**Genetic variants of erythropoietin (EPO) and EPO receptor genes in familial erythrocytosis**

Danijela Vočanec\(^1,2\) | Tinkara Prijatelj\(^1,2\) | Nataša Debeljak\(^2\) | Tanja Kunej\(^1\)

\(^1\)Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
\(^2\)Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

**Correspondence**
Nataša Debeljak, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.
Email: natasa.debeljak@mf.uni-lj.si
Tanja Kunej, Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.
Email: tanja.kunej@bf.uni-lj.si

**Funding information**
Javna Agencija za Raziskovalno Dejavnost RS, Grant/Award Number: L3-9279, P1-0390 and P4-0220

**Abstract**
Objectives: Erythrocytosis is characterized by the expansion of erythrocyte compartment including elevated red blood cell number, hematocrit, and hemoglobin content. Familial erythrocytosis (FE) is a congenital disorder with different genetic background. Type 1 FE is primary FE caused by mutation in erythropoietin receptor gene (EPOR). Type 2-5 FE are secondary FEs caused by mutations of genes involved in oxygen sensing pathway important for erythropoietin (EPO) regulation. In the present study, we summarized associations between EPOR and EPO gene variations with development of FE and searched for genetic variants located within regulatory regions.

Methods: Publications reporting EPOR and EPO sequence variants associated with FE or clinical features of erythrocytosis were retrieved from PubMed and WoS. In silico, sequence reanalysis was performed using Ensembl genomic browser, release 89 to screen for variants located within regulatory regions.

Results: To date, 28 variants of the EPOR and seven variants of the EPO gene have been associated with erythrocytosis or upper hematocrit. Sequence variants were also found to be present within regulatory regions.

Conclusions: Role of variants in regulatory regions of the EPO gene should be further investigated.

**Keywords**
erythropoietin, erythropoietin receptor, familial erythrocytosis, regulatory regions, sequence variants

---

1 | **INTRODUCTION**

Erythrocytosis comprises a heterogeneous group of disorders characterized by the expansion of the erythrocyte compartment in the peripheral blood, clinically reflected by an increased hematocrit, hemoglobin or red blood cell (RBC) number. Erythrocytosis is classified as congenital or acquired and its mechanism may be primary or secondary.\(^2\-^5\)

---

Vočanec and Prijatelj contributed equally to the work.
promoting substances acting on hematopoietic progenitors, mainly EPO. Secondary congenital erythrocytosis is characterized by inappropriately normal or high serum EPO levels due to defects in the oxygen sensing pathway (ECYT2-5) or variants effecting hemoglobin oxygen affinity (ECYT6-7) (OMIM). Defects in oxygen sensing pathway can be associated with pathogenic variants in one of the factors regulating EPO production; including von Hippel-Lindau tumor suppressor (VHL) indicative for ECYT2, egl-9 family hypoxia-inducible factor 1 (EGLN1, synonym PHD2) indicative for ECYT3, and endothelial PAS domain protein 1 (EPAS1, synonym HIF2A) indicative for ECYT4. Recently, the EPO gene was added to the OMIM database as new cause for FE (indicative for ECYT5).

Glycoprotein hormone EPO is the main regulator of red blood cell production. EPOR dimerization and activation by EPO leads to signaling cascade triggering several genes responsible for proliferation, survival, and differentiation of erythroid progenitor cells, leading to a tight control of RBC production in the bone marrow. EPO and its receptor EPOR can therefore be involved in development of FE.

Erythropoietin receptor gene is located on 19p13.2. The 2459 bp long primary transcript variant 1 encodes 508 amino acids (aa) EPOR precursor, resulting in 484 aa long mature receptor. The mature receptor consists of an extracellular, a transmembrane, and a cytoplasmic region. Soluble (EPOR-S) and truncated (EPOR-T) isoforms associated with cancer have also been identified. Multiple EPOR mutations associated with ECYT1 have been described, lacking a signal termination due to truncation of the inhibitory domain SHP-1/SOCS-3. All patients with EPOR mutations display suppressed serum EPO levels, but still produce erythroid colonies due to constitutive receptor activation. EPOR mutations have been found in 15% of all hereditary erythrocytosis cases.

Erythropoietin gene is located at 7q22.1. Single 1340 bp long primary transcript variant 1 encodes 508 amino acids (aa) EPO precursor, resulting in 484 aa long mature receptor. The mature receptor consists of an extracellular, a transmembrane, and a cytoplasmic region. Soluble (EPO-S) and truncated (EPO-T) isoforms associated with cancer have also been identified. Multiple EPO mutations associated with ECYT1 have been described, lacking a signal termination due to truncation of the inhibitory domain SHP-1/SOCS-3. All patients with EPO mutations display suppressed serum EPO levels, but still produce erythroid colonies due to constitutive receptor activation. EPO mutations have been found in 15% of all hereditary erythrocytosis cases.

Erythropoietin gene is located at 7q22.1. Single 1340 bp long splice variant encodes a 193 aa erythropoietin precursor, resulting in 165 aa long mature hormone. EPO production is increased in hypoxia resulting from anemia or decreased cellular oxygen tension. Under normal oxygen tension, HIF-alfa is hydroxylated by EGLN1 (PHD2) and targeted by VHL for ubiquitin-mediated degradation, resulting in no EPO production. Under hypoxic conditions, hydroxylase activity is inhibited, therefore EGLN1 proteins are unable to hydroxylate HIF-alfa allowing it to escape VHL protein recognition and subsequent degradation. HIF-alfa can therefore form an active transcriptional complex with HIF-beta,aryl hydrocarbon receptor nuclear translocator (ARNT). Hypoxia-inducible factor (HIF) complex translocates to nucleus and binds to the hypoxia response element (HRE) of the target genes and upregulates expression of more than 200 genes, including EPO. Among members of HIF-alfa gene family, EPAS1 (HIF2A) is involved in regulation of EPO production.

Tissue-specific expression of EPO gene in adult kidneys or fetal livers is dependent on far upstream cis elements and an enhancer element downstream from the polyadenylation signal. EPO gene expression is also regulated via stimulatory hepatocyte nuclear factor 4 alpha (HNF4A), inhibitory GATA binding protein 2 (GATA2), and NF-kappa-B (NFkB). The motif 5'-RCGTG-3' has been reported as the core sequence for a HIF binding site.

| TABLE 1 Genetic variants of the EPOR gene causing truncation of EPOR protein in familial erythrocytosis |
|--------------------------------------------------------------------------------------------------|
| **Genetic variants of the EPOR gene causing truncation of EPOR protein in familial erythrocytosis** |
| **dbSNP HGVS name** | **Variant PUB accession number** | **Location of the variant in the EPOR gene** | **Number of patients reported** | **Geographic origin/ethnic origin** | **Remarks** |
| c.1252_1255del | p.Gly418Profs*34 | Exon 8 | One family, four affected members | Denmark | 59 aa truncation confirmed in three members |
| g.5938_5941del | Exon 8 | One family, four affected members | na | na | 84 aa truncation |
| c.1273G>T | p.Glu425* | Exon 8 | One family, four affected members | na | 84 aa truncation |
| p.Glu425* | Exon 8 | One family, four affected members | na | na | 55 aa truncation, shortest known truncation |
| | | | | | |
| **EPOR, erythropoietin receptor; Var_pub_as, variant as reported originally; listed only when different from Variant/DNA.** |
| dbSNP HGVS name (reference SNP ID number, if available) | Var_pub_as | Location of the variant in the EPO gene | Geographic origin/ethnic origin | Number of patients reported | Reference | Remarks |
|----------------------------------------------------------|------------|----------------------------------------|-------------------------------|---------------------------|-----------|---------|
| NM_000799.2:c.-1306C>A (rs1617640)                       | G>T        | Upstream                               | Jordan                        | 298 healthy male blood donors; divided into: Ht level greater or equal to 48 (181), and Ht between 42% and 47.5% (117) | Khabour et al<sup>28</sup> PMID: 23142128 | G allele was found at significant higher frequency among upper-Ht group; unconfirmed |
| NM_000799.2:c.-136G>A                                    | /          | 5′UTR                                  | United Kingdom (Great Britain) | One family, three affected members and one family, five affected members | Taylor<sup>29</sup> PMID: 25985138 | Co-segregated with the disease in two independent families |
| NM_000799.2:c.32delG                                      | /          | exon 2                                 | Norway                        | One family, nine affected members | Zmajkovic et al<sup>10</sup> PMID: 29514032 | Frameshift in exon 2, excess production of EPO from alternative promoter located in intron 1 |
| NM_000799.2:c.19delC, p.P7fs                             | /          | exon 2                                 | /                             | Female with affected father and parental grandmother. | Camps et al<sup>4</sup> PMID: 27651169 | Identified as novel erythrocytosis-associated gene variant in WGS500 and gene panel sequencing project; unconfirmed. |
| NM_000799.2:c.250G>C, p.G84R (rs137953994)               | /          | exon 4                                 | /                             | One male patient | /         |         |
| NM_000799.2:c.296A>G, p.E99G                              | /          | exon 4                                 | /                             | Two male patients | /         |         |
| NM_000799.2:c.772G>T (rs551238)                           | G3544T     | 3′UTR enhancer                        | Jordan                        | 298 healthy male blood donors; divided into: Ht level greater or equal to 48 (181), and Ht between 42% and 47.5% (117) | Khabour et al<sup>28</sup> PMID: 23142128 | G allele was found at significant higher frequency among upper-Ht group; unconfirmed |

EPO, erythropoietin; Hb, hemoglobin; HRE, hypoxia response element in 3′ UTR enhancer; Ht, hematocrit; Var_pub_as, variant as reported originally; listed only when different from Variant/DNA.
One of the main obstacles in the research field presents the fact that terminology regarding genetic variants is heterogeneous and dispersed across publications and databases. Additionally, identification of regulatory regions was performed on various previous genomic assemblies and needs to be updated according to the genomic locations from latest genomic browsers. The main aim of the present study was therefore to: (a) summarize the literature and databases for associations between EPOR and EPO genes and familial erythrocytosis or its clinical signs and (b) to perform in silico sequence reanalysis of the EPO gene to identify variants overlapping regulatory sites.

2 | MATERIAL AND METHODS

Literature databases (PubMed and Web of Science; WoS) were screened for gene variations in EPO and EPOR genes in association with FE and its clinical characteristics: increased RBC mass, elevated hemoglobin, and hematocrit. The literature was retrieved using the keywords such as erythropoietin, erythropoietin receptor, mutations, polymorphism, erythrocytosis, polycythemia, hematocrit, and elevated red blood cell number. The time span of the literature search was from 1/1993 to 4/2018. Nucleotide sequence, genomic coordinates, and variants were extracted from the Ensembl, release 89. Obtained data were complemented with additional relevant genomic information. Reference SNP ID number (rs ID) if available, synonyms and clinical data were obtained from: The Single Nucleotide Polymorphism database (dbSNP), The Human Gene Mutation Database (HGMD), www.erythrocytosis.org and Leiden Open Variation Database (LOVD), release 3.0. Gene names were unified according to the HUGO Gene Nomenclature Committee (HGNC) database. Collected genomic data were organized and unified according to the human genome variation society (HGVS) recommendations. Locations of regulatory regions were extracted from the published literature and compared with genomic location of sequence variants deposited in the Ensembl browser.

3 | RESULTS

The review revealed that 25 genetic variants of the exon 8 of EPOR gene associated with FE are deposited in the LOVD database. Literature review revealed three additional variants reported

![Figure 1](image-url)
4 | DISCUSSION

Most pathogenic genetic variants of EPOR the gene are causative for FE due to truncation of protein cytoplasmic region responsible for receptor negative regulation by SHP-1 and SOCS-3 binding to EPOR at 454 aa and 454-456 aa, respectively.\(^{30-32}\) For example, variant c.1362C>G at 454 aa causes deletion of the last 55 aa,\(^{27}\) while the variant rs281860299 at 382 aa results in deletion of the last 127 aa.\(^{33}\)

In contrast, EPO variants are spread all over the gene and several disease causing mechanisms seem to be involved. EPO variant c.-136G>A is pathogenic due to increased EPO regulation\(^{29}\) and variant c.32delG due increased EPO production from alternative promoter.\(^{10}\) Variants in promoter and enhancer regions of EPO gene have been associated with elevated hematocrit suggesting involvement in FE, since both regulatory regions have an important role in EPO expression. Variant rs1617640 and rs551238 of EPO gene have also been associated with diabetic retinopathy,\(^{34}\) and hematopoietic disorder myelodysplastic syndrome.\(^{35}\)

Identification of variants within EPO regulatory regions is of interest since they could contain variants that constitutively activate the EPO gene. Sequence analysis of the 3’HRE region revealed four variants; however, none of which affected the HIF-1-binding site, although one was present within HNF-4 consensus region.\(^{19}\)

Screening of the Ensembl database revealed two variants with unknown function located within regulatory regions. Variant rs796952255 is located in HBS, also termed as hypoxia response element (HRE). Variants located within HRE were previously termed as HRE-SNPs.\(^{36}\) Additionally, variant rs539229161 is located within HNF4A binding region (DR-2).

In conclusion, sequence variants of the EPO gene show potential for further functional analyses based on their location within previously reported regulatory regions, which might enable generating more targeted hypotheses for testing in the future and enable more efficient biomarker development.

ACKNOWLEDGEMENTS

This work was supported by the Slovenian Research Agency (ARRS) through the Research programmes L3-9279, P1-0390 and P4-0220. We thank Daniel Kriz for language editing.

AUTHOR CONTRIBUTION

DV and TP preformed analysis of the literature and regulatory sites and drafted the paper, ND and TK designed the study and edited the final version of the manuscript.

ORCID

Tanja Kunej [https://orcid.org/0000-0002-0465-1762]

REFERENCES

1. Kralovics R, Prchal JT. Genetic heterogeneity of primary familial and congenital polycythemia. Am J Hematol. 2001;68(2):115-121.
2. Bunn HF. Erythropoietin. Cold Spring Harb Perspect Med. 2013;3(3):a011619.
3. Hussein K, Percy M, McMullin MF. Clinical utility gene card for: familial erythrocytosis. Haematologica. 2012;101(11):1306-1318.
4. Camps C, Petousi N, Bento C, et al. Gene panel sequencing improves the diagnostic work-up of patients with idiopathic erythrocytosis and identifies new mutations. Haematologica. 2016;101(11):1306-1318.
5. Bento C, Percy MJ, Gardie B, et al. Genetic basis of congenital erythrocytosis: mutation update and online databases. Hum Mutat. 2014;35(1):15-26.
6. Arcasoy MO, Karayal AF, Segal HM, Sinning JG, Forget BG. A novel mutation in the erythropoietin receptor gene is associated with familial erythrocytosis. Blood. 2002;99(8):3066-3069.
7. Bento C, McMullin M, Percy M, Cario H. Primary familial and congenital polycythemia. GeneReviews®. 2016. http://www.ncbi.nlm.nih.gov/books/NBK395975/
8. McMullin MF. The classification and diagnosis of erythrocytosis. Int J Lab Hematol. 2008;30(6):447-459.
9. Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic Acids Res. 2015;43(D1):D789-D798.
10. Zmajkovic J, Lundberg P, Nienhold R, et al. A gain-of-function mutation in EPO receptor causes dominantly inherited benign human erythrocytosis. Proc Natl Acad Sci U S A. 1993;90(10):4495-4499.
11. de la Chapelle A, Träskelin AL, Juvonen E. Truncated erythropoietin receptor causes dominantly inherited benign human erythrocytosis. Blood. 2007;100:10342-10345.
12. Debeljak N, Sytkowski AJ. EpoR. UCSD-Nature Molecule Pages. 2007. A000863.
13. Braunstein EM, Moliterno AR. Back to biology: new insights on in vivo regulation of the erythropoietin gene. Blood. 2002;99(4):132-134.
14. Weidemann A, Johnson RS. Nonrenal regulation of EPO synthesis. Blood. 1995;86(1):17-24.
15. Imagawa S, Yamamoto M, Miura Y. Negative regulation of the erythropoietin gene expression by the GATA transcription factors. Blood. 1997;90(4):2199-2207.
16. Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. Blood Rev. 2013;27(1):41-53.
17. Jelkmann W. Regulation of erythropoietin production. J Physiol. 2011;589(Pt 6):1251-1258.
18. Ebert BL, Bunn HF. Regulation of the erythropoietin gene. Blood. 1999;94(6):1864-1877.
19. Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet. 2015;47(7):717-726.
20. Klingmüller U, Lorenz U, Cantley LC, Neel BG, Lodish HF. Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. Cell. 1995;80(5):729-738.
21. Hörtner M, Nielsch U, Mayr LM, Heinrich PC, Haan S. A new high affinity binding site for suppressor of cytokine signaling-3 on the erythropoietin receptor. Eur J Biochem. 2002;269(10):2516-2526.
22. Sasaki A, Yasukawa H, Shouda T, Kitamura T, Dikic I, Yoshimura A. CIS3/SOCS-3 suppresses erythropoietin (EPO) signaling by binding the EPO receptor and JAK2. J Biol Chem. 2000;275(38):29338-29347.
23. Al-Sheikh M, Mazurier E, Gardie B, et al. A study of 36 unrelated cases with pure erythrocytosis revealed three new mutations in the erythropoietin receptor gene. Haematologica. 2008;93(7):1072-1075.
24. Yang X, Deng Y, Gu H, et al. Candidate gene association study for diabetic retinopathy in Chinese patients with type 2 diabetes. Mol Vis. 2014;20:200-214.
25. den Dunnen JT, Dalglish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat. 2016;37(6):564-569.
26. Petersen KB, Hokland P, Petersen GB, et al. Erythropoietin receptor defect: a cause of primary polycythaemia. Br J Haematol. 2004;125(4):537-538.
27. Chauveau A, Luque Paz D, Lecucq L, et al. A new point mutation in EPOR inducing a short deletion in congenital erythrocytosis. Br J Haematol. 2016;172(3):475-477.
28. Khabour OF, Bani-Ahmad MA, Hammash NM. Association between polymorphisms in erythropoietin gene and upper limit haematocrit levels among regular blood donors. Transfus Clin Biol. 2012;19(6):353-357.
29. Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet. 2015;47(7):717-726.
30. Slemc L, Kunej T. Transcription factor HIF1A: downstream targets, and personalized genomic medicine. Blood Rev. 2014;28(4):162–167. https://doi.org/10.1111/ijrh.12949
How to cite this article: Vočanec D, Prijatelj T, Debeljak N, Kunej T. Genetic variants of erythropoietin (EPO) and EPO receptor genes in familial erythrocytosis. Int J Lab Hem. 2019;41:162–167. https://doi.org/10.1111/ijlh.12949