Abstract: PB3265

Title: NGS PITFALLS – “OLD” TESTS ARE STILL VALUABLE

Abstract Type: Publication Only

Topic: Enzymopathies, membranopathies and other anemias

Background:

Pyruvate kinase (PK) deficiency is a rare cause of chronic non-spherocytic hemolytic anemia characterized by a highly heterogenous phenotype. The disease, caused by mutations in the *PKLR* gene, follows an autosomal recessive inheritance pattern, with a predominance of missense mutations. Nevertheless, various mutation types, including large deletions, insertions, nonsense, frameshift, splicing, and promoter region mutations, have been reported.

Aims:

In this case report, we endeavour to highlight some of the challenges and potential pitfalls involved in the diagnostic workup of PK deficiency, specifically focusing on cases associated with rare promoter region mutations.

Methods:

Data were retrospectively collected from the patient’s electronic record.

Results:

This case details a female child with severe symptomatic hemolytic anemia, diagnosed at 5 years of age, despite a previously unremarkable clinical and family history. The peripheral blood smear showed no morphological red blood cell changes, and PK measurement suggested a PK deficiency. Both parents were assymptomatic with normal red cell analysis, and both had a PK assay showing half the value of the normal control (compatible with carrier status). Molecular analysis was performed by next-generation sequencing (NGS) with a panel of all known genes associated with hemolytic anemias, and identified only an heterozygosity in the *PKLR* gene for the c.1456C>T (p.Arg486Trp) mutation in both the propositus and her father. As the phenotype and enzyme assay were indicative of PK deficiency, multiplex-ligation dependent probe amplification (MLPA) was performed to explore large deletions or duplications, yielding negative results. Next, expanding sequencing coverage to the promoter region uncovered the c.-72A>G mutation in both the child and her mother, confirming a compound heterozygosity status in the former.

Summary/Conclusion:

Promoter region mutations, comprising a mere 1% to 2.4% of all *PKLR* mutations, present a diagnostic challenge. This rare and intricate case underscores the limitations of NGS techniques, emphasizing the necessity of performing functional assays and, when facing suspicion of PK deficiency, extending investigations to the promoter region in cases of asynchronous clinical and laboratory findings.

Keywords: Red blood cell, Promoter, Pyruvate kinase deficiency, Hemolytic anemia