

Biallelic pathogenic variants in the mitochondrial form of phosphoenolpyruvate carboxykinase cause peripheral neuropathy

Neal Sondheimer,^{1,2,3,*} Alberto Aleman,⁴ Jessie Cameron,³ Hernan Gonorazky,⁴ Nesrin Sabha,² Paula Oliveira,² Kimberly Amburgey,⁴ Azizia Wahedi,² Dahai Wang,² Michael Shy,⁵ and James J. Dowling.^{1,2,4,6,*}

Summary

Phosphoenolpyruvate carboxykinase (PCK) plays a critical role in cytosolic gluconeogenesis, and defects in *PCK1* cause a fasting-aggravated metabolic disease with hypoglycemia and lactic acidosis. However, there are two genes encoding PCK, and the role of the mitochondrial resident PCK (encoded by *PCK2*) is unclear, since gluconeogenesis is cytosolic. We identified three patients in two families with biallelic variants in *PCK2*. One has compound heterozygous variants (p.Ser23Ter/p.Pro170Leu), and the other two (siblings) have homozygous p.Arg193Ter variation. All three patients have weakness and abnormal gait, an absence of PCK2 protein, and profound reduction in PCK2 activity in fibroblasts, but no obvious metabolic phenotype. Nerve conduction studies showed reduced conduction velocities with temporal dispersion and conduction block compatible with a demyelinating peripheral neuropathy. To validate the association between *PCK2* variants and clinical disease, we generated a mouse knockout model of *PCK2* deficiency. The animals present abnormal nerve conduction studies and peripheral nerve pathology, corroborating the human phenotype. In total, we conclude that biallelic variants in *PCK2* cause a neurogenetic disorder featuring abnormal gait and peripheral neuropathy.

Introduction

Phosphoenolpyruvate carboxykinase (PCK) plays a critical role in intermediary metabolism, converting the tricarboxylic acid cycle intermediate oxaloacetate into the gluconeogenic precursor phosphoenolpyruvate. There are two human genes encoding PCK. Cytosolic phosphoenolpyruvate carboxykinase (*PCK1*) encodes a protein with clear roles in hepatic gluconeogenesis, and loss-of-function variants cause a syndrome (MIM 261680) dominated by hypoglycemia during fasting.¹ Mitochondrial phosphoenolpyruvate carboxykinase (*PCK2*) encodes a protein with 70% identity to *PCK1* that is targeted to the mitochondrial matrix and expressed primarily in pancreas, kidney, liver, and fibroblasts.²

The cellular role of PCK2 is unclear, as gluconeogenesis is cytosolic. The mitochondrial PCK2 has kinetic properties similar to those of the cytosolic form and can substitute for PCK1 when targeted to the cytosol.³ One hypothesis specific to its function in the mitochondrial matrix is that PCK2 is required for the consumption of mitochondrial guanosine triphosphate (GTP) and the regulation of pancreatic insulin secretion.⁴ However, an animal model deficient in *Pck2*, while having impaired glucose-stimulated insulin secretion, was non-diabetic.⁵

While inherited defects in mitochondrial PCK2 were previously claimed in the medical literature (MIM 261650),^{6,7} none of the patients described were known

or found to have pathogenic variants in *PCK2*, making it unlikely that the disorder was correctly described.⁸ In this study, we identify three individuals with biallelic *PCK2* variants and a phenotype consistent with an inherited neurogenetic condition featuring abnormal gait and peripheral neuropathy.

Results

Case presentations

Using exome sequencing, we identified three patients in two families with a common phenotype and likely pathogenic variants in *PCK2* (Figure 1A). Patient 1 came to medical attention as a 3-year-old girl with ataxia and weakness, which was exacerbated by illness. Specifically, she experienced several episodes of severe gait instability resulting in transient loss of ambulation and necessitating hospitalization. Between episodes she was found to have mild, persistent gait ataxia and mild distal > proximal weakness.

Owing to the episodic nature of the presentation, particularly given exacerbation with illness, an inborn error of metabolism was suspected and investigated. Mild elevations in lactate were occasionally noted (2.4–3.5 mM during her initial hospitalization) but metabolic studies were otherwise unremarkable, and a 24-h fasting study provoked a ketotic response (β -hydroxybutyrate 0.8 mM at 24 h).

¹Departments of Paediatrics and Molecular Genetics, The University of Toronto, Toronto, ON, Canada; ²Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada; ³Divisions of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada; ⁴Divisions of Neurology, The Hospital for Sick Children, Toronto, ON, Canada; ⁵Department of Neurology, University of Iowa, Iowa City, IA, USA

⁶Lead contact

*Correspondence: neal.sondheimer@sickkids.ca (N.S.), james.dowling@sickkids.ca (J.J.D.)

<https://doi.org/10.1016/j.xhgg.2023.100182>.

© 2023 The Authors. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



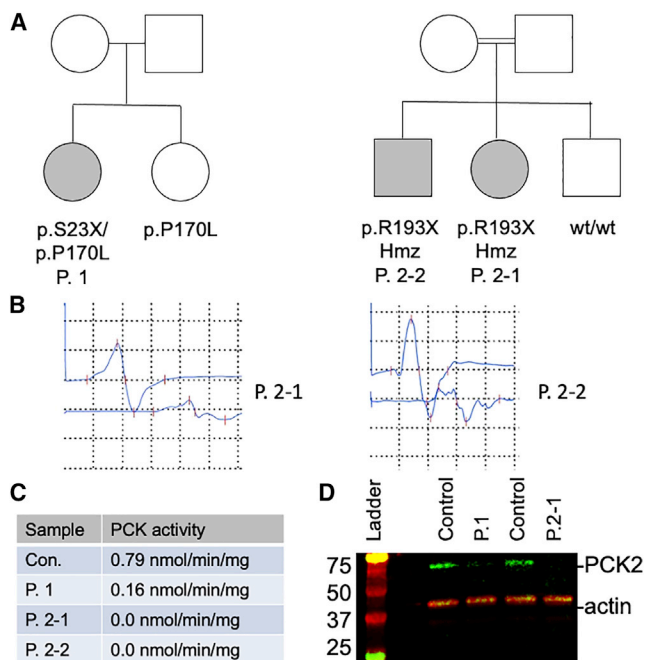


Figure 1. *PCK2* variants are associated with peripheral neuropathy

(A) Pedigrees from two families with biallelic variants in *PCK2*.

(B) Nerve conduction studies (NCS) from right tibial nerve. In patient P.2-1, NCS showed low proximal and distal compound muscle action potential (CMAP) amplitudes (2.5 mV and 0.79 mV, respectively) with reduced conduction velocity (31.1 m/s) and temporal dispersion. In patient P.2-2, NCS showed low proximal and distal CMAP amplitudes (0.89 mV and 0.25 mV, respectively) with reduced conduction velocity (32.3 m/s) and temporal dispersion.

(C) Reduction in *PCK2* activity in fibroblast lysates from patients harboring biallelic *PCK2* variants (Con., unrelated normal control).

(D) Western blot of fibroblast lysates from patients P.1 and P.2-1 and from two unrelated controls. Actin was provided as a loading control.

She was lost to follow-up for many years until she presented again in adulthood. She continues to experience gait ataxia but has maintained independent ambulation. No additional diagnostic data were able to be obtained; of note, nerve conduction studies have never been performed.

Whole-exome sequencing identified p.Ser23Ter (c.68C>G [NM004563.4]) and p.Pro170Leu (c.509C>T) variants in *PCK2* and no other potentially causative variants. An unaffected sibling carried the p.Pro170Leu variant only. The allele frequency of p.Ser23Ter is 1.87×10^{-3} and p.Pro170Leu is 4.78×10^{-5} in a database of large-scale sequencing projects (gnomAD v2.1.1, May 1, 2021 release).⁹ The prevalence of p.Ser23Ter is unexpectedly high for a loss-of-function allele. Of note, a single p.Ser23Ter homozygous individual is found enumerated in GnomAD.

A second family was identified after referral of a 12-year old girl (patient 2-1) for abnormal gait. Her gait changes were progressive, with symptom onset at age 10 years and with increasing clumsiness and falls over an approximate span of 2 years. Family history revealed that her older

brother (patient 2-2) was similarly affected and that the parents are first cousins. Physical examination of the proband was notable for distal muscle atrophy and bilateral pes cavus, distal muscle weakness, and absent reflexes in the knees and ankles. Sensory exam was abnormal in the lower extremities to all modalities. Of note, both the proband and her brother had evidence of asymmetry in terms of muscle atrophy and weakness. Unlike with individual 1, there was no clear demonstration of fluctuation in symptomatology or worsening with illness in either sibling.

Nerve conduction studies, performed on both siblings, showed a sensory-motor demyelinating peripheral neuropathy with evidence of temporal dispersion and conduction block (Figure 1B and Table S1). As with the physical exam, there were asymmetrical changes in nerve conduction. Metabolic studies, including lactate, pyruvate, amino acids, and urine organic acids, were unremarkable. The proband was initially diagnosed, based on the nerve conduction studies, with chronic inflammatory demyelinating polyneuropathy and treated with intravenous immunoglobulin. After failure of response to therapy, genetic testing was pursued. PMP22 deletion/duplication testing was normal, and a Charcot-Marie-Tooth disease multigene panel was unremarkable. Whole-exome sequencing identified homozygous p.Arg193Ter variants in *PCK2* in both siblings (c.577C>T [NM004563.4]), which had a population allele frequency of 8.14×10^{-5} . A third sibling in the family had normal gait and did not carry the variant. Of note, no other disease-associated pathogenic variants were identified in either sibling.

Validation studies: Patient fibroblasts show reduced *PCK2* levels and activity

Fibroblast samples were obtained from all patients. Since *PCK1* is not expressed in fibroblasts,² enzymatic studies of *PCK* activity in these cells reflects *PCK2* alone. There was a partial loss of fibroblast *PCK2* activity in fibroblasts from patient 1, and a complete loss of activity in cells from patients 2-1 and 2-2 (Figure 1C). Western blotting of fibroblast lysates confirmed a partial loss of detectable *PCK2* in patient 1 and a complete loss of protein in patient 2-1 (Figure 1D). Oximetry studies of intact fibroblasts showed no significant difference in basal or maximal oxygen consumption between control cells and cells from patient 2-1 (Figure S1), consistent with a lack of defect in mitochondrial oxidative phosphorylation.

Characterization of a *Pck2* knockout mouse line

To further investigate the connection between the phenotype and *PCK2* variants, we created and evaluated a mouse model of *Pck2* deficiency (*Pck2_em1_del* or *Pck2* KO). This mouse line, generated on a C57BL/6NCrl background, harbors a homozygous 252 bp deletion in *Pck2*, resulting in a p.Ser142Hisfs*21 nonsense mutation (see supplemental material and methods for details of the generation of this strain). Homozygous animals were viable with no overt phenotype but were obtained at slightly below expected

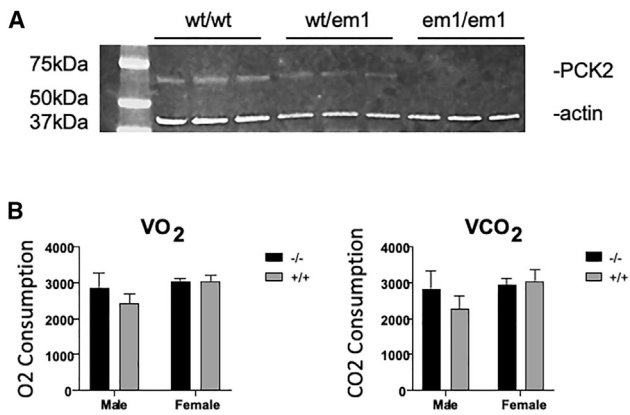


Figure 2. Characterization of mouse embryonic fibroblasts from *Pck2* knockout mice

(A) Western blotting of wild-type (wt), heterozygous (wt/em1), and *Pck2* knockout (em1/em1) animals. Actin was used as a control. Protein extracts were derived from mouse embryonic fibroblasts for the indicated genotypes.

(B) Metabolic studies of *Pck2* knockout compared with wild-type mice (n = 8 animals studied).

VO₂ p = 0.20 and VCO₂ p = 0.017 by two-way ANOVA without Bonferroni correction.

frequency (22%; 53/240), with a mild bias toward male survival (30% of males homozygous, 15% of females homozygous).

Murine embryonic fibroblast (MEF) lines were created from wild-type, heterozygous, and homozygous *Pck2* KO animals. Western blotting of the cell lines showed an absence of protein in the *Pck2* KO animals and a partial loss of protein in the heterozygotes (Figure 2A). Studies of PCK activity in lysates showed a corresponding loss of activity in *Pck2* KO MEFs compared with wild-type MEFs (wild type = 0.57 ± 0.04 nmol/min/mg protein, *Pck2* KO = 0.0 nmol/min/mg protein).

We performed a battery of phenotypic analyses on the *Pck2* KO animals, comparing them with wild-type littermates, with independent evaluation of both male and female animals because of the observed difference in viability. A small difference was seen in metabolic studies in the males, with increased volume of oxygen consumed (VO₂) and volume of CO₂ produced (VCO₂) in the KO animals that was no longer significant after correction for multiple testing (Figure 2B). Treadmill testing was used to evaluate the animal's gait. Minor differences were noted in gait, primarily in female animals, but these again were not significant after correction for multiple testing.

We then focused additional investigations on the peripheral nervous system. *Pck2* KO animals and wild-type littermates were evaluated by nerve conduction studies, where we observed in KO mice an asymmetric reduction of the conduction velocities (Figure 3A), mainly in males, associated with variable penetrance. Some of the KO mice also showed mild temporal dispersion, a finding not seen in any wild-type mice. Considering 40 m/s as the lower limit of normal for sural nerve conduction velocity, 6 of 15 *Pck2* KO mice had reduced values (i.e., <40 m/s) while

only 1 of 15 wild-type littermates showed a reduction (Table S2). Lastly, we examined for peripheral nerve pathology using both light and electron microscopy. No obvious structural abnormalities were consistently observed. Quantitative analysis of sciatic nerves, however, revealed variability in both axon and myelin sheath diameter (Figure 3B), with significant shifts to disproportionate amounts of small or large fibers (Figures 3C and S2). In total, these findings support the presence in KO mice of a demyelinating peripheral neuropathy of variable penetrance.

Discussion

In summary, our studies demonstrate that the loss of *PCK2* causes a neurogenetic disorder featuring abnormal gait with evidence of demyelinating peripheral neuropathy. This phenotype stands in contrast to the decompensating metabolic condition that has been associated with the loss of *PCK1*. The difference in phenotype is likely related to the targeting of PCK to the mitochondrial matrix and its expression in a different set of tissues. There is no evidence of any diabetic phenotype in the patients or animal model, although there were small changes in mitochondrial coupling in the male *Pck2* KO animals.

Previous studies of *PCK2* posited a role in pancreatic insulin release. However, none of our patients are diabetic, and patient 1 had an unremarkable glucose tolerance test as a child. Although *PCK2* is apparently highly expressed in liver and pancreas,² we observed no deficits in our patients or in the KO mice corresponding to hepatic or endocrine or exocrine pancreatic function. We conclude that a loss of mitochondrial PCK activity is not diabetogenic.

The clinical phenotype, at least in family 2 and also supported by the mouse studies, is in keeping with a recessive form of Charcot-Marie-Tooth disease (CMT type 4) or hereditary motor and sensory neuropathy. A key unanswered question of this study concerns the mechanism through which the loss of mitochondrial PCK activity causes peripheral neuropathy. Previous studies have implicated *PCK2* in the maintenance of neuronal progenitor state in cell culture,¹⁰ and loss of this function may explain the histopathological finding of excessive variability of axon diameter. However, it is unclear how this finding relates to the demyelination changes observed in our patients or to the temporal dispersion of nerve conduction signals seen in both patients and mice. From a pathomechanistic perspective, *PCK2* deficiency fits with the broader subgroup of peripheral neuropathies due to abnormalities in mitochondrial structure and/or function, a group that includes mutations in *MFN2* (CMT2A), *SURF1* (CMT4K), and *PDK3* (CMTX6).

Of note, neither isoform of PCK is highly expressed in the central nervous system, although *PCK2* is detectable in many brain regions where *PCK1* expression is absent. A study of dogs with paroxysmal dyskinesia has recently implicated a heterozygous variant in the GTP-binding domain of *PCK2* as causative.¹¹ One possible avenue for

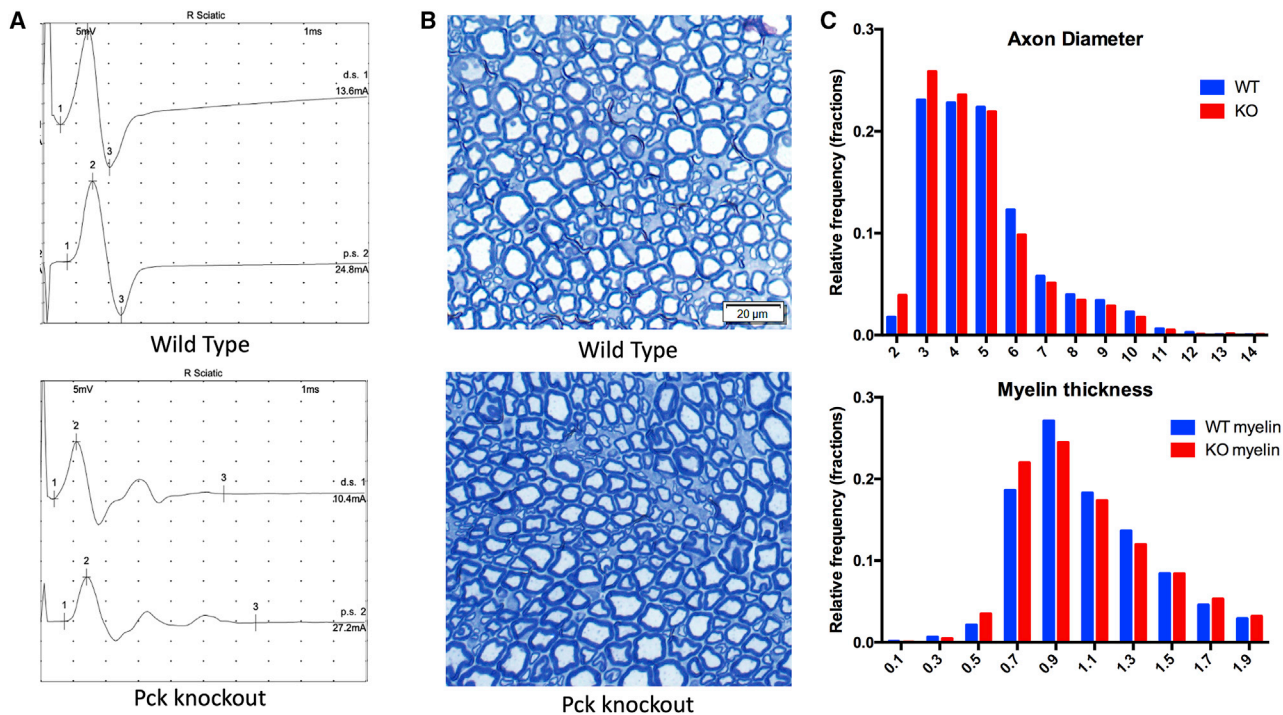


Figure 3. Peripheral nervous system changes in *Pck2* knockout mice

(A) Sciatic nerve conduction studies of *Pck2* knockout mice reveal reduced conduction velocities and temporal dispersion (Table S2) ($n = 15$ per genotype). Shown are representative tracings from a wild-type littermate and a *Pck2* knockout mouse.

(B) Representative semi-thin sections of sciatic nerve. No overt abnormalities were seen, although there was the appearance of an increased proportion of small fibers.

(C) Quantification of axon diameter and myelin thickness as represented by frequency distribution plots. Difference in distribution between genotypes was $p = 0.0001$ (Wilcoxon rank test, $n = 8$ per genotype with >50 fibers measured per nerve/animal).

future studies is investigating whether the clearance of GTP in the mitochondrial matrix by PCK may be important for the maintenance of neuronal health.

The phenotype described in our patients is relatively mild in that all three individuals have maintained the ability to ambulate independently. Given the relatively high allele frequency of loss-of-function variants in *PCK2*, particularly the p.Ser23Ter allele, combined with mild phenotypic presentations, we posit that deficiency in *PCK2* may be a fairly common genetic cause of peripheral neuropathy that is significantly underdiagnosed. As the sequencing of patients with ataxia, gait disturbance, and/or abnormal nerve conduction studies becomes more common, we suspect that *PCK2* variants will be increasingly recognized as a cause of human neurological disease.

Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.xhgg.2023.100182>.

Acknowledgments

We thank the patients and families for agreeing to participate in this study. This study was funded in part through a Rare Disease Models and Mechanisms grant from the Canadian Institutes of Health Research (N.S., M.S.). Additional support was provided by the Mogford Campbell Family endowed chair fund (J.J.D.). We

thank the Toronto Center for Phenogenomics for assistance with the *Pck2* knockout mouse model.

Declaration of interests

N.S. is an employee of Synlogic, and K.A. is a part-time employee of Deep Genomics.

Received: July 22, 2022

Accepted: January 18, 2023

References

- Vieira, P., Cameron, J., Rahikkala, E., Keski-Filppula, R., Zhang, L.H., Santra, S., Matthews, A., Myllynen, P., Nuutinen, M., Moilanen, J.S., et al. (2017). Novel homozygous *PCK1* mutation causing cytosolic phosphoenolpyruvate carboxykinase deficiency presenting as childhood hypoglycemia, an abnormal pattern of urine metabolites and liver dysfunction. *Mol. Genet. Metabol.* 120, 337–341.
- Modaresi, S., Brechtel, K., Christ, B., and Jungermann, K. (1998). Human mitochondrial phosphoenolpyruvate carboxykinase 2 gene. Structure, chromosomal localization and tissue-specific expression. *Biochem. J.* 333, 359–366.
- Escós, M., Latorre, P., Hidalgo, J., Hurtado-Guerrero, R., Carrodegua, J.A., and López-Buesa, P. (2016). Kinetic and functional properties of human mitochondrial phosphoenolpyruvate carboxykinase. *Biochem. Biophys. Rep.* 7, 124–129.

4. Stark, R., Pasquel, F., Turcu, A., Pongratz, R.L., Roden, M., Cline, G.W., Shulman, G.I., and Kibbey, R.G. (2009). Phosphoenolpyruvate cycling via mitochondrial phosphoenolpyruvate carboxykinase links anaplerosis and mitochondrial GTP with insulin secretion. *J. Biol. Chem.* *284*, 26578–26590.
5. Abulizi, A., Cardone, R.L., Stark, R., Lewandowski, S.L., Zhao, X., Hillion, J., Ma, L., Sehgal, R., Alves, T.C., Thomas, C., et al. (2020). Multi-Tissue acceleration of the mitochondrial phosphoenolpyruvate cycle improves whole-body metabolic health. *Cell Metabol.* *32*, 751–766.e11.
6. Robinson, B.H., Taylor, J., and Sherwood, W.G. (1980). The genetic heterogeneity of lactic acidosis: occurrence of recognizable inborn errors of metabolism in pediatric population with lactic acidosis. *Pediatr. Res.* *14*, 956–962.
7. Clayton, P.T., Hyland, K., Brand, M., and Leonard, J.V. (1986). Mitochondrial phosphoenolpyruvate carboxykinase deficiency. *Eur. J. Pediatr.* *145*, 46–50.
8. Bodnar, A.G., Cooper, J.M., Holt, I.J., Leonard, J.V., and Schapira, A.H. (1993). Nuclear complementation restores mtDNA levels in cultured cells from a patient with mtDNA depletion. *Am. J. Hum. Genet.* *53*, 663–669.
9. Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alfoldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P., et al. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* *581*, 434–443.
10. Álvarez, Z., Hyroššová, P., Perales, J.C., and Alcántara, S. (2016). Neuronal progenitor maintenance requires lactate metabolism and PEPCK-M-directed cataplerosis. *Cerebr. Cortex* *26*, 1046–1058.
11. Nessler, J., Hug, P., Mandigers, P.J.J., Leegwater, P.A.J., Jagannathan, V., Das, A.M., Rosati, M., Matiassek, K., Sewell, A.C., Kornberg, M., et al. (2020). Mitochondrial PCK2 missense variant in shetland sheepdogs with paroxysmal exercise-induced dyskinesia (PED). *Genes* *11*, 774–815.