# Compound heterozygosity in *PKLR* gene for a previously unrecognized intronic polymorphism and a rare missense mutation as a novel cause of severe pyruvate kinase deficiency

*Shruti Bagla,[1] Kanta Bhambhani,[1] Manisha Gadgeel,[1] Steven Buck,[1] Jian-Ping Jin[2] and Yaddanapudi Ravindranath[1]*

*[1]Division of Hematology/Oncology, Department of Pediatrics, Wayne State University – School of Medicine, and Children's Hospital of Michigan and [2]Department of Physiology, Wayne State University, School of Medicine, Detroit, MI, USA*

*Correspondence: YADDANAPUDI RAVINDRANATH - ravi@med.wayne.edu*
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**Supplementary Data**

**DNA isolation:** Peripheral blood was collected in EDTA tubes and centrifuged for 5-10 minutes to separate the buffy coat, plasma and RBC fractions. Buffy coat was carefully collected from the top of RBC fraction. DNA was isolated from the buffy coat using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to manufacturer's protocol.

**Polymerase chain reaction and Sanger sequencing:** For each PCR, 10ng of genomic DNA was amplified with 250nM of forward and reverse primers and AmpliTaq Gold 360 PCR 2x Master Mix (Thermo Fisher Scientific, Rockford, IL, USA). Standard PCR experiment was run according to the manufacturer's protocol. Annealing temperature was calculated for each primer pair according to their melting temperature. PCR product was further purified using PCR purification kit (Qiagen, Hilden, Germany). Primer sequences are listed in supplementary table below.

For Sanger sequencing, DNA samples and primers were submitted to the Applied Genomics Technology Center Sequencing Core at Wayne State University.

**RNA isolation:** RNA was isolated from buffy coat using manufacturer's guidelines (RiboPure RNA purification kit, blood; Thermo Fisher Scientific, Rockford, IL, USA).

**qPCR:** For each sample, 500ng of total RNA was reverse transcribed using Superscript IV Reverse Transcriptase Kit (Thermo Fisher Scientific, Rockford, IL, USA). Random hexamers were used for cDNA synthesis, and reaction was carried out in two steps according to the manufacturer's guidelines.

For regular PCR for mRNA sequencing, cDNA was diluted five-fold with nuclease-free water (120ul NFW in 30ul cDNA reaction mix). Primer sequences for *PKLR* around exon 7 were designed with NCBI Primer design tool and ordered from Integrated DNA Technologies. PCR protocol is described above.

For real-time PCR, Taqman gene expression assays were ordered from Applied Biosystems for *PKLR* (Hs00176075_m1) and *GAPDH* (Hs02758991_g1) as the endogenous control. Standard Taqman reaction was run on ABI Q3 instrument.

**Supplementary Table 1: List of primers for *PKLR* gene**

| Primer | Sequence | Assay |
|---|---|---|
| **E7F** | GGA AGG ACA CGG CAT CAA GA | For Sanger sequencing of cDNA |
| **E7R** | CTT GTC TCT GCC CTC GTT GG | |
| **R2** | GAT CTC CGG TCC CTT GGT GTC C | PCR only |
| **R1** | TCC ACG GAG CGA GAT GC | |
| **F2** | TCC TGG AAC ACC TCT GCC TAC TG | |
| **F3** | TTC GGT CAT GGG TCT CTA AGT | |
| **F1** | TGC CTC TGA CAA CCC AAC A | |
| **E2F1** | GCT ATG GCA GAC ACC TTC CT | |
| **I2R1** | CAT ACT CAG CTA GAC TGT CAG CTC | |

## Supplementary Table 2: Proband's genomic sequence within *PKLR* gene

*PKLR* intron 2 sequence:

GTAAGCACTCCCATCCCCCTGCAGCCACACAGGGCCTATTGGTATTTCTTGAGGTGCT[T/A]C
TTCATCTTTTGTCTCCTTTGAGACTTCTCCATGTTTGACACAGTCATT[C/T]ATTTAACAAAA
ATTTGTTGAGCATATAGTAGACAAGATTTTGGGCCCTGGGAGTAGATCAGTGAAAAAAAC
AGACAAAAATCCCTACCCTTGGGGAGCTGACAGTCTAGCTGAGTATGACAATAAATAGTA
AGCACAATAAATTATTTAAAATAAGTAAATTATTTATTCCGTTAGAAAGTGAGGCCGGGC
ATGGTGGCTCATGCCTGTAATCGCAGCATGTTGGGAGGCCCAGGTGGGCAGATCACTTGA
GGTCAGGAGTTCGAGACTAGCCTGACCAACATGGAGAAACCCCGTCTCTACTAAAAATAC
AAAATTAGCCGGGCATGGTGGTGCGTGCCTGCAATCCCAGCTACTCAGGAGGCTGAGGCA
GGAGAATCGCTTGAACCCAGGAGGCGGAGACTGTGGTGAGCCGAGATCACACCATTGCAT
TCCAGCCTGGGCAACAGGAGAAAAACTCCATCTCACAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAGTGGGCTGGGCTCAGTGGCTCATGCCTGTAATCCCAGCACTTTAGGAGGCCAAG
GTTGGCAGATCGCTTGAGCCCAGGAGTTTGAGACCAGTCTGGGTAAATGGCAAAACCCAT
CTCTACAAAAAATACAAAACTTAGTTGAGTGTGGTGGTGCATGCCTGTAGTCCCAGCTAC
TCAGGAGGCTGAGGTGGGAGGATCACTTAAGCCCAGGAGGTCACGGCTGCAGTGAGTCAT
GATCGAGCCACTGTACTCCAGCCTAGGTGACAGACGAGACCCTAGAGAGAAAGAGAGAAA
GAAAGAAAGAAGGAAAGAAAGAAAGAAAGAGAGAGAGAAAGAAGGAAGGAAGGAAGGAGG
GAGGGAGGGAGGGAAGGAAGGAAGGAAAGAAAGCAAGCAGGCAAGAAAGAAAGAAAGAAA
AGAAAGAAGGAAGGAAGGAAGGAAGGAAAGAAAGAAAGAAAGAGAAAGAAAGAAAGAAAG
AAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAG
AAAGGAGTGAAAGTTGGCCGGGCATGGTGGCTCTTGCCTATAATCCCAGCACTTTGGGAG
GCTGAGGCAGGTGGATCACCTGAGGTCAGGGGTCCGAGACCAGCCTGGCTAATGTGGTGA
AACTCTGTTTCTACTAAAAATACAAAAAATTAGCCAGGCATGGTGGCATGTGCCTATAAT
CCCAGCTACTCGGGAGGCTGAGGCAGGGGAATCGCTTGAACCCGGGAGACAGAGATTGCA
GTGAGCCAAGATCACGCCATTGCACTCCAGTTTGGGCAACAAGAGCGAAACTCTGTTTGT
TTGTTTGTTTGTTTTTAAAAAAAGAAAAAAAAGCTGGGCGCGGTGGCTCACGCCTGTAAT
CCCAGCACTTTGGGAGGCCGAGGCGGGCGGATCACCTGAGGTCAGGAGTTCGAGACCAGC
CTCAACATGGAGAAACCCCGTCTCTACTAAAAATACAAAAAATTATCCGGGCATGGTGGT
GCATGCCTGTAATCCCAGCTACTCAGGAGGCTAAGGCAGGAGAATTGCTTGAACCTGGGA
GGCGGAGGTTGCGGTGAGCCAAGATCGTGCCATTGCACCCCAGCCTGGGCAACAAGAGCG
AAACTCCGTCTCAAAAAAAAAAAAGGCCAGGCGTGGTGTTTCATGCCTGTAATCCCAGCA
CTTTGGGAGGCCGAGGCAGACTGATCACGAGGTCAAGAGATCGATACCATCCTGGCCAAC
ATGGTGAAACCCCGTCTCTAATAAAAATACAAAAATTAGCTGGGCGTGGTGGCACATGCC
TGTAGTCCCAGCTACTCGGAAGGCTGAGGCAGGAGAATCACTTGAACTGGAAGGCAGGGA
GCAGAGATCGCACCTCTGTCCTCCAGCCTGGTGACAGAGCGAGATTCCATCTAAAAAAAA
AAGAAAAGAAAAGAAAGAAAAAGTGAAAGTTTTAAGTGCTATGGGGAAAGAAATCAAGTG
TGATAAGGGCATCAGGAGTACGAGGGTGAAGACAGGTTGCAGCCCTAAATAGGGAGGTCA
GTTATTATTGAGTAAATGAGACCAAAGGGAAGACTTGAAGGAGATGAGAGAATTAGCCAT
GCAGAGACCTGGGGGGAAGAGAATTCCAGGAAGAAGGATCAGTCAGTGTCAAGGCCTAAGT
CAAGAAGGTTAGAAGAGCTAGAAACCATCAAGGGGGAAGAGCGGCAGACATGAAGAGACT
GTGTACGGACAGGAAGATCAGGTGCAGCTTTGTAGGCCATTTTTAGAATTTGAGTTGCGT
TTTCCTCCGAGTGAAATGAGAAACTGCTGCAGGGTTTTGAGCAGGGGAATGACAAGCTCT
TATTTATGTTTTAATAGAGCCTCCCCTCCCCTGCTGCTGCACTGAAAATAGATGGGGGGT
GGGGGGAAGGGTGCCCCTGCAGTATTTCTGGCTCAGACCAGTTGGCGGCAGTGAGGTGGT
GAGAAGTGGTCAGATTCTGAATGTATTTTGCAGGTAGAGCTGACAGGATTTGCTAATTGA
TCGGATATGGGGTATGGAGTGTGGGCGAGAAAGAAAGGAGTCAAAGGTGACAGGTGACTC
TGAGGTTTTTGCCATAAGGAACTGGAACGATGCATTAGCCACTGAGCAGGAAAGACTGTG
GTGGAAGGGGTTGGGGGGAGAGCAGAAGTTTGCTTGGGACATGTTGAGTCTGAGATGCTT
ATTAGCCATCCAAATGTGATAGAGGGGGCAGTCAGGTACACAACTCTGGGTTTGGGAGAA
AGGTCTGGATTGGAGAGACATTTGGGAGTTGGCTACATATAGATGGTATCTCAAGCCATG
AGACTAGTTGAGACCACCAAAGGAGTGTAGGTGAAATGACGGAGAAGAGAACAGGGTATC
TAACGTTAAGGGGAGGAACAAGGAAAGGAGACTGGGAAAGAGCAGCTAGTGAGGTAGGAG
TCAACCAAGAGAGGGTGCAGCCCTGGAGGTCAGGTGAAGACAGTGTGTCAAAGAAGGATG

TATGTTCAGGCTGCTGATGGGCCAATAGAGGAGGACTGAGCATTGAGCATTACATGGAGC
AAAAACACAGAGGTTCTTGGTGACCTTGACAAGAACAGATGCAGTGGAGAGATGGAGGCC
AACGCCTGAGTGGGGTGGGTTCAAGGGAGAATGGGGGAGGGGAACTAGAGGTAGCAAGTA
CAGATAACTCTTGCGATGAGTTTTGCTGCAAGTGAGAGCAAATACTGGGACTGAGGGGAA
ACCAGGAGATTAAGAGAATTTTTTTTTAATGATGAAAGAAATAACAGTAGGTTTACCTGC
GGATGGGGATGATCCAGTAAAAAAGGAAAACATGGCTGGGCACGGTGACTCACACCTGTA
ATCCCAGCATTTTGGGAGGGCGAGGTGGATGGATCACCTGAGGTCAAGAGTTCAAGACCA
ACCTGGCCAGCATGGTGAAACCCCTTCTCTACTAAAAATACAAAAAAATTAGCCAGGCGT
GGTGGTGCACACCTGTAATCCCAGCTACTCAGGAGGCTGAAGCAGGATAATCACTTGAAC
TGGGGAGGCAGAGGTTGCAGTGAGCTGAGATGGTGCCACTGCACTCCAGCCTGGGCGACA
AGAGTGAAACTCCCCCCGTCTCAAAAAAAAAGAAAGAAAAAGAAAAGGAAAACATGGGTG
TCACAGAGGACCACAGAATGGCTTAATTTATGTCCTTGAGCAGGTGAGAAGGAGGAGACC
TAGTGCACAAGTGGAAAGACAGATAGGGACCGAAGAATGAGCCAACCTGCTCTCGGCTAG
CAGCTGCCCCATAGCAGTGCAGGCATGGGATAGAACTCAGCTCTCCTCAGTCCATGAGGC
CTCCTAGCTCTAAAAGCCCGCACCCAAACGCCCTCACCTGGCTCCCAGCCCCTGCCCTAC
ACCCCATACCCTGGGGTAGCCGGGCAAGCAGCACTTACATACCCATGCCCATACAGTGCC
CATACATGCCCATACAGTGACCTCAGGCCTGGCGGAGGGCACTCCCCTCCGATTTCCACA
CTGCTGCCTCCCCAAGGGGATGGATGTTGGCTTGAGAGGGAAGGGGAGTCTGTGATCTGT

GGGCAGGGGTTGCATCAGGGAATAAAG[A/G]TCAGGTAACAGGACGCCTGTGGCGTGAGGCGT
TCTGAGAATGGTAATGGGTTGGGTTTGGTTGCCTCTCATGTTCTGGGGGAACGTTGTCTG
AACGTGAATCTCTGGTTCTAG

*PKLR* exon 7 sequence:

GTTTGATGAAATCCTGGAGGTGAGCGAC[G/A]GCATCATGGTGGCACGGGGGGACCTAGGCAT
CGAGATCCCAGCAGAGAAGGTTTTCCTGGCTCAGAAGATGATGATTGGGCGCTGCAACTT
GGCGGGCAAGCCTGTTGTCTGTGCCACACAG