transplant, with the issue of multisystem involvement, particularly immune dysregulation, carefully considered in selected patients. Post-transplant malignancies are a well-recognised long-term complication of cardiac transplant, and, in the absence of any other malignancies having been reported in patients with RBCK1-related disease, we feel that this is the most likely explanation for the development of Hodgkin lymphoma in patient.
The RBCK1 pathology in our series was similar to the previously described cases, with florid vacuolation and polyglucosan storage in the skeletal and cardiac muscles affecting the majority of fibres. The fibres containing polyglucosan inclusions were often significantly depleted of normal glycogen. The marked slow fibre predominance may suggest a physiological adaption to early involvement and loss of type II/fast glycolytic fibres. Unusually, some of the residual fast fibres were significantly hypertrophied. The inclusions were consistently strongly immunoreactive for ubiquitin and p62. While this suggests induction and/or inefficient ubiquitin-proteosome and autophagic degradation of polyglucosan the precise pathomechanism of polyglucosan storage in RBCK1 deficiency remains unknown.6 It probably involves unknown mechanisms independent of IL-1, TNF, and NF-κB, as polyglucosan storage is not seen in other diseases with enhanced or impaired NF-κB immunity.1,18,19

Muscle MRI findings, to our knowledge reported here for the first time in RBCK1-related disorders, showed a distinct pattern of selective involvement predominantly affecting vasti, sartorius, adductor magnus and, to a lesser extent, hamstring muscles within the thigh. Marked signal changes in corresponding areas on STIR sequences indicated marked oedema suggestive of ongoing inflammation. Interestingly, the muscle MRI pattern showed some similarities, in particular prominent adductor magnus involvement, with the muscle MRI pattern reported in acid maltase deficiency.20

Very early onset enteropathies and intestinal infections or IBD-like disorders have been described in monogenic diseases of immunodeficiency and/or auto-inflammation including defects in RBCK1.21 In our series, F1 presented with persistent diarrhoea and enteritis since early childhood. She underwent multiple GI biopsies, as well as a bronchial biopsy due to recurrent chest infections over a period of 10 years that showed variable chronic inflammation on routine haematoxylin and eosin staining. Retrospective staining of these biopsies with PAS/PAS-diastase revealed numerous small polyglucosan bodies within the lamina propria. Given the emerging phenotypic variability of RBCK1-associated disease, patients with early onset IBD/IBD-like disease with or without recurrent infections and/or myopathy should be screened for variants in RBCK1, especially with evidence of polyglucosan storage in a GI biopsy. As PAS/PAS-diastase stains are not performed routinely, there is a case for inclusion of these two stains along with haematoxylin and eosin for screening GI biopsies.

As yet, we do not have a complete understanding of the pathogenesis of RBCK1-related disorders, an explanation for the variability in phenotypic expression, nor an effective treatment. Early recognition of the condition, which may predominantly present in different organ systems, is important for individual and family counselling, cardiac monitoring and consideration of transplant.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Rahul Phadke and Renata S. Scalco performed the medical notes review, literature review, and drafted the manuscript. Elaine Murphy assessed patients, drafted, and revised the manuscript. Roshni Vara, Vasiliki Nakou, Heinz Jungbluth, Max Sugarman, Ana Jovanovic, Mark Roberts, Halima Amer, Reecha Sofat, and
Aine Merwick assessed patients, revised, and reviewed the manuscript. Rahul Phadke, Anders Oldfors, Marco Novelli, Andrew King, Istvan Bodi, and Michael Ashworth reviewed the pathology slides and generated the images. David M. Lowe reported the immunology findings, assessed patients, and reviewed the manuscript. Carola Hedberg-Oldfors and Anders Oldfors performed the genetic investigations and reviewed the manuscript. All authors approved the final version of this manuscript.

ETHICS STATEMENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. The study included only tests derived from routine clinical practice and therefore did not require specific ethics committee approval at our institution. Additional written informed consent was obtained from all patients (or an appropriate consultee) for whom potentially identifying information and imaging is included in this article. Written consent was obtained from all patients (or an appropriate consultee) for this publication.

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