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Case Report

Phenotypic and genotyping spectrum of two Iranian cases with *RBCK1*-associated polyglucosan body myopathy

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Glycogen storage diseases (GSDs) are a group of metabolic disorders affecting glycogen metabolism, with polyglucosan body myopathy type 1 (PGBM1) being a rare variant linked to *RBCK1* gene mutations. Understanding the clinical diversity of PGBM1 aids in better characterization of the disease. Two unrelated Iranian families with individuals exhibiting progressive muscle weakness underwent clinical evaluations, genetic analysis using whole exome sequencing (WES), and histopathological examinations of muscle biopsies. In one case, a novel homozygous *RBCK1* variant was identified, presenting with isolated myopathy without cardiac or immune involvement. Conversely, the second case harbored a known homozygous *RBCK1* variant, displaying a broader phenotype encompassing myopathy, cardiomyopathy, inflammation, and immunodeficiency. Histopathological analyses confirmed characteristic skeletal muscle abnormalities consistent with PGBM1. Our study contributes to the expanding understanding of *RBCK1*-related diseases, illustrating the spectrum of phenotypic variability associated with distinct *RBCK1* variants. These findings underscore the importance of genotype–phenotype correlations in elucidating disease mechanisms and guiding clinical management. Furthermore, the utility of next-generation sequencing techniques in diagnosing complex neurogenetic disorders is emphasized, facilitating precise diagnosis and enabling tailored genetic counseling for affected individuals and their families.

Key words: cardiomyopathy, glycogen storage disease, polyglucosan body myopathy, *RBCK1*, whole-exome sequencing.

INTRODUCTION

Glycogen storage disease (GSD) is a group of disorders, that intervene in glycogen synthesis or degradation.[1] Most of the manifestations of these disorders are related to enzyme deficiency and include exercise intolerance associated with rhabdomyolysis episodes or permanent muscle weakness.[1] However, other organ involvement varies based on the severity of enzyme deficiency and the specific enzyme involved. Polyglucosan body disease (PBD) is a type of glycogen storage disease with skeletal myopathy and cardiomyopathy with or without an immune disorder.[2] Eight different human genes are mainly responsible for causing it: *RBCK1, GYG1*, *GBE1*, *PFKM*, *EPM2A*, *EPM2B*, *PRDM8*, and *PRKAG2*.[1]

Polyglucosan body myopathy type 1 (PGBM1, OMIM #615895) is a rare autosomal recessive disorder caused by *RBCK1* gene variants, and is associated with a range of clinical presentations, including myopathy, cardiac involvement, autoinflammation, and immunodeficiency.[3] The *RBCK1* gene (OMIM #610924) encodes a protein called RANBP2-Type and C3HC4-Type Zinc Finger Containing 1, which is involved in glycogen metabolism and protein quality control pathways. Variants in *RBCK1* can lead to the sequestration of key enzymes of glycogen metabolism in polyglucosan bodies, resulting in glycogen depletion

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and muscle function impairment.[4] Symptoms of PGBM1 typically appear before adulthood; however, a case of adult-onset PGBM1 has been reported with a novel compound heterozygous *RBCK1* gene variant.[5]

Herein, we report two confirmed PGBM1 cases based on whole exome sequencing (WES), in which one of them presented a novel variant in the *RBCK1* gene without any evidence of cardiomyopathy and immune disorder. Our report provides further insight into the genotype-phenotype associations of *RBCK1*-related diseases.

CLINICAL SUMMARY

Subjects

Two affected individuals from two unrelated Iranian families who suffered from progressive muscle weakness were included. The disease seemed to be sporadic, albeit consanguineous marriage was noted in the parents of family 1, and the parents of family 2 came from nearby small villages, which suggests a common ancestry (Fig. 1A, B). The informed consent form was signed by the patients or their parents. The study protocol was confirmed by the ethics committee of the institution.

Patient 1-III1

An 11-year-old girl was referred to the neuromuscular clinic for an evaluation of her gait difficulty. The patient was born to consanguine parents, and her cousin had similar symptoms. The individual had achieved normal neurodevelopmental milestones, with no delays observed in social or mental development. While school aged, she experienced progressive difficulty running, jogging, and climbing stairs. A detailed neurological examination demonstrated mild bilateral ptosis without ophthalmoparesis and facial weakness. Manual muscle testing revealed a Medical Research Council (MRC) score of 4 for the neck flexor, shoulder abductor, and elbow flexor muscles, while the hip flexor and knee extensor muscle strengths were 3/5. Additionally, she exhibited a positive Gower's sign. The distal muscle strength was normal. No signs of dysphonia, dysphagia, contracture, calf hypertrophy, or hyperlaxity were observed. She reported moderate improvement after taking Co-Q10 daily for three months.

In the laboratory data, the lymphocyte count and immunoglobulin levels in the blood, as well as creatine kinase (CK) level (55 IU/L), were within normal limits. Thyroid function tests (TFT), liver function tests (LFT), and viral markers were also normal. The patient and her parents did not report any additional symptoms beyond muscular issues. Electrodiagnostic studies were performed When the patient was 10 years old. The nerve conduction studies (NCS) findings, including motor amplitudes and velocities, were within normal limits; however, a short, multi-phase and early recruitment of motor unit potentials (MUPs) was reported in proximal tested muscles such as deltoids, biceps brachii, and vastus medialis, without any spontaneous activity. The patient did not complain of cardiac manifestation and her electrocardiogram (ECG) and echocardiography did not show abnormal findings. The results of the cardiac echocardiography indicated an ejection fraction of 60%.

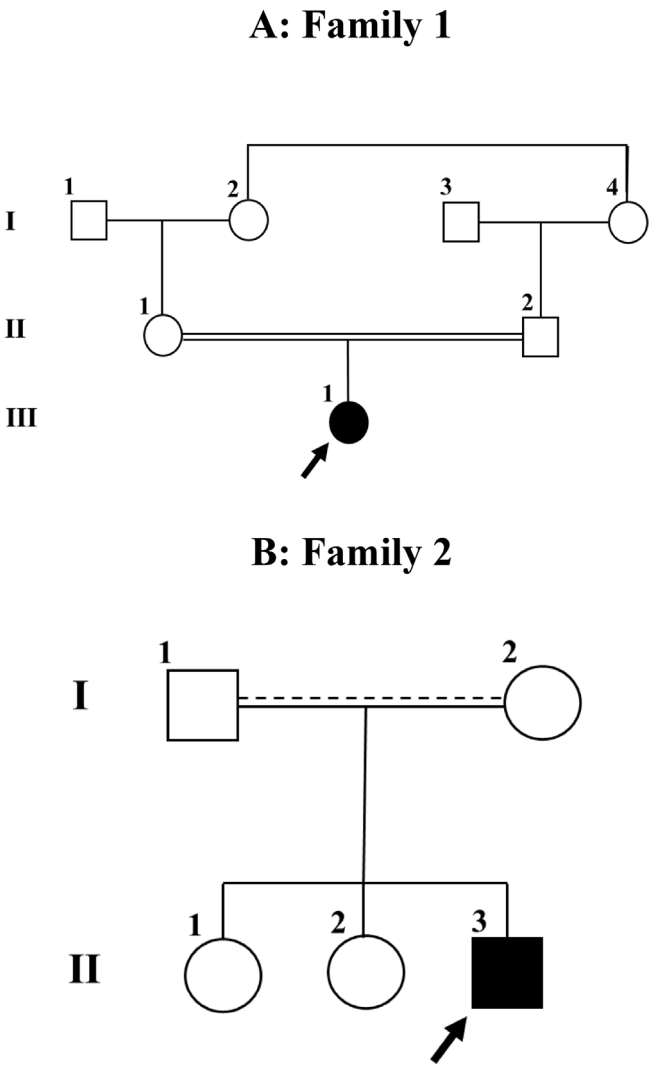

Fig 1 (A) Pedigree of family 1 and (B) pedigree of family 2, who carried homozygous variants c.736_743del (p.Ala246SerfsTer51) and c.1111dup (p.Cys371LeufsTer13) in the *RBCK1* gene, respectively. The arrow denotes the probands. The dashed line shows the parents originated from nearby small villages, suggesting a common ancestry. Blank circles and squares: normal individuals; dark circles and squares: PGBM1-affected individuals.

Patient 2-II3

A previously healthy 13-year-old boy, born to non-consanguine parents, presented with early fatigue and

exercise intolerance starting at age eight years. He had developed proximal muscle weakness over the past eight months. The muscle weakness began in the proximal part of the lower extremity and progressed to the proximal portion of the upper extremity three months before the initial visit.

The patient's father reported that he had suffered from repeated upper respiratory tract infections and prolonged (over two months) submandibular lymphadenopathy (LAP) two years previously. He underwent a lymph node biopsy and subsequently received chemotherapy after being diagnosed with B-cell lymphoma, and he had no further recurrence of the disease. On the first visit to the neuromuscular clinic, a neurologic examination revealed normal mental status and sensory function. In the cranial nerve examination, he showed mild bilateral ptosis with normal extraocular movement. He demonstrated weakness in bilateral arm abduction, elbow flexion, and elbow extension (MRC score + 4/5), along with hip flexion, knee extension, and knee flexion (MRC score – 4/5). Furthermore, we noticed bilateral scapular winging and a positive Gower's sign. The routine laboratory results, including TFT, LFT, and CK level (154 IU/L), were all within normal duration, low amplitude MUAPs, as well as spontaneous activity and myotonic limits. The electrodiagnostic (EDX) study revealed early recruitment and short discharges in proximal muscles. Cardiac ejection fraction (EF) was 45%, and there was evidence of left ventricular (LV) dilation, diastolic and systolic dysfunction (LV end diastolic and systolic diameters were 59 and 49 mm, respectively), and impaired global contractility with normal LV wall thickness. Mild-to-moderate mitral valve regurgitation (MR) was detected, while right ventricular (RV) systolic and diastolic function were normal. The above findings were in favor of dilated cardiomyopathy in echocardiography. The echocardiography was repeated due to progressive dyspnea after six months, which showed a significant decrease in EF (10%), along with severe global hypokinesia and severe MR. Detailed clinical data and genetic findings of both patients are summarized In Table 1.

Table 1 Clinical, laboratory, and genetic findings of patients with *RBCK1*-related myopathy

Patient ID	Sex	AAO (y)	AAD (y)	Consanguinity	Family History	DDMM	Follow-up Duration (m)	Initial Presentation	Cardiomyopathy	Auto Inflammation	Immunodeficiency	CK Level (IU/L)	Variant (NM_031229.4)	ACMG classification (Varsome)
P1-III1	F	6	11	+	–	–	36	Mild Symmetric Ptosis Proximal UL and LL weakness LL > UL	–	–	–	55	†c.736_743del, p.Ala246SerfsTer51	Likely pathogenic (PVS1, PM2)
P2-II3	M	8	13	–	–	–	18	Mild Symmetric Ptosis Proximal UL and LL weakness LL > UL	+	Recurrent URT Infection	Primary B-Cell Lymphoma	154	c.1111dup, p.Cys371LeufsTer13	Pathogenic (PVS1, PP5, PM2)

Abbreviations: AAD, age at diagnosis; AAO, age at onset; CK, creatin kinase; DDMM, delayed developmental motor milestone; F, female; LL, lower limb; M, male; UL, upper limb; URI, upper respiratory tract infection. †Novel variant.

PATHOLOGY FINDINGS

Histopathological analysis was conducted on open muscle biopsies (left biceps brachii for P1 and left vastus lateralis for P2) prior to genetic testing. The skeletal muscle's morphology was investigated using light microscopy. Hematoxylin and eosin (HE) staining revealed the presence of subsarcolemmal and cytoplasmic vacuoles, along with some endomysial fibrosis (Figs 2A, 3A). The Gomori trichrome stain of Patient 2 showed some materials that appeared pale green-gray in color (Fig. 3B). In both

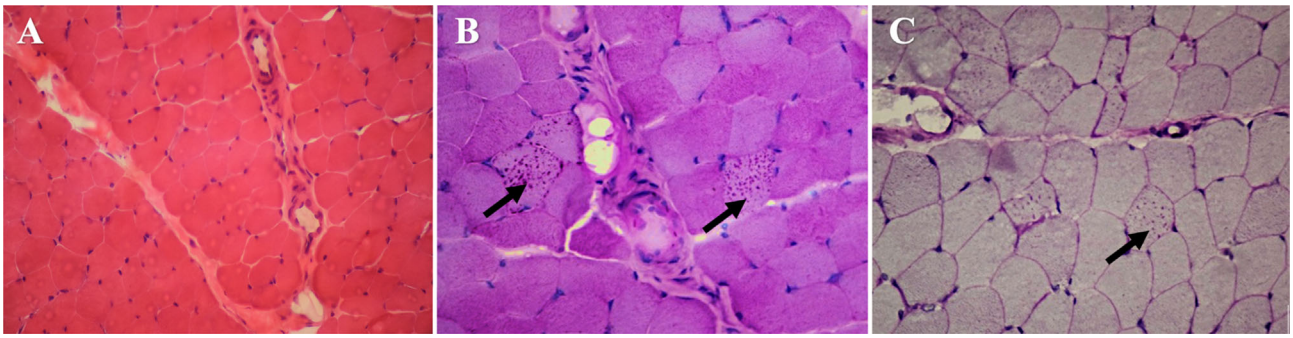

Fig 2 Histopathological findings in P1 from biceps brachii biopsy. (A) HE × 400 staining displays mild subsarcolemmal vacuoles and endomysial fibrosis. (B, C) Staining of PAS (×400) shows that PAS-positive materials (arrows) accumulate in some fibers, which are resistant to diastase (arrow).

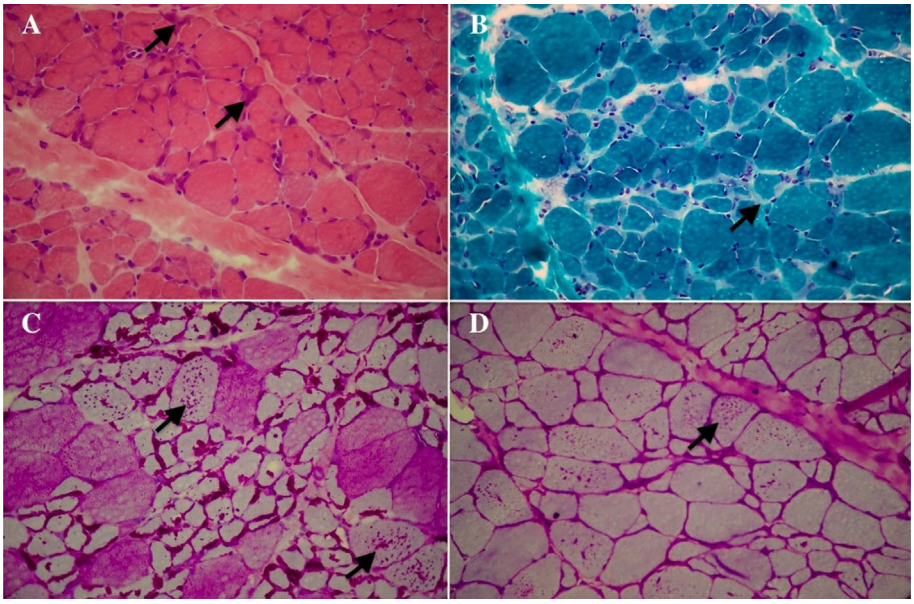

Fig 3 Morphological characteristics of vastus lateralis muscle in P2. (A) Numerous subsarcolemmal and cytoplasmic vacuoles and endomysial fibrosis are seen in hematoxylin and eosin (HE × 400) staining (arrows). (B) Gomori trichrome × 400 demonstrates the pale green-gray materials (arrow). (C, D) The PAS-stained × 400 section indicates abundant PAS-positive vacuoles filled with glycogen-storage materials that are preserved after diastase (arrows).

patients, the inclusions contained periodic acid-Schiff (PAS)-positive materials (Figs 2B, 3D), which were resistant to diastase digestion, as shown in Figure 2C and 3D, respectively. The NADH staining of patients showed some lobulated-like features as well as some reticular intermyofibrillar network patterns. In addition, all cytoplasmic vacuoles were clear. As shown in Figures 2 and 3, Patient 2 showed more prominent pathological findings than Patient 1.

GENETIC ANALYSIS

For identifying the underlying genetic defect, DNA was extracted from the peripheral blood of probands and their family members using the standard salting-out protocol. WES was performed using Illumina HiSeq4000 on the DNA of the probands. WES data was analyzed as previously reported workflows.[6] The candidate variants were amplified by polymerase chain reaction (PCR) and Sanger sequenced using the ABI Prism3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) in the probands and their family members for co-segregation analysis.

A novel homozygous variant, c.736_743del (p.Ala246SerfsTer51) in *RBCK1* (NM_031229.4), was identified in Patient 1-III1. It results in a reading frame shift causing a premature termination signal after 50 amino acid changes. The variant was co-segregated with the disease status in the family and predicted as likely pathogenic based on the American College of Medical Genetics and Genomics (ACMG) classification (rules PVS1 and PM2). This variant has not been reported in generalist polymorphism databases (ExaC, dbSNP, and the 1000 Genomes Project) and was also not detected among the exome data

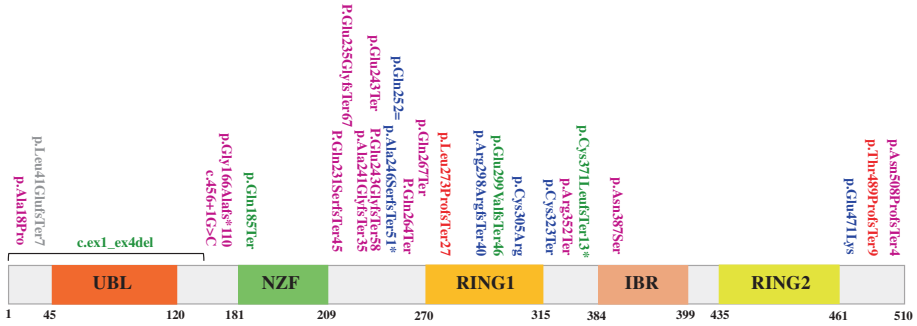

Fig 4 Schematic representation of the location of different domains in the human *RBCK1* gene. IBR, in-between RING domain; NZF, Npl4 zinc finger; UBL, ubiquitin-like domain. The greens show variants with all of the myopathy, cardiomyopathy, inflammation, and immune disorder phenotypes; the purples show variants with muscle and cardiomyopathy; the blues are associated with just muscle myopathy; the reds are related to all of the mentioned phenotypes except immune disorder; and for the gray one, all of the phenotypes except muscle myopathy are reported. The stars represent our variants.

of the 1000 unrelated Iranian individuals (Iranome database: www.iranome.com/).

The WES data analysis for Patient 2-II3 revealed a homozygous known *RBCK1* variant, c.1111dup (p.-Cys371LeufsTer13). According to the ACMG guidelines, the variant is classified as pathogenic (rules PVS1, PP5, and PM2) and is also listed in the ClinVar variant database. This variant was also co-segregated with the disease status in available members of the family.

DISCUSSION

We report two cases of pathogenic mutations in the *RBCK1* gene, expanding the number of known families with this condition; one of them had novel mutation. The *RBCK1* gene, located in 20p13, encodes a protein that has been found to interact with protein kinase C (PKC) and might function as a transcription factor. This protein consists of an N-terminal domain, an NZF domain (Npl4 zinc finger) for M1-polyubiquitin binding, and an RBR catalytic domain (RING-IBR-RING domain) in the C-terminal (Fig. 4).[7]

So far, there have been reports of 30 *RBCK1* homozygous and compound heterozygous variants in at least 27 cases (according to The Human Gene Mutation Database [HGMD] Professional 2023.4). These individuals with *RBCK1* variants exhibit a variety of symptoms, with specific clinical phenotypes being prominent. Some individuals experience muscle involvement, which is considered a hallmark of the disease, while others might suffer from immune system disorders and even cognitive impairment. The reason for such a wide range of clinical presentations remains unclear.[8,9] Phadke *et al.*, in their review, identified several patients with *RBCK1* variants who presented with significant immunological impairments, including recurrent infections and autoimmune manifestations. The authors provided detailed clinical descriptions and genetic analyses, highlighting the correlation between N-terminal *RBCK1* variants and immune system dysfunction.[1] However, the case report by Krenn *et al.* demonstrated that mutations outside the N-terminal part of *RBCK1* might cause immunological dysfunction later in the disease course. The mentioned studies emphasized the role of *RBCK1* in immune regulation and its effect on autoimmunity.[10] Sun *et al.* described a 15-year-old boy with the *RBCK1* variant who had been diagnosed with cardiac insufficiency twice. The cardiac symptoms masked the muscle involvement. Additionally, the authors documented several *RBCK1* patients with dilated cardiomyopathy.[11]

It has been assumed that the exact location of the variant in the gene can predict the prevailing phenotype, so that variants in the N-terminal region of *RBCK1* mainly lead to immunological impairment and autoinflammation, whereas the variants in the central or C-terminal part are more associated with (cardio) myopathy phenotypes.[8,10]

A literature review revealed that all genetic mutations associated with muscle involvement, cardiomyopathy, inflammation, and immune disorders are either nonsense variations or copy number variations (Fig. 4). Furthermore, other variants giving rise to muscle and cardiomyopathy phenotypes are predominantly found in the middle section of the protein, and the mutations associated exclusively with the phenotype of myopathy only are located within the middle or C-terminal regions of the protein (Fig. 4).

Regarding pathological findings, our cases were similar to the previously described patients. In skeletal muscle staining, there is abnormal vacuolization, polyglucosan storage, and some reticular intermyofibrillar network patterns. In Gomori trichrome staining, we observed normal-looking mitochondria surrounded by polyglucosan bodies. The vacuoles contained material, that strongly reacted to PAS staining, while the surrounding cytoplasmic areas

appeared to have depleted glycogen. These intense PAS-positive inclusions were highly resistant to α-amylase digestion, while the less intense PAS-positive normal glycogen was no longer visible.[1,12] Previous reports suggested that polyglucosan (PG) bodies were not found in younger cases but were present in older cases, indicating that they might develop gradually and contribute to the onset of muscle weakness.[13] This might support our findings that Patient 1, who experienced weakness at a younger age than Patient 2, did not have remarkable PG bodies. However, there was no significant difference in AAO and AAD between the two patients. The findings emphasize the importance of repeated muscle biopsies when a diagnosis has not been previously established.

Our study focuses on two Iranian patients whose genetic testing revealed two different homozygous variants in the middle of the *RBCK1* gene, with two different phenotypes. Patient 1-III1 showed myopathy with no signs of cardiac or immune involvement over 36 months of follow-up, while Patient 2-II3 presented with myopathy, cardiomyopathy, inflammation, and immunodeficiency, which might have been caused by his genetic variant increasing the risk of developing immunological dysfunction, such as recurrent upper respiratory tract infection and prolonged LAP. Moreover, *RBCK1* has been shown to play a role in the NF-κB signaling pathway, an important regulator of the immune system.[14,15]

In summary, our study expanded the genetic and phenotypic spectrum of *RBCK1*-related disease through the presentation of patients with biallelic pathogenic variants. Identifying the phenotypic spectrum improves our understanding of disease progression, offering clinicians valuable insights and enabling patients and their families to receive appropriate genetic counseling. This highlights the clinical utility of next-generation sequencing (NGS) techniques in complicated neurogenetic disorders.[12]

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AUTHOR CONTRIBUTIONS

MB and YN designed the manuscript. MR, MB, YN, and PS provided the outlines for the study's presentation. MR, and AG wrote the manuscript. MB and MMT supervised the study process and edited the final manuscript. SA, MM, and AG assisted in the manuscript's genetic section and writing the pertinent section. wrote the manuscript. All authors have reviewed the manuscript's data analysis process and writing and approved the final article.

DISCLOSURE

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

INFORMED CONSENT

Informed consent was obtained from participants and their parents.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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