Report:

Background - Deficiency of human erythrocyte R isozyme (RPK) is, together with glucose 6-phosphate dehydrogenase deficiency, the most common cause of the nonspherocytic hemolytic anemia. To provide a molecular framework to the disease, we have undertaken the molecular analysis of this human enzyme.

Results - We have solved the 2.7 Å resolution crystal structure of human RPK in complex with fructose 1,6-bisphosphate, the allosteric activator, and phosphoglycolate, a substrate analogue, and we have functionally and structurally characterized eight mutants (G332S, G364D, T384M, D390N, R479H, R486W, R504L, R532W; see Figure) found in RPK-deficient patients. The mutations target distinct regions of RPK structure, including domain interfaces and catalytic and allosteric sites. The mutations affect to different extent thermostability, catalytic efficiency and regulatory properties. These studies are the first to correlate the
clinical symptoms with the molecular properties of the mutant enzymes. Mutations greatly impairing thermostability and/or activity are associated to severe anemia. Some mutant proteins exhibit moderate changes in the kinetic parameters, which are sufficient to cause mild-to-severe anemia, underlining the crucial role of RPK for erythrocyte metabolism.

**Conclusions** - Prediction of the effects of mutations is difficult since there is no relation between the nature and location of the replaced amino acid and the type of molecular perturbation. Characterization of mutant proteins may serve as a valuable tool to assist with diagnosis and genetic counseling.
Reference

Valentini, G., Chiarelli, L.R., Fortin, R., Dolzan, M., Galizzi, A., Abraham, D.J., Wang, C., Bianchi, P.,
basis of nonspherocytic hemolytic anemia. J. Biol. Chem. 277, 23807-23814.