PDXK Mutations Cause Polyneuropathy Responsive to Pyridoxal 5'-Phosphate Supplementation

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Objective: To identify disease-causing variants in autosomal recessive axonal polyneuropathy with optic atrophy and provide targeted replacement therapy.

Methods: We performed genome-wide sequencing, homozygosity mapping, and segregation analysis for novel disease-causing gene discovery. We used circular dichroism to show secondary structure changes and isothermal titration calorimetry to investigate the impact of variants on adenosine triphosphate (ATP) binding. Pathogenicity was further supported by enzymatic assays and mass spectroscopy on recombinant protein, patient-derived fibroblasts, plasma, and erythrocytes. Response to supplementation was measured with clinical validated rating scales, electrophysiology, and biochemical quantification.

Results: We identified biallelic mutations in PDXK in 5 individuals from 2 unrelated families with primary axonal polyneuropathy and optic atrophy. The natural history of this disorder suggests that untreated, affected individuals become wheelchair-bound and blind. We identified conformational rearrangement in the mutant enzyme around the ATP-binding pocket. Low PDXK ATP binding resulted in decreased erythrocyte PDXK activity and low pyridoxal 5'-phosphate (PLP) concentrations. We rescued the clinical and biochemical profile with PLP supplementation in 1 family, improvement in power, pain, and fatigue contributing to patients regaining their ability to walk independently during the first year of PLP normalization.

Interpretation: We show that mutations in PDXK cause autosomal recessive axonal peripheral polyneuropathy leading to disease via reduced PDXK enzymatic activity and low PLP. We show that the biochemical profile can be rescued with PLP supplementation associated with clinical improvement. As B₆ is a cofactor in diverse essential biological pathways, our findings may have direct implications for neuropathies of unknown etiology characterized by reduced PLP levels.

Peripheral neuropathies are among the most common neurological disorders worldwide, affecting approximately 20 million people in Europe and the USA alone, with few disease-modifying treatments established for these conditions. Inherited forms of peripheral neuropathies affecting both the motor and sensory nerves, known as Charcot–Marie–Tooth disease (CMT), are the most common genetic neuromuscular disorders, affecting approximately 1 in 2,500 people. However, only 25% of all autosomal recessive (AR) CMT cases have causal variants identified, and treatment is only supportive. Most known genes associated with AR CMT cause disease either by affecting the cytoskeleton or axonal trafficking.

Subjects and Methods

Study Participants

Patients with polyneuropathy and optic atrophy were identified by neurogeneticists at the National Hospital for Neurology and Neurosurgery London and Children’s Hospital of Eastern Ontario. The affected cases were recruited along with unaffected family members under institutional review board/ethics-approved research protocols (University College London Hospitals: 04/N034; Children’s Hospital of Eastern Ontario: CTO/1577) with informed consent. All cases had extensive genetic, metabolic, and mitochondrial investigations carried out that excluded acquired and other inherited causes of polyneuropathy and optic atrophy.

Phenotype and Clinical Measures

All cases had comprehensive phenotyping performed by neurogenetics specialists including clinical assessment, electrophysiology, neuroimaging, and videography. Motor function was quantified using the Medical Research Council (MRC) muscle scale, a standardized scale for the assessment of the peripheral nervous system (PNS) that grades muscle power from 0 to 5 in 6 validated movements and provides a total score of a possible 60 points. The disease severity and progression were assessed using the validated Neurological Impairment Scale (NIS) and the Charcot-Marie-Tooth Neuropathy Score version 2 (CMTNS2). CMTNS2 is a reliable and valid composite of symptoms (3 items), signs (4 items), and neurophysiology (2 items). It is designed to measure length-dependent motor and sensory impairment in genetic neuropathies. The NIS forms part of the standard minimum dataset for the Functional Independence Measure and UK Functional Assessment Measure. It records the severity of functional impairment (rated 0–3) across 13 domains mapped onto the International Classification of Functioning. The score range is 0 to 50. The affected individuals from Family 1 had MRC scales, CMTNS2, and NIS assessment before and after replacement therapy.

Genome-wide Sequencing and Haplotype Analysis

DNA was extracted from peripheral blood. Whole genome sequencing (WGS) was performed by deCODE genetics (Reykjavik, Iceland), using DNA from the 2 affected siblings from Family 1. A HiSeq4000 instrument (Illumina, San Diego, CA) was used to generate 100bp paired-end reads. Alignment was performed using BWA (http://bio-bwa.sourceforge.net/) with GRCH37 as a reference. Variants were called using the GATK14–17 Unified Genotyper-based pipeline14–16 workflow. All variants were annotated using ANNOVAR18 and filtered using custom R scripts. Shared regions of homozygosity were identified using HomozygosityMapper19 and Bcftools/RoH.20 Only novel or very rare variants with a minor allele frequency of <0.01 in the 1000 Genomes Project,21 NHLBI GO Exome Sequencing,22 and Exome Aggregation Consortium database23 were considered for analysis.
were included. Coding/splicing homozygous variants that were within the autozyome of the affected cases were prioritized.

Whole exome sequencing (WES) was performed in Family 2 using DNA from both affected siblings and their unaffected mother as part of the Care4Rare Canada Consortium. Exonic DNA was selected using the SureSelect Clinical Research Exome V1 kit (Agilent Technologies, Santa Clara, CA), then sequenced on an Illumina NextSeq 500 with 2 × 150bp chemistry. Read alignment, variant calling, and annotation were done as outlined for previous FORGE and Care4Rare Canada projects. Average coverage for the exomes was 182×, 190×, and 244×, and >97% of consensus coding sequence exons in all exomes were covered at >10×.

**Sanger Sequencing**

The variants identified by WGS and WES were confirmed by Sanger sequencing. The region was amplified using the following primers: 5′-AGGAGGATCAGGGATGGGAG-3′ and 5′-CTCTCATAT CCTGCTCCCCA-3′ in Family 1 and 5′-CITGCGCTCTAT GTTGGAC-3′ and 5′-AGAGTAGTACGCGGCAACTC-3′ in Family 2. The promoter region of PDXK was amplified using the following primers: 5′-GGCGTTTCTCCGTGGATC-3′ and 5′-ACG CCTCCTTCTGACCT-3′. Genomic DNA was extracted from dried blood spots (DBSs) from affected individuals from Family 1 and controls using the QIAamp DNA Micro Kit (Qiagen, Valencia, CA) and from blood from Family 2. Sequencing reactions were performed using the BigDye Terminator 1.1 system (Thermo Fisher Scientific, Paisley, UK) followed by sequencing using an ABI DNA Analyser (Thermo Fisher Scientific). Electropherograms were analyzed using the Sequencer software package (Gene Codes Corporation, Ann Arbor, MI).

**Gene Co-expression Analysis**

We generated gene co-expression networks for central nervous system (CNS) and PNS tissue-specific transcriptomic data generated by the Genotype-Tissue Expression Consortium (GTExv6 gene expression data are accessible from https://www.gtexportal.org/). We analyzed each of the tissue sample sets separately by filtering genes on the basis of an Reads Per Kilobase Million (RPKM) >0.1 (observed in >80% of the samples for a given tissue). We then corrected for batch effects, age, gender, and RNA integrity numbers (RIN) using ComBat. Finally, we used the residuals of these linear regression models to construct gene co-expression networks for each tissue using WGCNA and postprocessing with k-means to improve gene clustering. Gene modules were functionally annotated with gProfiler R package using the Gene Ontology (GO) database without electronic inferred annotations and accounting for multiple testing with gSCS. The top-down plot figure was done with Cytoscape 3.5.1. We initially selected the 50 most connected genes within the black module, for the coexpression network of tibial nerve, based on adjacency values. We included all connections between genes. Then we filtered out the connections with weight <0.05. Finally, we applied a Kamada–Kawai layout algorithm to the remaining genes and edges to dispose them spatially at the canvas so their relative positions reflected similarity in connections.

**Plasmid Constructs**

The human PDXK wild-type (WT) and PDXK mutant p.Ala228Thr cDNA were purchased from Invitrogen (Carlsbad, CA) and cloned into the plasmid pGEX6p-1 (GE Healthcare, Piscataway, NJ) using the sites BamHI and NotI (New England Biolabs, Ipswich, MA).

**Protein Purification**

*E. coli* strain Rosetta2 (DE3; Novagen, Darmstadt, Germany) was transformed with the plasmids pGEX6p-1-PDXK and pGEX6p-1-PDXK p.Ala228Thr and grown at 37°C to an OD600nm of 0.8 and then induced with 0.5mM isopropyl β-D-1-thiogalactopyranoside for 4 hours. Cells were lysed, using a cell disruptor (Avestin, Ottawa, Ontario, Canada), in lysis buffer containing 25mM hydroxyethylpiperazine ethane sulfonic acid (HEPES), pH 7.4, 400mM KCl, 4% Triton X-100, 1mM 1,4-dithiothreitol (Sigma-Aldrich, Gillingham, UK), and SIGMAFAST Protease Inhibitor Cocktail Tablets, EDTA-Free (Sigma-Aldrich). The samples were supplemented with 5% polyethyleneimine (Sigma-Aldrich) and clarified using a 45Ti rotor (Beckman Coulter, Brea, CA) at 100,000 × g for 30 minutes prior to incubation overnight at 4°C with glutathione agarose beads (Pierce, Rockford, IL) previously washed in lysis buffer containing 1% Triton X-100. The glutathione agarose beads containing the immobilized proteins GST-PDXK and PDXK p.Ala228Thr were subsequently washed in lysis buffer containing 1% Triton X-100 and incubated with 10mg/ml DNAse and RNase (Sigma-Aldrich) for 1 hour at room temperature (RT), prior to washing in lysis buffer containing no Triton X-100. The GST tag was cleaved using 100U of PreScission Protease (GE Healthcare) for 2 hours at RT. After elution, the proteins were dialyzed in 25mM HEPES, pH 7.4, 100mM KCl, 1mM 1,4-dithiothreitol overnight at 4°C. Protein concentration was determined using the BCA Protein Assay Kit (Thermo Fisher Scientific).

**Circular Dichroism**

To investigate conformational differences between WT PDXK and the disease-linked mutant p.Ala228Thr, circular dichroism (CD) analyses were performed. The extinction coefficients of both proteins (ε230 = 31,400M–1cm–1) were calculated using the ProtParam tool within the ExPASy Portal. Proteins were prepared as described above and dialyzed overnight at 4°C in 30mM tris, pH 7, 100mM KCl, 3mM MgCl2, 0.5mM tris (2-carboxyethyl)phosphine (TCEP). Samples were concentrated to 0.2mg/ml using Amicon Ultra 0.5ml centrifugal filters (Merck, Darmstadt, Germany).

Ultraviolet (UV) and CD spectra were acquired on the Applied Photophysics (Leatherhead, UK) Chirascan Plus spectrometer across the 400 to 190nm wavelength region. Ten-millimeter (400–230nm) and 0.5mm (260–190nm) strain-free rectangular cells were employed. The instrument was flushed continuously with pure evaporated nitrogen throughout the experiment. The following parameters were employed: 2nm spectral bandwidth, 1nm step size, and 1-second instrument measurement time-per-point. All spectra were acquired at 25°C, and buffer baseline corrected. Light scattering correction was
applied on the UV spectra of both proteins using the Chirascan Pro-Data Software (Applied Photophysics). Where possible, spectra were smoothed with a window factor of 4 using the Savitzky–Golay method for better presentation.

The far-UV CD spectra of the 2 proteins were corrected for concentration and pathlength and expressed in terms of $\Delta \varepsilon$ (M$^{-1}$cm$^{-1}$) per amino acid residue. Protein secondary structure content was assessed using the Principle Component Regression method based on 16 known protein structures embedded in the PLSPlus/IQ routine in GRAMS32 AI software (Galactic Software, Salem, MA).

**Isothermal Titration Calorimetry**

The affinity of a nonhydrolyzable analogue of adenosine triphosphate (ATP), called adenosine 5'-3-thiotriphosphate tetralithium salt (ATPyS), for both PDXK WT and p.Ala228Thr mutant, was examined using a MicroCal Isothermal Titration Calorimeter ITC200 instrument (Malvern Panalytical, Malvern, UK). Both proteins were prepared as described above in 30mM tris, pH 7.0, 100mM KCl, 3mM MgCl$_2$, 0.5mM TCEP (isothermal titration calorimetry [ITC] buffer). An initial 0.2M stock of ATPyS was prepared in water and diluted with ITC buffer to a final concentration of 250μM. The concentration was adjusted using the UV absorbance at 295 nm, and the extinction coefficient of 15.4μM$^{-1}$cm$^{-1}$ suggested by the ligand provider (Jena Bioscience, Jena, Germany).

The experiments were conducted at 25°C following standard procedures as described previously. For microliters of 250μM ATPyS was titrated into a 330μl solution of 25μM WT or p.Ala228Thr protein. The ITC experiment consisted of 20μl injections with a spacing of 180 seconds. Heat produced by titrant dilution was obtained by a control experiment, titrating into buffer alone, under the same conditions.

The MicroCal-Origin 7.0 software package was used to fit the integrated heat data obtained for the titrations corrected for heats of dilution, using a nonlinear least-squares minimization algorithm based on an independent binding sites model. ΔH (reaction enthalpy change in kcal/mol), Ka (1/Kd; equilibrium association constant per molar), and n (molar ratio of the proteins in the complex) were the fitting parameters. The reaction entropy was calculated using the relationships $\Delta G = \Delta H - T\Delta S = -RT\ln(1/Kd)$.

**Western Blotting**

Fibroblast samples from affected, carriers, and healthy people were obtained with consent. After reaching 90% confluence, the cultured cells were harvested and lysed in ice-cold lysis radioimmunoprecipitation assay buffer containing 50mM tris-HCl pH 8.0, 150mM NaCl, 0.5% DOC, 0.1% Triton X-100, 0.1% sodium dodecyl sulphate (SDS), and SIGMAFAST Protease Inhibitor Cocktail for 30 minutes. The lysate was then centrifuged at 16,000 x g at 4°C, and the supernatant transferred to a new tube. The protein concentration was measured using the BCA Protein Assay Kit (Thermo Fisher Scientific) according to manufacturer’s protocol. Equal amounts of protein (30μg) from affected and healthy individuals were separated on a 10% SDS–polyacrylamide gel electrophoresis gel (NuPAGE, Invitrogen) and transferred onto an Immobilon membrane (Millipore, Billerica, MA). After blocking the membrane with 2% fat-free milk in phosphate-buffered saline–Tween for 1 hour at RT, the membrane was incubated with anti-PDXK antibody (Abcam, Cambridge, UK; 38208, 1:500) for 1 hour at RT. The membranes were subsequently washed and incubated with horseradish peroxidase–conjugated goat antirabbit IgG (Bio-Rad Laboratories, Hercules, CA; 17210, 1:5,000) for 1 hour at RT. Blots were developed using ECL Prime (GE Healthcare), visualized via a ChemiDoc Touch Imaging System (Bio-Rad Laboratories), and analyzed using Image Lab 5.2 software (Bio-Rad Laboratories). For the quantification, the signal intensity of each band corresponding to the PDXK WT and PDXK p.Ala228Thr mutant protein was normalized to the signal intensity of the corresponding reversible Ponceau staining as a reliable loading control. The amount of PDXK WT and mutant protein was expressed as a percentage of the control sample. The experiment was repeated 3 times.

**Measurement of Pyridoxal Kinase Activity**

The pyridoxal (PL) kinase activity present in DBSs was determined by adapting the protocol used by Wilson et al., with the following modifications. Three-millimeter discs punched from DBSs were incubated for 10 minutes at 37°C with shaking at 300rpm in a reaction buffer containing 20mol/l potassium phosphate adjusted to pH 6.1, 10μmol/l PL, and 300μmol/l MgATP (all purchased from Sigma-Aldrich). The same method was adapted for the investigation of activity of the purified recombinant PL kinase. Five microliters of 20ng/μl purified PL kinase protein lyasate was placed in an ultraperformance liquid chromatography (UPLC) 96-well sample plate (Waters, Ekktrée, UK). Subsequently, 115μl of reaction buffer containing potassium phosphate (pH 6.1), PL, and MgATP was added to create a final concentration of 20mol/l potassium phosphate, with PL and MgATP concentrations adjusted as appropriate. Investigation of PL kinase kinetics as a function of PL concentration used an MgATP concentration of 300μmol/l. A PL concentration of 50μmol/l was utilized for the determination of PL kinase kinetics as a function of MgATP concentration. After reaction buffer addition, the plate was incubated for 10 minutes at 37°C in an Eppendorf (Hamburg, Germany) Thermomixer C with shaking at 300rpm prior to addition of 120μl of a reaction stop mix identical to that used for the determination of pyridoxamine (PM) 5'-phosphate (PMP) oxidase activity from DBSs.

DBSs were collected from 4 affected cases and 22 controls (age = 15–92 years). PL kinase activity of the recombinant proteins and that of DBSs were determined by using UPLC–tandem mass spectrometry (MS/MS) to determine the formation of PL 5'-phosphate (PLP) after incubation of these with the enzyme substrate PL and expressed as μmol PLP 1h$^{-1}$ from recombinant protein and as pmol PLP (3mm DBS) 1h$^{-1}$ from DBSs.

Data collection and statistical analysis were performed using Waters MassLynx and GraphPad (San Diego, CA) Prism 6.0 software packages.
**UPLC-MS/MS Measurement of the B₆ Vitamers**

B₆ vitamer concentrations (pyridoxine, PL, PLP, PM, PMP) and pyridoxic acid were quantified by UPLC-MS/MS using stable isotope-labeled internal standards.³⁵ ³⁶

**High-Performance Liquid Chromatograph Measurement of B₆ Vitamers**

PL phosphate was measured by high-performance liquid chromatography (HPLC) with fluorescence detection using a Chromsystems Instruments & Chemicals (Gräfelfing, Germany) kit as described previously.³⁵ Pyridoxic acid concentration was calculated using an external calibration standard.

**PLP Measurements before and after PLP Replacement**

Plasma PLP concentrations before and after PLP replacement were measured using 2 independent HPLC and UPLC-MS/MS methods as described above to validate the results. The difference between groups was tested with the use of a 1-way analysis of variance test, followed by Tukey–Kramer test.

**Measurement of Neurofilament Light Chain Concentration**

Plasma neurofilament light chain (NFL) concentration was measured by digital enzyme-linked immunosorbent assay using the commercially available NF-Light kit on a single molecule array (Simoa) platform (HD-1 Analyzer) according to instructions by the manufacturer (Quanterix, Lexington, MA). All measurements were performed in duplicates, in 1 round of experiments using 1 batch of reagents. Intra-assay coefficients of variation were <10%.

**Results**

Here, we describe 5 affected cases from 2 unrelated families (Fig 1A) with early, childhood onset, sensorimotor, length-dependent, predominantly axonal polyneuropathy and adult onset optic atrophy (Table 1).

**Disease Characterization**

Family 1 is of Cypriot origin (affected individuals F1-II-5, currently 80 years old, F1-II-6, currently 75 years old, and F1-II-9, died at 71 years old from an unrelated medical condition). Three affected siblings presented with difficulties running, distal wasting, and weakness in the lower limbs before the age of 10 years, progression to upper limbs by the age of 12 years, and onset of visual loss in their 40s. The disease progressed, and at the age of 79 years, case F1-II-5 required the assistance of 2 people for sit-to-stand, mobilizing with the assistance of 2 plus Zimmer frames for transfers and short distances, and using a wheelchair for all other activities. Neurogenic-type pain, particularly in the lower limbs, was difficult to control despite multiple lines of symptomatic treatment. Case F1-II-6 (age = 74 years) could only walk short distances with assistance from 1 individual. Neurological examination showed bilateral distal wasting from mid forearm and below the knee, with wasting of the thenar, hypothenar, and intrinsic muscle of the hands and distal lower limb muscles, pes cavus, and absent reflexes throughout in both patients (see Fig 1B). The MRC muscle score was 44 (of 60) in cases F1-II-5 and F1-II-6. Strikingly, both patients had severely reduced sensation in all limbs and in all modalities, with profound joint position sense loss up to the knee and vibration loss up to the iliac crest in both cases (see Table 1 and Supplementary Table 1). Vision was reduced (including color vision) and fundoscopy confirmed bilateral optic atrophy in both cases (see Fig 1C). The remaining cranial nerve examination was normal. The third sibling F1-II-9 (now deceased) was similarly affected.

Family 2 is of Scottish (paternal) and Italian (maternal) origin with 2 affected sisters (Patient F2-II-1, currently 31 years old; Patient F2-II-2, currently 29 years old) who presented with childhood onset of a mild decrease in balance, which progressed to difficulty with running in adolescence, and then to mild distal hand weakness in the 3rd decade. Both sisters ambulate independently without aids, and neither has symptoms of visual loss at their present ages. Neurological examination showed mild symmetrical distal wasting in hand intrinsic muscles and prominent distal lower limb muscle wasting with pes cavus and mild hammertoes. There was mild distal weakness in hand intrinsic muscles, and in the upper limbs, and in the toes and distal lower legs and feet. The MRC scores were 56 (F2-II-1) and 54 (F2-II-2). Both sisters were areflexic, with reduced pinprick sense in the lower extremities and decreased vibration sense to the knees (see Table 1 and Supplementary Table 1). Color vision was reduced. Fundoscopy confirmed bilateral optic atrophy in both cases; the remainder of the cranial nerve examination was normal.

Nerve conduction studies (NCS) in all affected cases revealed a progressive, length-dependent, sensorimotor, predominantly axonal neuropathy (Supplementary Table 2). Early in the disease (as seen in Family 2), the NCS show a predominantly axonal neuropathy affecting mainly the lower limbs with absent sensory responses. Mild conduction slowing with normal motor responses was recorded in the upper limbs. The natural history of this disorder in the older individuals from Family 1 (F1-II-5 and F1-II-6) with data recorded over 20 years of disease duration suggests that untreated, the neuropathy progresses in a length-dependent fashion and is characterized by prolonged distal motor latency (DML), severe reduction of compound muscle action potentials (CMAPs), and absent sensory responses affecting upper and lower limbs, as confirmed on NCS. Interestingly, both patients present with significantly more prolonged DML in the median compared to ulnar nerve.
Although it is possible that this is secondary to a longstanding median compression lesion at the wrist, we cannot exclude that this finding is disease-related. The reduced CMAPs were associated with reduction of conduction velocities in the intermediate range mainly in the median nerves. Electromyography in all cases tested was consistent with chronic denervation with no myopathic changes.

To further ascertain the exact type of neuropathy, we performed a nerve biopsy in case F1-II-6. It showed diffuse and severe depletion of both small and large myelinated axons with regenerating clusters but no "onion..."
| Phenotype/Case | F1-II-5 | F1-II-6 | F1-II-8 | F2-II-1 | F2-II-2 |
|---------------|--------|--------|--------|--------|--------|
| Demographics  |        |        |        |        |        |
| Gender        | M      | F      | F      | F      | F      |
| Age at examination, yr | 79 | 74 | Died at 71 years from leukemia | 31 | 29 |
| Age at onset, yr | 7 | 9 | 7 | 5 | 2 |
| Progression   |        |        |        |        |        |
| Symptoms at onset | Lower limb weakness and wasting | Lower limb weakness and wasting | Lower limb weakness and wasting | Lower limb weakness and wasting | Lower limb weakness and wasting |
| Upper limb weakness | At 12 years | At 12 years | At 17 years | 20s | 20s |
| Optic atrophy  | At 40 years | At 47 years | At 50 years | 31 | 29 |
| Neurological examination | | | | | |
| Fundoscopy    | Pale optic discs bilaterally | Pale optic discs bilaterally | NA | Mild optic disc pallor bilaterally | Mild optic disc pallor bilaterally |
| Other cranial nerves | Normal | Normal | NA | Normal | Normal |
| Skeletal deformities | Pes cavus, hammer toes, clawing of hands | Pes cavus, hammer toes | NA | Normal | Normal |
| Power         | Severe weakness of dorsiflexion/plantar flexion, long finger extensors and intrinsic muscles of the hands | Severe weakness of dorsiflexion/plantar flexion, long finger extensors and intrinsic muscles of the hands | NA | Moderate-severe weakness of dorsiflexion/plantar flexion, long finger extensors and mild weakness of intrinsic muscles of the hands | Moderate-severe weakness of dorsiflexion/plantar flexion, long finger extensors and mild weakness of intrinsic muscles of the hands |
| MRC power score | 44 | 44 | NA | 56 | 54 |
| Reflexes      | Absent throughout; mute plantar responses | Absent throughout; mute plantar responses | NA | Absent; mute plantar responses | Absent; mute plantar responses |
| Sensation     | Reduced pain to midcalf and wrists | Reduced pain to ankles and elbows | Reduced pain to ankles | Reduced pain to upper calf and base of fingers | Reduced pain to upper calf and base of fingers |
| Romberg sign  | Present | Present | Present | Present | Present |
| Coordination  | Normal | Normal | Normal | Normal | Normal |
| Visual acuities | Unable to count fingers | 6/12 in both eyes | NA | NA | NA |
| Color vision  | Unable to distinguish any color | Groudy impaired, 3/17 Ishihara plates | NA | 15/17 Ishihara plates | 3/17 Ishihara plates |
| Peripheral vision | Normal | Normal | Normal | Normal | Normal |
| Cognitive function | Normal | Normal | Normal | Normal | Normal |
| Seizures      | Absent | Absent | Absent | Absent | Absent |
| Investigation results | | | | | |
| MRI head      | Normal | Normal | Normal | NA | NA |
| Optic nerve and chiasm CT | Normal | Normal | NA | NA | NA |
| VEPs          | Severely attenuated bilaterally with anomalous waveform on flash VEPs | Severely attenuated bilaterally with anomalous waveform on flash VEPs | NA | NA | NA |
| Somatosensory evoked potentials | NA | Poorly formed due to severe polyneuropathy | NA | NA | NA |
| Nerve conduction study | Severe sensorimotor axonal neuropathy | Severe sensorimotor axonal neuropathy | Severe sensorimotor axonal neuropathy | Severe sensorimotor axonal neuropathy | Severe sensorimotor axonal neuropathy |
| Electromyography | Chronic denervation in a length-dependent pattern; no myopathic changes | Chronic denervation in a length-dependent pattern; no myopathic changes | Chronic denervation in a length-dependent pattern; no myopathic changes | Chronic denervation in a length-dependent pattern; no myopathic changes | Chronic denervation in a length-dependent pattern; no myopathic changes |
| Renal function | Normal | Normal | Normal | Normal | Normal |
| Biochemical profile | | | | | |
| Liver function | Normal | Normal | Normal | Normal | Normal |
| GI tract      | Normal colonoscopy | Normal colonoscopy | Normal | Normal | Normal |
| Plasma amino acids | Normal | NA | Normal | NA | NA |
| Vitamin B12, normal range = 67–265nmol/L | NA | 176 | 149 | 183 | 211 |
| Plasma amino acids tested: methionine, isoleucine, leucine, tyrosine, phenylalanine, ornithine, lysine, histidine, and arginine.

CT = computed tomography; F = female; GI = gastrointestinal; M = male; MRC = Medical Research Council; MRI = magnetic resonance imaging; NA = not available; VEP = visual evoked potential.
bulbs.” This confirms a longstanding axonopathy with no overt demyelination (see Fig 1D).

All cases reported here had pale optic discs on clinical examination. We performed visual evoked potentials (VEPs) to further describe the involvement of the optic nerve in the course of disease. We found that VEPs in Case F1-II-6 had decreased considerably in amplitude from 7.9 and 7.4 in each eye to 1.8 and 1.9 over a 20-year period, indicating axonal loss and bilateral visual pathway involvement (see Supplementary Table 2). Similar results were confirmed in case F1-II-5. Family 2 has not been investigated with VEPs.

Identification of Disease-Causing PDXK Variants

Previous extensive genetic, metabolic, and mitochondrial investigations failed to identify any acquired or known causes of inherited polyneuropathy in all cases. WGS and homozygosity mapping in Family 1 revealed a shared homozygous region from 42 to 45Mb on chromosome 21. This region included a shared homozygous variant in the 2 affected siblings that was carried by unaffected family members in heterozygous state: PDXK (NM_003681) c.682G>A (p.Ala228Thr). This variant was absent in 150 Cypriot controls. The p.Ala228Thr is absent from the gnomAD database in homozygous state though reported in 5 heterozygous European alleles from 250146 (total allele frequency of 0.00002793).23

Independently, homozygosity mapping in Family 2 revealed a 1.35Mb region of homozygosity on chromosome 21. WES in Family 2 identified a shared homozygous variant in this region, the c.659G>A (p.Arg220Gln) in the 2 affected sisters, carried by each parent. This variant is absent from gnomAD in homozygous state and reported in 21.1% of helical and strand content, respectively, were estimated in case-derived transcriptomic data (see Fig 2B).38 This showed different co-expression profiles in brain compared to peripheral nerve tissue. Weighted gene co-expression analysis demonstrated that in 9 of 11 brain regions analyzed, PDXK was coexpressed with genes relating to synaptic transmission and neuronal identity, whereas co-expression data from the tibial nerve showed that PDXK was located within a module enriched for genes involved in oxidation-reduction processes (GO: 055114; false discovery rate−corrected $p = 2.36 \times 10^{-5}$; see Fig 2C and Supplementary Table 3).

Assessment of the Impact of PDXK Variants on Protein Structure and Enzymatic Activity

Arginine 220 and alanine 228 are evolutionarily conserved amino acids in eutherians (see Fig 2D). Arginine 220 is located in the β9 in the vicinity of the ATP-binding site.39 Alanine 228 is located in the ATP-binding pocket of PL kinase (see Fig 2E), a critical region of the kinase, part of the loop region between strand β11 and helix α7 that rotates away from the active site, allowing binding of the ATP adenine.39 Although the Ala228 side-chain does not contact ATP directly, its backbone is involved in recruiting ATP to the catalytic pocket.

Far-UV CD spectra analysis of both WT and p.Ala228Thr revealed that, whereas both proteins were folded, there were small but significant differences in their secondary structure (Fig 3A). WT PDXK had an estimated α-helix and β-strand content of 31.2% and 19%, respectively, similar to previous X-ray studies (PDB accession number 3KEU).40 In the mutant protein, 26.0% and 21.1% of helical and strand content, respectively, were estimated, suggesting altered protein conformation. Furthermore, near-UV CD analysis revealed distinctive CD fingerprints for WT and p.Ala228Thr proteins, indicating conformational rearrangements of aromatic side-chains around the catalytic pocket, most likely hindering the enzyme’s ability to bind ATP.

We performed ITC confirming that p.Ala228Thr affected the ATP-binding ability of PDXK. Interaction of WT PDXK with the nonhydrolyzable ATP analogue ATPγS generated well-interpolated, sigmoid-shaped curves, based on an independent and equivalent binding site model centered on 1:1 stoichiometry. By contrast, no association between p.Ala228Thr and ATPγS was observed (see Fig 3B).

Whereas no reduction in the expression of the mutant protein (p.Ala228Thr) compared to WT PDXK in case-derived fibroblasts was evident (see Fig 3C), kinetic studies with human recombinant PDXK showed an approximate 2-fold increase in the $K_m$ of the p.Ala228Thr mutant protein (31.9μmol/l) for PL compared to WT protein (14.5μmol/l) and a reduction in the
V_{\text{max}} \text{ of the mutant protein (p.Ala228Thr) to 0.95\,\mu\text{mol/l/h compared to 2.52\,\mu\text{mol/l/h for WT PDXK.}}
Upon variation of MgATP concentration, sigmoidal kinetics were observed (WT: k_{0.5} = 53.4\,\mu\text{mol/l,}
V_{\text{max}} = 16.8\,\mu\text{mol/h; p.Ala228Thr: k}_{0.5} = 174.4\,\mu\text{mol/l,}
V_{\text{max}} = 6.3\,\mu\text{mol/h), indicating cooperative binding between the 2 substrates, ATP and PL (see Fig 3D, E).}

Analysis of erythrocyte PDXK activity from cases carrying the p.Arg220Gln and p.Ala228Thr substitutions further confirmed the deleterious effect of these variants on PL kinase activity (see Fig 3F). The PDXK activity in the 4 affected cases was reduced (1.1 and 0.8pmol DBS^{-1}h^{-1} in Family 1 and 1.19 and 0.5pmol DBS^{-1}h^{-1} in Family 2) relative to controls (2.6–14.7pmol DBS^{-1}h^{-1}, mean = 8.0).
FIGURE 3: PDXK mutations lead to reduced pyridoxal (PL) kinase enzymatic activity and low PL 5'-phosphate (PLP). (A) Circular dichroism analyses of recombinant PDXK wild-type (WT) and p.Ala228Thr mutant proteins. The left and right panels show the normalized far-ultraviolet (UV) and near-UV spectra of the 2 proteins, respectively. A clear difference in secondary structure content between the 2 proteins is observed from the far-UV experiment. CD = circular dichroism. (B) Analysis of the interaction of nonhydrolyzable analogue adenosine 5'-(3-thiotriphosphate) tetralithium salt (ATPγS) with PDXK WT and p.Ala228Thr mutant proteins by isothermal titration calorimetry. The left panel shows the titration of ATPγS (250 μM) into a PDXK WT solution (25 μM). The thermogram shows that the interaction was entropically and enthalpically favored, with $\Delta H = -3.27 \pm 0.42 \text{kcal/mol}$, $\Delta S = -4.42 \pm 0.48 \text{kcal/mol}$, $K_D = 2.33 \pm 0.25 \mu M$, and $\Delta G = -7.69 \pm 0.06 \text{kcal/mol}$. The stoichiometry was 0.80 μM, indicating that each molecule of PDXK binds to 1 molecule of ATPγS. The right panel reports the titration of ATPγS (250 μM) into a PDXK p.Ala228Thr solution (25 μM). The experiment showed no interaction under the experimental conditions tested, suggesting that the mutation affected the ability of the kinase to bind the analogue substrate ATPγS. (C) Western blot analysis shows normal expression of the PDXK protein in cases compared to controls. (D) Activity of recombinant WT and p.Ala228Thr PL kinase protein measured as PLP formation. Conditions: 0–100 μmol/l PL, 300 μmol/l MgATP, 20 mmol/l potassium phosphate, pH 7.0, 37°C, 10-minute incubation with 100 ng recombinant protein. Points displayed are a mean of 3 repeats. $V_{max}$: WT = 2.17 μmol/l/h, p.Ala228Thr = 2.52 μmol/l/h, $V_{max}$: WT = 14.53 μmol/l, p.Ala228Thr = 31.93 μmol/l. MUT = mutant. (E) Kinetics of recombinant WT and p.Ala228Thr PL kinase protein upon variation of PL concentration. PL kinase activity of recombinant human WT and p.Ala228Thr PDXK protein is measured as PLP formed after incubation with the substrate PL. Incubations were performed in the presence of variable concentrations of MgATP (0–500 μmol/l) and 50 μmol/l PL. Kinetics were sigmoidal, and parameters established were as follows. WT: $k_{0.5} = 53.4 \mu M$, $V_{max} = 16.8 \mu M$/h; p.Ala228Thr: $k_{0.5} = 174.4 \mu M$, $V_{max} = 6.3 \mu M$/h. Results indicate a dramatic reduction in the catalytic efficiency of the p.Ala228Thr PDXK protein. $n = 3$ at each data point. (F) Erythrocyte PDXK activity in dried blood spots (DBSs) from cases homozygous for the p.Ala228Thr and p.Arg220Gln versus controls (age = 15–92 years). Patients homozygous for p.Ala228Thr and p.Arg220Gln have lower activity than all controls. Activity measured as PLP formed after incubation of a 3mm DBS punch with PL. Each sample was analyzed in duplicate, and the mean is shown. There was no correlation of PDXK activity with age. (G, H) Comparison of plasma PLP concentrations (retention time = 2.78/2.84 minutes) in control (red) and cases carrying the PDXK mutation (blue) p.Arg220Gln (G) and p.Ala228Thr (H) show a significant reduction of PLP in the case samples (7.8 and 9 nmol/l, respectively) versus control (control range = 25–75 nmol/l).
Heterozygote activity \((n = 1, \text{F-1-III-1})\) was within normal range \((4.9 \text{pmol DBS}^{-1} \text{h}^{-1})\). No correlation of DBS PL kinase activity with age was identified in controls. When segregated according to gender, a difference was identified between control males and females \((9 \text{ males: mean } = 9.2, \text{ range } = 5.9–14.7; 12 \text{ females: mean } = 7.1, \text{ range } = 2.6–11.7)\) that approached significance \((p = 0.1099 \text{ by untailed t test})\). An insertion located in the erythroid-specific promoter region of PL kinase has been associated with reduced erythrocyte PDXK activity.\(^{21}\) Sequencing this region showed that the reduced activity of all affected individuals was not attributable to this insertion (Supplementary Table 4).\(^{42}\)

Finally, we measured plasma PLP concentrations in all affected individuals. Plasma PLP was greatly reduced in cases carrying p.Arg220Gln and p.Ala228Thr compared to age-matched controls (see Fig 3G, H).

To quantify the axonal damage, we measured the NFL protein in plasma from patients carrying PDXK mutations. NFL, a major axonal cytoskeletal protein, is released into cerebrospinal fluid and blood during axonal breakdown,\(^{43}\) providing a dynamic biomarker of axonal damage in neurodegenerative disorders, including inherited neuropathies.\(^{44}\) Both p.Arg220Gln and p.Ala228Thr variants were associated with high NFL (Case F2-II-1, 49.6pg/ml; Case F1-II-5, 25pg/ml), within the range of NFL concentrations published for other axonal CMTs.\(^{44}\)

**Supplementation with PLP Is Associated with Biochemical Correction and Clinical Improvement**

Based on clear pathogenicity of the PDXK mutations leading to low PLP, Case F1-II-5 and F1-II-6 were treated with oral PLP. High doses of pyridoxine are known to be neurotoxic\(^{45}\); therefore, we used PLP, which is hydrolyzed to PL, to increase the upstream PL and force the flux with an enzyme with a high \(K_m\) based on our results with recombinant p.Ala228Thr protein kinetics. We initiated PLP replacement with 50mg/day PLP, a dose that was commonly used in adults taking PLP as a vitamin
supplement with no reported side effects. We aimed to achieve plasma PLP concentration above the normal range by PLP replacement, and this was achieved with 50mg/day.

Two weeks after initiating the supplementation, plasma PLP levels had increased considerably without any side effects. PLP levels remained stable at 1, 3, 12, 18, and 24 months on PLP replacement (Fig 4A, B, Table 2), with improvement in symptoms (Supplementary Table 5). Clinical improvement was noted after the first 3 months of PLP replacement in the absence of any additional physiotherapy. Sensory examination and vision remained unchanged in both cases. Main motor improvements were in elbow and wrist flexion/extension and hip and knee flexion/extension, with modest improvement in finger extension (see Supplementary Table 5), leading to 8- and 6-point improvement in the MRC sum score at 18 months on PLP replacement in Case F1-II-5 and Case F1-II-6, respectively. At last examination, 24 months on PLP replacement, patients were ambulating with high-stepping gait, unaided (Supplementary Video). Furthermore, neuropathic pain has completely subsided; neither case requires additional analgesic medication.

These results led to an improvement of 5 points in the NIS score in Case F1-II-5 and 4 points in Case F1-II-6 based on motor, pain, and fatigue subsets. Similarly, the CMT neuropathy score on PLP replacement improved by 4 points and 2 points in Cases F1-II-5 and F1-II-6, respectively (see Supplementary Table 5).

Electrophysiological studies performed 18 months after PLP supplementation have demonstrated no disease progression, with no significant changes in the motor and sensory responses. Whereas the distal motor responses were not significantly different from previous studies, the electromyogram of the proximal muscles showed motor unit recruitment and interference pattern improvement in the proximal muscles (quadriceps and tibialis anterior muscles, biceps and flexor carpi radialis) with activity present in the tibialis anterior that was unrecordable on previous studies.

The clinical response to PLP replacement was further supported by measuring NFL protein in plasma before and after PLP replacement from the PDXK cases in Family 1. A reduction of NFL levels to the normal control range (median = 13pg/ml, range = 11–18pg/ml; see Fig 4C) occurred upon restoration of PLP levels and continued to improve with PLP supplementation. At 24 months with PLP supplementation, the NFL level was 2.5 times lower than before supplementation.

### Discussion

Here, we show that biallelic mutations in PDXK lead to peripheral neuropathy with optic atrophy. We have shown previously that several inherited disorders affecting B₆ vitamer metabolism, or resulting in inactivation of PLP, are implicated in neurological conditions. These include PNPO (Mendelian Inheritance in Man [MIM]: 603287), ALDH7A1 (MIM: 266100), and PROSC deficiency.

#### TABLE 2. Plasma B₆ Vitamer Profiles for Patients with PROSC, PNPO, and PDXK Deficiency Supplemented with PLP

| Age       | B₆ (dose) | PLP | PL | PA | PN | PNP | PMP | PM |
|-----------|-----------|-----|----|----|----|-----|-----|----|
| Control rangeᵃ | 4.3–16 years | None | 46–321 | 5–18 | 16–139 | nd–0.6 | nd | nd–9 | nd |
| PROSCᵇ | 3 years | PLP (70mg QDS) | 2,769ᵇ | 796ᵇ | 2,043ᵇ | 0.5 | nd | nd | nd |
| PROSCᵇ | 6 months | PLP (45mg/kg/day) | 2,166ᵇ | 1,695ᵇ | 700ᵇ | nd | nd | nd | nd |
| PNPOᵈ | 2 years | PLP (30mg/kg/d) | 580ᵈ | 427ᵈ | 793ᵈ | 575ᵈ | 43ᵈ | 18ᵈ | 193ᵈ |
| PNPOᵈ | 10 years | PLP (30mg/kg/d) | 633ᵈ | 5,798ᵈ | 7,926ᵈ | 599ᵈ | 77ᵈ | 101ᵈ | 2,731ᵈ |
| PDXKᵉ | 79 years | PLP (50mg/d) | 415ᵉ | 1,550ᵉ | 1,617ᵉ | 31ᵉ | nd | nd | nd |

All units are given as nmol/L, except for PNP, which is given in concentration units.

ᵃControl range as described in Footitt et al⁴⁶.
ᵇData from Darin et al⁴⁷.
ᶜValues outside the reference range.
ᵈData from Footitt et al 2013. PLP doses received by PROSC and PNPO patients were larger than the doses given to the pyruvate kinase–deficient patients (II-5 and II-6).
ᵉValues at 12 months on treatment with PLP.
⁹nd = not detected; PA = 4-pyridoxic acid; PL = pyridoxal; PLP = pyridoxal 5’-phosphate; PM = pyridoxamine; PMP = pyridoxamine 5’-phosphate; PN = pyridoxine; PNP = pyridoxine 5’-phosphate; QDS = 4 times per day.
TABLE 3. Vitamin B₆–Related Disease Models

| Model                        | Phenotype                                      | Mechanism                                                                 | Effect on B₆ Pathway                  | Response to PLP Supplementation |
|------------------------------|------------------------------------------------|----------------------------------------------------------------------------|--------------------------------------|----------------------------------|
| Pharmacological models       |                                                |                                                                           |                                      |                                  |
| Isoniazid, L-dopa, gentamicin, D-penicillamine | Axonal peripheral neuropathy⁵                 | Interaction between the reactive aldehyde group of PLP, with nucleophilic acceptors such as amine, hydrazine, hydroxylamine, or sulfdyl groups rendering PLP inactive | Decrease in serum PLP             | Prevents onset of peripheral neuropathy |
| Methylxanthines              | Axonal peripheral neuropathy⁷,⁸                 | Directly inhibit enzymes involved in B₆ metabolism                         |                                      |                                  |
| Carbamazepine, vigabatrin, sodium valproate | Axonal peripheral neuropathy⁴–⁷                | Potent hepatic inducers of cytochrome P450, induce enzymes involved in the catabolism of PLP |                                      |                                  |
| B₆ antivitamin ginkgotoxin (4’-O-methylpyridoxine) | Epileptic convulsions, leg paralysis, and loss of consciousness⁹   | Analogue of PLP; inhibition of pyridoxal kinase by serving as an alternate substrate for the enzyme | Decrease in PLP formation | NA                              |
| Animal models                |                                                |                                                                           |                                      |                                  |
| Mouse                        | Prewaning lethality, MGI: 1351869               | PDXXK knockout                                                           | NA                                   | NA                               |
| Mouse                        | Abnormal walking track patterns, axonal peripheral neuropathy with intact myelin on nerve biopsy⁹ | PLP dietary-deficient                                                   | Decrease in serum PLP             | Complete reversal of symptoms with PLP supplementation |
| Rat                          | Tissue-specific PDXXK response to B₆ deficiency¹⁰,¹¹ | B₆ diet deficient                                                        | PDXXK activity rapidly decreases in the liver, muscle, and plasma compared to the brain | Reversal with PLP supplementation |
| Caenorhabditis elegans       | Sensory–motor integration deficit in neuromuscular behavior¹² | PDXXK knockout                                                           | NA                                   | Rescued by expression of a WT transgene |
| Drosophila                   | Motility and eye dysfunction with compromised climbing ability, ommatidial array disruption, and decreased longevity¹³ | PDXXK knockdown by RNA interference                                      | NA                                   | Rescued by expression of a WT transgene |

PLP = pyridoxal 5’-phosphate; MGI = Mouse Genome Informatics; NA = not applicable; WT = wild-type.

(MIM: 604436)⁵¹,⁵² where the lack of PLP in the brain leads to early onset, vitamin B₆-dependent epilepsy refractory to anticonvulsants. In most cases, delayed vitamin B₆ supplementation results in early death or severe handicap in these disorders. The PDXXK activity is tissue-specific under PLP-deficiency conditions. In rats, B₆ deficiency leads to rapid decreases in PDXXK activity in the peripheries (liver, muscle, and plasma), with maintained PDXXK activity and B₆ supply in the brain.⁵³,⁵⁴ This could explain the absence of seizures in humans with PDXXK deficiency who present predominantly with peripheral nerve disease and normal CNS function. Furthermore, our PDXXK co-expression network analyses from peripheral nerve suggest a link to a module enriched for genes involved in oxidation-reduction processes in which mitochondria play a significant role. PLP is a cofactor for several mitochondrial enzymes, but it is not certain how PLP is transported into mitochondria in humans; in yeast, there is evidence for involvement of Mtm1p.⁵⁵ Although we acknowledge that coexpression networks generated from bulk RNA-seq of peripheral nerves are complex to interpret, there is growing evidence for the presence and importance of axonal RNA transport.⁵⁶–⁵⁸ Thus, our findings are interesting given the strong phenotypic similarity (optic atrophy and axonal polineuropathy) to other disorders.
caused by mutations in genes involved in mitochondrial functions associated with neurodegeneration, such as \( \text{OPA1} \) (MIM: 605290), \( \text{DHTKD1} \) (MIM: 614984), \( \text{MFN1} \) (MIM: 608506), and \( \text{MFN2} \) (MIM: 608507). Interestingly, within the \( \text{PDXK} \) regulon from the tibial nerve, we found \( \text{DHTKD1} \) already linked to Mendelian disorders and associated with primary peripheral axonal neuropathy.\(^{59,60}\) This suggests that within this regulon there may be other genes involved in neuronal maintenance that could cause a similar disease phenotype to be discovered.

Clinically, the \( \text{PDXK} \)-related neuropathy presented with mixed sensory and motor involvement in early childhood and was associated with optic atrophy later in adulthood. Electrophysiologically, one of the earliest signs of the disease was the absence of sensory responses even when the motor responses were within normal limits, as seen in Family 2. We note that the patients with longstanding disease (Family 1) had significant standing and walking difficulties despite a mild proximal motor deficit most likely due to the combination of severe distal motor involvement with severe proprioceptive impairment, extending to the proximal joints, exacerbated by the severely impaired vision.\(^{61}\)

Based on our collective experience in rare diseases, we acknowledge that it is difficult to implement randomized case–control studies for these patients and the subsequent limitations when designing therapeutic interventions. We note that some clinical improvements seen in the patients reported here, such as pain and fatigue, are susceptible to placebo effects. Furthermore, the severity of the polyneuropathy in the cases that received PLP supplementation and the absence of more proximal nerve conduction studies are limitations for the characterization of the neurophysiological phenotype and response to treatment. However, although we are limited in our ability to fully explain the mechanism of clinical improvement, the supplementation with PLP was associated with normalization of the biochemical profile and improved clinical outcomes in individuals F1-II-5 and F1-II-6. The clinical improvement was supported by the normalization of PLP levels, stable electrophysiological picture, and NFL normalization after treatment. Furthermore, animal models of \( \text{PDXK} \) knockout, \( \text{B}_6 \) dietary deficiency, and pharmacological models all showed significant decreases in circulating PLP levels, with reversible adverse effect(s) on the peripheral/sensory nerve similar to the human phenotype observed here (Table 3). The severe sensory involvement recorded at least 20 years before treatment most likely accounted for the absence of a clinical effect on the sensory function in the 2 patients from Family 1. We are limited in our ability to fully quantify the potential for electrophysiological improvement at this stage in the disease due to severe axonal loss, the short interval of PLP replacement, and the absence of more proximal nerve conduction studies. Therefore, larger studies with treatment started earlier in the disease are warranted to assess the full potential benefit from treatment.

**Conclusions**

We show that biallelic mutations in \( \text{PDXK} \) cause autosomal recessive axonal peripheral polyneuropathy leading to disease via reduced \( \text{PDXK} \) enzymatic activity and low PLP. We show that the biochemical profile in affected individuals can be rescued with PLP supplementation and that this is associated with improvement in clinical scales. Therefore, we recommend that \( \text{PDXK} \) mutations should be screened for in patients with autosomal recessive early onset polyneuropathy, and supplementation with PLP should be started promptly with long-term monitoring of clinical outcomes. Furthermore, \( \text{B}_6 \) vitamers can be linked to diseases through a wide range of processes and genes involved in the vitamin \( \text{B}_6 \) pathway. Therefore, our results can be extended to other neuropathies of different etiology characterized by reduction of PLP levels or reduction of PLP-dependent enzyme activities. Collectively, these data expand the genetic causes of primary polyneuropathy and the heterogeneity of inborn errors of vitamin \( \text{B}_6 \) metabolism and identify PLP as a potential therapeutic target for this disorder.

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Author Contributions
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Potential Conflicts of Interest
Nothing to report.

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