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Title: Identification and Breakpoint Mapping of Intragenic Deletions in Patients with Anemia Due to Pyruvate Kinase Deficiency
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Background: Pyruvate kinase (PK) deficiency, the most common glycolytic enzymopathy resulting in hemolytic anemia, is due to recessively inherited mutations in the PKLR gene. Most cases are due to homozygous or compound heterozygous missense mutations. A few patients have been described with intragenic deletions of the PKLR gene. Deletion detection using commercially available multiplex ligation-dependent probe amplification (MLPA) assays has been unreliable, making diagnosis difficult. Recently, mutation type has been correlated with response to a newly released PK-stabilizing agent and mutation detection has been recommended before its prescription. The objective of this study was to develop and apply techniques for identifying intragenic deletions in the PKLR gene in genomic DNA from PK-deficient patients.

Methods: Genomic DNA from 12 unrelated pyruvate kinase-deficient patients with single or no PKLR coding region mutations were studied. A multiplex long range polymerase chain reaction assay (LR-PCR) and a digital droplet PCR assay were developed to screen for intragenic deletions. The LR-PCR assay utilized sets of overlapping primers spanning the PKLR locus. The ddPCR assay was designed to quantify the copy number of each exon of the PKLR gene, then optimized for compatibility for single plate thermocycling to maximize ease of use. Breakpoint mapping was carried out by Sanger sequencing of subcloned PCR products spanning the deleted region.

Results: LR-PCR screening identified intragenic deletions in 11 patients, 10 with exon 11 deleted and one with exons 4-10 deleted. ddPCR identified deletions in all 12 patients, 10 with exon 11 deleted, one with exons 4-10 deleted, and one with exons 1-3 deleted not detected by LR-PCR. Breakpoint mapping in exon 11 deletion patients showed 9 were identical to the “Gypsy” mutation and 1 was novel. The patient with the exon 4-10 deletion had the previously reported “Vietnamese” deletion. The novel deletion of exons 1-3 had breakpoints in the 5’ untranslated region and intron 3.

Conclusions: These assays expand the armamentarium of molecular diagnostic tests available for disease diagnosis in patients with PK deficiency. Similar assays can be developed for other disease loci with frequent intragenic mutations.

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