



Category: Clinical Genomics

Next-generation sequencing-based molecular diagnosis of chronic non-spherocytic hemolysis in erythrocytic enzymopathies

Manu Jamwal¹, Anu Aggarwal¹, Prashant Sharma¹, Deepak Bansal², Pankaj Malhotra³, Arindam Maitra⁴ and Reena Das¹

¹Department of Hematology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, INDIA

²Department of Pediatrics (Hemato-Oncology), Post Graduate Institute of Medical Education and Research, Chandigarh 160012, INDIA

³Department of Internal Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, INDIA

⁴National Institute of Biomedical Genomics, Kalyani, West Bengal 741235, INDIA

Presenting author: manujamwal25@gmail.com

Abstract

Mutations in genes encoding red blood cell enzymes are often inherited in an autosomal recessive manner and can lead to chronic nonspherocytic hemolytic anemia (CNSHA) in homozygotes and compound heterozygotes. Usual clinical manifestations include jaundice, cholelithiasis and splenomegaly with normocytic normochromic hemolysis. Phenotypes range from fully-compensated hemolysis (without anemia) to transfusion-dependent states. Definitive diagnosis requires biochemical testing of enzyme levels, which for rarer enzymes are often difficult and not easily available. Molecular diagnosis using a gene-by-gene approach is expensive, time-consuming and cumbersome. Targeted resequencing can expedite the molecular diagnosis in cases where the hemolysis remains unexplained after routine laboratory tests. Ten patients with clinical and laboratory evidence suggestive of hemolytic anemia, but negative family history, were enrolled. Various biochemical and molecular tests were used to exclude glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassemias, hemoglobinopathies, autoimmune hemolysis, hereditary spherocytosis and pyruvate kinase (PKLR) deficiency. Common *G6PD* and *PKLR* variants were excluded by molecular tests. DNA Libraries were prepared using TruSight One™ panel and sequenced on MiSeq™ Sequencing System. MiSeq Reporter™ and VariantStudio™ v2.1 were used for analysis, classification, and reporting of genomic variants reporting genomic variants. All 10 patients' diagnoses were resolved by targeted resequencing: two had G6PD deficiency, two had glucose-6-phosphate isomerase (GPI) deficiency and six unexpectedly had pyruvate kinase deficiency despite pyruvate kinase enzyme activity assays previously being normal in all. All the mutations were predicted deleterious by PolyPhen, SIFT, Provean, mutpred and Mutationtaster software. The mutations were validated in parents and/or siblings (where available) to establish the mode of inheritance. Our data demonstrates the high clinical utility of next-generation sequencing for molecular diagnosis of CNSHA due to red cell enzymopathies. This is important as a molecular diagnosis aids genetic counselling and future antenatal diagnosis, and also streamlines management, avoiding unnecessary further investigations. Our results also caution that pyruvate kinase deficiency may be missed by conventional biochemical testing approaches.

Citation: Jamwal, M., Aggarwal, A., Sharma, P., Bansal, D., Malhotra, P., Maitra, A. and Das, R. Next-generation sequencing-based molecular diagnosis of chronic non-spherocytic hemolysis in erythrocytic enzymopathies [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 83. <https://doi.org/10.24870/cjb.2017-a70>