

MISSENSE MUTATION N487S AND P488S OF THE ERYTHROPOIETIN RECEPTOR ARE NOT ASSOCIATED WITH CONSTITUTIVELY ELEVATED HEMOGLOBIN CONCENTRATION IN BLOOD DONORS

Chantal Brönnimann, Christoph Gassner, Beat M. Frey
Stiftung Zürcher Blutspendedienst SRK, Schlieren

Background: Various conditions cause erythrocytosis (EC) that may be discovered by chance at checking for blood donation. Primary EC (pEC) may be triggered by acquired mutations of JAK2, MPL or EPOR. Alternatively, deficient oxygen sensing/delivery conditions such as mutations of the VHL, PHD2 or HIF2a gene as well as hemoglobinopathies with increased oxygen affinity such as hemoglobin Chesapeake or hemoglobin Kempsey may be considered. However, secondary forms of EC (sEC) are more prevalent and their underlying condition (pulmonary or cardiac affections, paraneoplasia or high altitude related) may easily be diagnosed by anamnestic and physical examination. In contrast, following exclusion of inherited conditions by personal and familial history, pEC may need further investigation of molecular defects pointing to smoldering clonal disorders. Deletional and insertional mutations of the inhibitory, intracellular domain of the erythropoietin receptor (*EPOR*, exon 7 and 8) are known to cause increased sensitivity to endogenous erythropoietin (EPO) leading to pEC.

Methods: 12 patients with pEC (P1), requiring regular phlebotomy and found negative for JAK2, VHL, BCR-ABL mutations, aberrant EPO concentrations, hemoglobinopathies and secondary clinical causes for EC were assessed for mutations of exon 7/8 of *EPOR* by sequencing. Similarly, 24 healthy platelet donors (P2) with high-normal hemoglobin (157 – 182 g/l) were assessed for *EPOR* ex 7/8 mutations. Finally, 315 (171 males, 144 females) healthy blood donors (P3) with random distribution of hemoglobin concentrations (Hb) were assessed for N487S/A1460G and P488S/C1462T of exon 8 of *EPOR*, which were found in P2.

Results: P1 revealed only wild type sequence of exon 7 and 8 of *EPOR*. In P2 one donor carried N487S/A1460G and another donor carried P488S/C1462T. Both individuals were active platelet donors and had Hb values of 176 g/l. In P3, 8 carriers with N487S (4 males, 3 females) and 3 carriers with P488S (2 males, 1 female) were found. The median Hb (mHb) of mutation carriers was in males 152.5 g/l (144 – 180 g/l) and in females 138 g/l (135 – 145 g/l). There was no significant difference in mHb between carrier and non-carrier of *EPOR* mutations.

Conclusions: Although it has been shown that deletional and insertional *EPOR* mutations leading to truncation of the inhibitory domain of *EPOR* and by this way may cause pEC, our findings suggest that the missense mutations N487S and P488S of the inhibitory domain of *EPOR* do not compromise its function. These mutations may represent *EPOR* polymorphisms that do not sufficiently explain pEC. However, these mutations may represent a first hit in the development of clonal disease, since they affect the binding site of Grb2[1] and may therefore disturb the intracellular *ERK/MAPK* signal pathway.

1. Le Couedic, J.P., et al., *Missense mutation of the erythropoietin receptor is a rare event in human erythroid malignancies*. *Blood*, 1996. 87(4): p. 1502-11.