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**Abstract Topic:-** Clinical Genetics

**Abstract Title:-** Molecular characterization of unexplained cases of congenital anaemia using next-generation sequencing panel

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**Aims:-**To diagnose unexplained cases of congenital anemia using next-generation sequencing panel.

**Methods:-** The suspected patients of congenital anemias were screened for hemoglobinopathies , membranopathy, and enzymopathy. Patients are then subjected to DNA Sanger sequencing and Targeted next generation sequencing, if required. The library was generated using the Twist Lib prep Kit. The raw reads were analysed by in-house bioinformatics pipelines following GATK guidelines. The VCF was annotated by wANNOVAR software. The damaging effects of the variants were predicted using tools like SIFT, Polyphen-2, MutPred, Provean, and Mutation Assessor. The effect of the variants on the protein structure was studied using PyMol, Chimaera, and the Swiss PDB Viewer. cDNA was synthesised by the Taqman reverse transcription kit, and qPCR was done on Step-One PCR to perform the functional validation of the novel variants.

**Results:-** We have diagnosed a total of 134 cases of congenital anaemia,, including 45 cases of Pyruvate kinase deficiency, 45 cases of glucose phosphate deficiency, 3 cases of adenylate kinase deficiency, 1 case of hexokinase deficiency, 4 cases of pyrimidine 5’ nucleotidase deficiency, 3 cases of aldolase deficiency, and 5 cases of phospho glycerate kinase deficiency. 1 case of CDA type 1B, 11 cases of congenital sideroblastic anaemia, 11 cases of diamond blackfan anaemia, and 5 cases of rare aetiology The variants are classified as variants of unknown clinical significance (VOUS) as per the ACMG guidelines. The pathogenicity of the novel variants was assessed using different prediction tools, and all of them showed damaging effects on the protein structure. Most of the cases remained undiagnosed for a long period of time and were initially treated as thalassemia major in view of the transfusion requirement. NGS made the diagnosis accurate. The qPCR suggested the reduction of 40-50 % mRNA level of mutated variant as compared to that of wild type.

**Conclusions:-** This study explores the rare enzyme deficiencies, including hexokinase, glucose 6-phsophate isomerase, phosphofructose kinase, aldolase, triose phosphate isomerase, phosphoglycerate kinase, pyruvate kinase and pyrimidine 5’ nucleotidase deficiency., along with identifying extremely rare causes of congenital anemias. No curative therapy is available however patient can be managed as per the clinical symptoms. Blood transfusions, chelation therapy, splenectomy and supportive care are mainstays in managing the conditions with recent advancements in gene therapy and allosteric activator showing promise for future therapeutic options.

**Keywords:-** Congenital anemia, unexplained anemia, next generation sequencing, qRT PCR, gene expression