


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

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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.¹ Erythrocytosis is classified as congenital or acquired and its mechanism may be primary or secondary.²⁻⁵

Primary erythrocytosis results from a molecular defect in the hematopoietic progenitor cells, leading to cell hypersensitivity to erythropoietin (EPO) and consequently in serum EPO levels below normal. The primary cause of increased RBC number may be polycythemia vera (PV) (acquired) or familial erythrocytosis (FE) (congenital). The mutation of a Janus kinase 2 (*JAK2*) gene is indicative for PV. The erythropoietin receptor (*EPOR*) mutation is the indicator for primary FE type 1 (ECYT1), also known as congenital erythrocytosis (CE) or primary congenital familial polycythemia.^{6,7} Secondary erythrocytosis results from aberrant regulation of erythropoiesis

Vočanec and Prijatelj contributed equally to the work.

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TABLE 1 Genetic variants of the *EPOR* gene causing truncation of EPOR protein in familial erythrocytosis

dbSNP HGVS name	Var_pub_as	Location of the variant in the EPOR gene	Geographic origin/ ethnic origin	Number of patients reported	Reference	Remarks
c.1252_1255del p.Gly418Profs*34	g.5938_5941del	Exon 8	Denmark	One family, four affected members	Petersen et al ²⁶ PMID: 15142125	58 aa truncation confirmed in three members
c.1273G>T p.Glu425*	G5959T	Exon 8	na	One family, four affected members	Kralovics and Prchal ¹ PMID: 11559951	84 aa truncation
c.1362C>G p.Tyr454*	/	Exon 8	France	One patient (30-y-old female)	Chauveau et al ²⁷ PMID: 26010769	55 aa truncation, shortest known truncation

EPOR, erythropoietin receptor; Var_pub_as, variant as reported originally; listed only when different from Variant/DNA.

promoting substances acting on hematopoietic progenitors, mainly EPO.⁴ 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)⁸ (OMIM).⁹ 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).^{9,10}

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.¹¹ Hormone 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.¹² 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.¹³

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- α 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- α allowing it to escape VHL protein recognition and subsequent degradation. HIF- α can therefore form an active transcriptional complex with HIF- β , 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*.² Among members of HIF- α gene family, EPAS1 (HIF2A) is involved in regulation of EPO production.¹⁴

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 poly-adenylation signal.² *EPO* gene expression is also regulated via stimulatory hepatocyte nuclear factor 4 alpha (HNF4A), inhibitory GATA binding protein 2 (GATA2), and NF- κ -B (NFKB).¹⁵⁻¹⁷ The motif 5'-RCGTG-3' has been reported as the core sequence for a HIF binding site.^{14,16,18,19}

TABLE 2 Genetic variants of the EPO gene associated with erythrocytosis or hematocrit level

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 ²⁸ 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 ²⁹ 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 ¹⁰ 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 ⁴ 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 ²⁸ 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.

in^{1,26,27} (Table 1). Mutations result in premature termination and, consequently, these mutant receptors lack portions of the C-terminal EPOR cytoplasmic domain responsible for negative regulation.

Out of 11 tested sequence variants of the *EPO* gene, seven were associated with erythrocytosis or upper-limit hematocrit (Table 2).^{4,10,28,29} Four tested sequence variants were not associated with FE or clinical signs.^{19,28} The first pathogenic variant of the *EPO* gene is located in the 5' UTR region and it was reported by the WGS500 project. It was identified in two independent families with erythrocytosis and is sufficiently substantiated by segregation data to assume its disease causing effect, most probably due to increased *EPO* regulation.²⁹ Three variants are located within exons 2 and 4 in patients with FE⁴; however, the causative for FE needs to be confirmed. Pathogenic variant c.32delG located in exon 2 causative for FE was recently identified. This pathogenic variant introduces a frameshift in exon 2 and interrupts *EPO* mRNA translation from primary transcript variant 1. Increased transcription of *EPO* mRNA is initiated from an alternative promoter located in intron 1.¹⁰ Two sequence variants located within regulatory regions; rs1617640 and rs551238 were identified in blood donors with significantly increased hematocrit,²⁸ however their causative for FE needs to be reviewed.

We also analyzed if retrieved *EPO* gene sequence variants are located within regulatory regions. Genetic locations of binding regions for EPAS1 (HIF2A), ARNT (HIF1B), and HNF4A binding sites were retrieved from publications^{16,18,19} and mapped to the sequence according to the latest genome assembly. Downstream from the EPAS1 and ARNT binding site (HIF binding site, HBS), the *EPO* 3' enhancer contains a series of CA repeats (HIF ancillary sequence, HAS) and two tandem consensus steroid hormone response elements 5'–YGACCY–3' separated by 2 bp, binding sites for HNF4A (nuclear receptor half site, DR-2). The analysis revealed that seven variants associated with erythrocytosis or hematocrit level (Table 2) are not located within regulatory regions (Figure 1). We additionally screened Ensembl database and retrieved two SNPs located within regulatory regions (rs796952255 and rs539229161; Figure 1). These two SNPs have unknown function and were also not yet tested in association with FE.

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* regulation²⁹ 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 retinopathy³⁴ 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 performed 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.

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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>