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RESEARCH REPORT

# A Hemizygous Deletion Within the *PGK1* Gene in Males with PGK1 Deficiency

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**Abstract** Phosphoglycerate kinase-1 (PGK1) deficiency is a rare X-linked disorder caused by pathogenic variants in the *PGK1* gene. Complete loss-of-function variants have not been reported in this gene, indicating that residual enzyme function is critical for viability in males. Therefore, copy number variants (CNVs) that include single exon or multiple exon deletions or duplications are generally not expected in individuals with PGK1 deficiency. Here we describe a 64-year-old male presenting with a family history (three additional affected males) and a personal history of childhood-onset metabolic myopathy that involves episodes of muscle pain, stiffness after activity, exercise intolerance, and myoglobinuria after exertion. Biochemical analysis on a muscle biopsy indicated significantly reduced activity (15% compared to normal) for phosphoglycerate kinase (PGK1), a glycolytic enzyme encoded by *PGK1*. A diagnosis of PGK1 deficiency was established by molecular analysis which detected an approximately 886 kb deletion involving the polyadenylation site in the 3′UTR of the *PGK1* gene. RNA analysis showed significantly reduced *PGK1* transcript levels (30% compared to normal). This is the first deletion reported in the *PGK1* gene and is the first pathogenic variant involving the 3′UTR polyadenylation site of this gene. Our report emphasizes the role of 3′UTR variants in human disorders and underscores the need for exploring noncoding regions of disease-associated genes when seeking a molecular diagnosis.

## Introduction

Phosphoglycerate kinase-1 (PGK1) deficiency (MIM# 300653) is a rare X-linked disorder that is clinically heterogeneous and presents with hemolytic anemia, muscular defects, and neurological dysfunction. The disease exists in two major forms: the hemolytic subtype, in which affected individuals have hereditary non-spherocytic hemolytic anemia (HNSA), and the myopathic form, characterized by progressive muscle weakness, pain, and cramping (Beutler 2007). Both forms can be accompanied by intellectual disability or other neurological manifestations (Fermo et al. 2012).

PGK1 deficiency is caused by pathogenic variants in the *PGK1* gene. *PGK1* encodes a phosphoglycerate kinase (EC.2.7.2.3) that catalyzes a critical ATP-generating step of glycolysis. A single pathogenic variant in a hemizygous copy of the *PGK1* gene causes disease in males; female carriers are typically asymptomatic or demonstrate chronic, mild hemolytic anemia (Valentine et al. 1968). At least 25 different *PGK1* pathogenic variants, all of which are

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sequence variants, have been reported in the literature. These include 16 missense variants, 2 small (less than 5 basepairs) deletions within exons, and 4 splice site variants, all of which are expected to reduce, but not completely abolish, expression levels, stability, or enzymatic efficiency of the PGK1 protein (Stenson et al. 2017). Complete loss-of-function variants have not been reported in this gene, indicating that residual enzyme function is critical for viability in males (Chiarelli et al. 2012; Pey et al. 2014). Therefore, copy number variants (CNVs) that include single exon or multiple exon deletions or duplications are generally not expected in patients with PGK1 deficiency. Here we report a familial PGK1 deficiency caused by a novel 886 kb deletion located downstream of the translation termination site and involving the 3′UTR polyadenylation (poly-A) sequence; the entire *PGK1* coding region is otherwise intact. This is the first pathogenic CNV and the first 3′UTR variant ever reported in *PGK1*.

## Clinical Report

A 64-year-old Caucasian male presented with a childhood-onset metabolic myopathy characterized by episodes of muscle pain, muscle stiffness, exercise intolerance, and myoglobinuria after activity. One episode of rhabdomyolysis occurred during the fifth decade of life. The patient is asymptomatic at rest and does not require assistance with daily activities or assistive devices for mobility, but rests between activities that require exertion. Creatine kinase (CK) levels have reportedly been elevated since childhood and ranged from 600 to 7,000 U/L for the past year. He has mild proximal weakness of shoulder girdle and hip girdle muscles. EMG findings indicated a nonirritable myopathy. Dried blood spot test showed normal acid alpha-glucosidase level arguing against Pompe disease. Muscle biopsy showed normal routine histology, arguing against a dystrophic myopathy or a mitochondrial myopathy.

Given the concern for a metabolic myopathy, a comprehensive biochemical evaluation of the patient's muscle tissue was performed. Biochemical analysis of a muscle biopsy revealed significant deficiency of PGK1 enzyme activity (18 μmol/min/g tissue compared to a normal reference mean of 116.5 μmol/min/g; reference range of 48–184), indicating a possible diagnosis of PGK1 deficiency. Biochemical analysis of all enzyme levels in the myoglobinuria profile panel including myophosphorylase, phosphorylase b kinase, phosphofructokinase, phosphoglycerate mutase, carnitine palmitoyltransferase, lactate dehydrogenase, myoadenylate deaminase was normal. This residual PGK1 activity of 15% is comparable to those reported in individuals with a clinical diagnosis of PGK1 deficiency (Chiarelli et al. 2012). Congruent myopathic symptoms were noted in the patient's maternal uncle, brother, and sister's son, consistent with an X-linked disorder (Fig. 1a). No history of anemia or neurological dysfunction was noted in the proband or other affected family members.

### Molecular Analysis and RNA Quantification

*PGK1* Sanger sequencing and deletion/duplication analysis by array CGH were performed at EGL Genetics (Tucker, GA, USA), a CAP and CLIA-certified clinical laboratory. Deletion breakpoint mapping analysis was performed using sequence-specific primers as described previously (Ankala et al. 2012). RNA was isolated from peripheral blood and analyzed by RT-PCR. All samples were run in triplicate and products were visualized on an agarose gel. RNA quantification and relative *PGK1* RNA expression (normalized to β-actin) were performed with imaging software (Image Studio Lite, version 3.1; Li-Cor Biosciences, Lincoln, Nebraska, USA) according to the manufacturer's instructions.

### Results

Sequence analysis of the coding region and flanking intronic sequences of *PGK1* did not detect any sequence variants (benign, pathogenic, or otherwise). Subsequently, analysis for intragenic deletions or duplications was performed using array CGH. No CNVs were detected within the protein-coding region of the *PGK1* gene; however, an 886 kb deletion with genomic breakpoints at nucleotide positions g.77,381,971 and g.78,268,131 (hg19 reference; X chromosome; ClinVar accession number SCV000678240) and located approximately 600 bp downstream of the translation termination site of the *PGK1* gene was detected (Fig. 1b, c). This deletion encompasses the terminal portion of the 3′UTR and includes the poly-A signal sequence (ATTAAA) of the gene (Thierry-Mieg and Thierry-Mieg 2006). It also includes five additional genes, *TAF9B*, *CYSLTR1*, *ZCCHC5*, *LPAR4*, and *P2RY10,* none of which have been associated with human diseases. A deletion involving this region of the human genome has not been reported in the general population (MacDonald et al. 2014) or in individuals with disease (Stenson et al. 2003). The proband's affected brother was also found to carry this deletion (Fig. 2a). Quantitative analysis of RNA extracted from the peripheral blood sample of the affected brothers showed significantly reduced (37–40%) transcript levels of *PGK1* when compared to that of a gender-matched unaffected control (Fig. 2b). No other family members were available for testing. While the segregation analysis shown here is limited, all other lines of evidence including

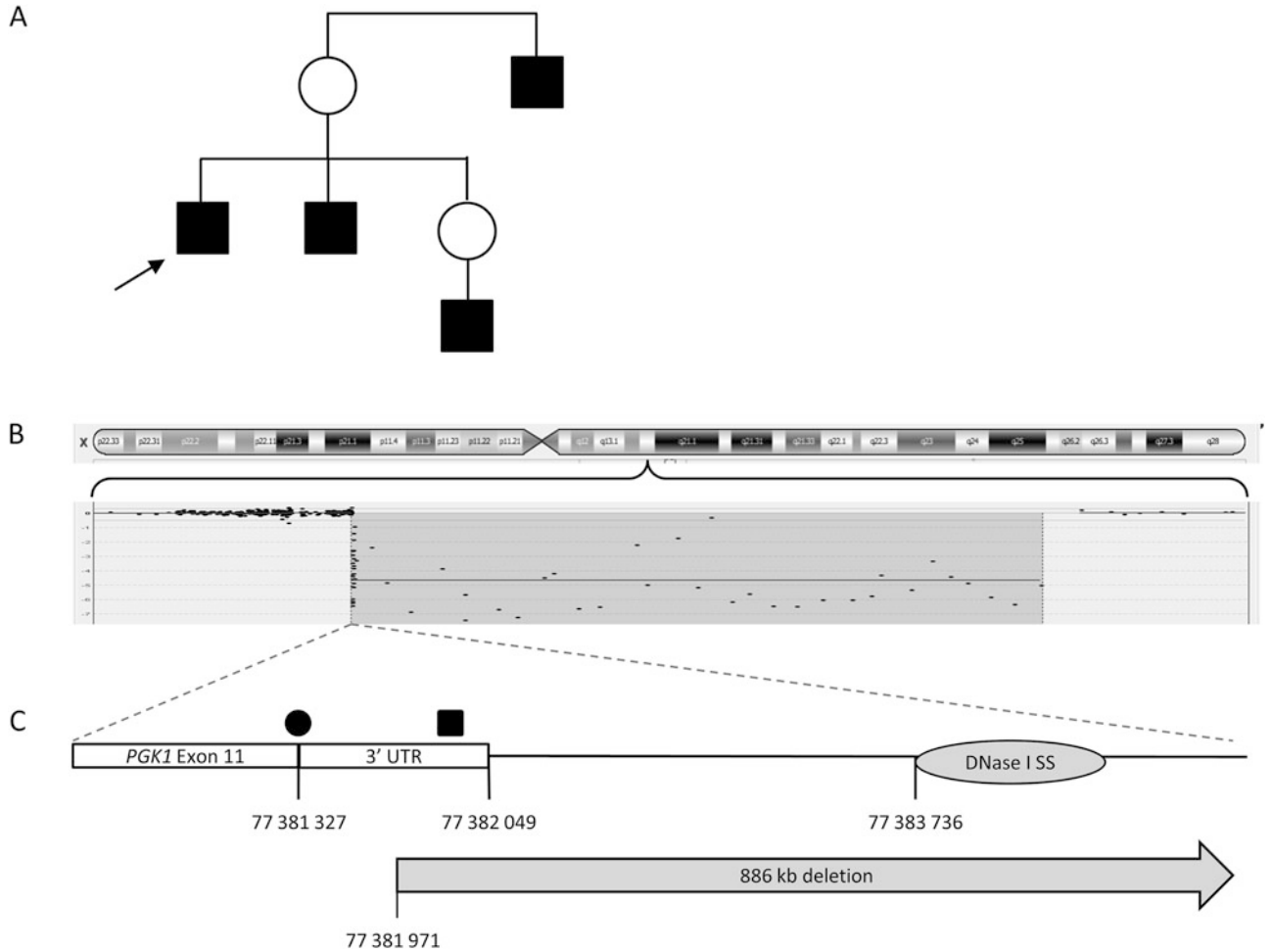


**Fig. 1** (**a**) The proband (arrow) reports that his maternal uncle, brother, and sister's son share similar myopathic symptoms (black squares), consistent with an X-linked disorder. (**b**) Deletion and duplication analysis by CGH detected an 866 kb deletion in the noncoding region downstream of *PGK1*. (**c**) The 5′ breakpoint of the deletion occurs 600 bp downstream of the PGK1 translation termination codon (black circle). The deletion (gray arrow) encompasses a portion of the 3′UTR, including the polyadenylation site (ATTAAA; black square) and a DNase sensitive site (DNase I SS; gray oval) that may act as a *PGK1* transcription regulatory element. Numbers indicate genomic location of labeled sites (hg19, chromosome X). Genetic elements are not to scale

biochemical (reduced enzyme activity), molecular (reduced transcript levels), population data (absent in population), and family history (X-linked inheritance) suggest that the observed deletion is potentially pathogenic.

## Discussion

After a comprehensive analysis involving clinical evaluation, enzyme activity assays on muscle biopsy, molecular investigation of genomic DNA, and RNA quantitation, we establish a diagnosis of PGK1 deficiency in a 64-year-old male with a childhood-onset metabolic myopathy. We report the first pathogenic variant within the 3′UTR of the *PGK1* gene: an 886 kb deletion involving the poly-A site. Our report of the first pathogenic CNV within the *PGK1* gene expands the mutation spectrum of the gene and establishes the clinical utility of deletion/duplication analysis for PGK1 deficiency as a follow-up test for individuals with a clinical diagnosis of PGK1 deficiency but negative sequencing results.

3′UTRs are known to contain important sequence elements that collectively determine the fate of mRNA, from its post-transcriptional modifications, stability, and half-life to its export from the nucleus and successful translation into a full-length polypeptide. An important component of this region is the poly-A signal sequence, which is critical for proper transcription termination, post-transcriptional pre-mRNA cleavage, and subsequent placement of the poly-A tail (Chen et al. 2006). Several variants that disrupt this sequence have been associated with disease. These include single nucleotide variants (SNVs) in the poly-A hexamer of the *FOXP3* gene that causes IPEX syndrome (Bennett et al. 2001) and of the *IL2RG* gene that causes severe combined immunodeficiency syndrome (SCID; Hsu et al. 2009).

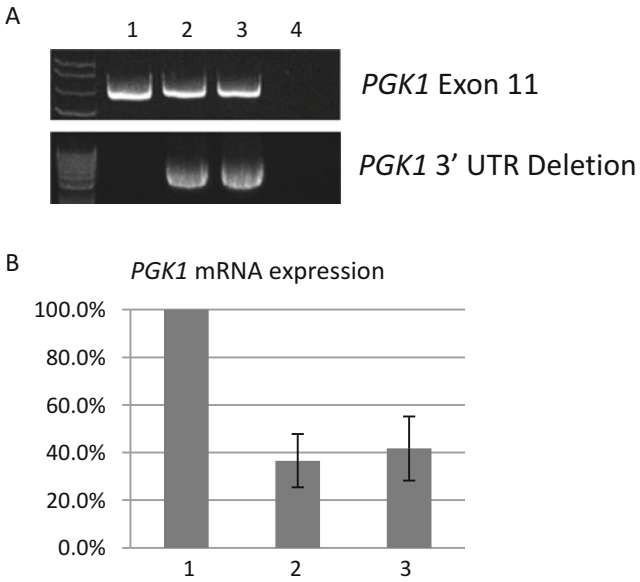


**Fig. 2** *PGK1* 3′UTR deletion is associated with decreased *PGK1* mRNA expression. (**a**) PCR analysis showing that the coding region (gel image at the top) of the last exon (exon 11) of *PGK1* is intact in (lane 1) a control sample, (lane 2) the proband, and (lane 3) the proband's affected brother. Lane 4 is a blank (no DNA) control. Breakpoint-junction primers designed to amplify across the deletion (intact control sample would not give a product) demonstrate that the affected brothers (lanes 2 and 3), but not the control (lane 1), carry the 3′ UTR deletion (gel image at the bottom). (**b**) Relative RNA analysis demonstrates that compared to a normal control (1), *PGK1* mRNA expression was decreased to 37% in the proband (2) and to 42% in the proband's affected brother (3). Percentages represent an average of three separate experiments

Additionally, deletion variants within the poly-A signals of the *HBA2* and *HBB* genes have been reported to cause α-thalassemia and $\beta^{+}$-thalassemia, respectively (Prior et al. 2007; Rund et al. 1992). Such disruption of the poly-A signal has been reported to result in read-through transcripts extending past the normal poly-A cleavage site, resulting in aberrant transcription termination and RNA splicing and processing (Rund et al. 1992). Although these longer and abnormal transcripts are reported as unstable and likely targeted by nonsense mediated decay (Chen et al. 2006), they are also translatable in vivo and can contribute to residual protein expression (Rund et al. 1992). This likely explains the residual PGK1 enzyme activity and metabolic myopathy in our study individuals.

Variants outside the poly-A signal sequence have also been identified as causative of disease. These include the 13 bp deletion and the c.*32A>C variant in the 3′UTR of the β-globin mRNA (*HBB* gene) which result in thalassemia by affecting nuclear processing of the mRNA or by decreasing mRNA stability, respectively (Bilenoglu et al. 2002; Hino et al. 2012). Alternatively, similar variants that disrupt 3′UTR sequences may alter recruitment of *trans*-acting factors which regulate gene expression or modify the 3′UTR secondary structure, thereby leading to disease (Chen et al. 2006). Gene expression (temporal and/or spatial) may also be altered by variants that disrupt chromosomal structure around a gene and/or of the *cis*-acting long-range regulatory elements (reviewed in Maston et al. 2006). Similarly, the deletion detected in this study may also alter *PGK1* gene expression as it includes a known DNase I sensitive site (Fig. 1c; Riley et al. 1991). This site has been shown to be specific to the actively expressing *PGK1* allele and is suggested to play a role as a regulatory element in mediating chromatin configuration around the *PGK1* gene and in regulating its expression. The possible mechanisms (of position effect) by which this potential regulatory element may alter *PGK1* gene expression are illustrated in the supplementary data (Fig. S1).

For a metabolic disease like PGK1 deficiency that is very rare and has a wide clinical spectrum that ranges from death in early childhood to being asymptomatic (Beutler 2007), making a molecular diagnosis and understanding the genotype–phenotype correlation are important. Given the high clinical variability, it is highly likely that the disease in general is underdiagnosed. In the current study, the negative sequencing result for *PGK1*, an X-linked gene (with only one copy in a male) would have likely ruled out a deletion and potentially evaded the diagnosis, had the subsequent CNV analysis not been performed. Therefore, it is critical that a comprehensive genetic evaluation (that includes SNV and CNV analysis) be made, especially when there is a strong clinical suspicion of a genetic disorder. Occasionally, mosaicism for CNVs has been reported in males with a milder presentation of an otherwise lethal X-linked disorder (Maddalena et al. 1988), which further emphasizes the need for complete genetic analysis.

Our findings further demonstrate the importance of investigating the noncoding regions of the genome. The promoter region, protein-coding exons, noncoding exons that comprise the 5′ and 3′ untranslated regions (UTRs), and interspersing introns together constitute the basic structure of a functional eukaryotic gene. However, given the lack of complete understanding of the sequence context of each individual gene and transcript in the human genome, molecular diagnostics is most often confined to variants within the protein-coding region. Currently, CNVs and SNVs within these noncoding and regulatory regions, that potentially modify gene expression and cause diseases, typically escape most routine molecular diagnostic tests. However, as investigation of the roles of 5′ and 3′ UTRs and other regulatory elements continues, and reports of disease-causing variants within these regions emerge (Ma et al. 2015), the need for interrogating these regions will increase. The rapidly reducing costs of sequencing are expected to further facilitate this, thereby allowing for increased clinical diagnostic yield.

## Synopsis

This report of a PGK1 deficiency case caused by a novel hemizygous deletion in the *PGK1* gene demonstrates the need to include exon level copy number analysis in the diagnostic workup to fully exclude this disorder.

## Author Contributions

Andrea Behlmann contributed to analysis and interpretation of data and drafted the chapter.

Namita Goyal contributed to conception and design and revised it critically.

Xiaoyu Yang contributed to conception and design and drafted the chapter.

Ping Chen contributed to analysis and interpretation of data and revised it critically.

Arunkanth Ankala contributed to conception and design and revised it critically and is the Guarantor for the study.

## Competing Interest

Andrea Behlmann, Namita Goyal, Xiaoyu Yang, and Ping Chen declare no conflict of interest. Arunkanth Ankala is employed by Emory University and is a laboratory director at EGL Genetic Diagnostics, LLC, a clinical genetics laboratory which performs testing described in this paper.

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## Ethics Approval/Patient Consent

No individually identifiable patient information is used.

## Animal Usage

None.

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