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Mutation analysis of *EPO* and *EPOR* genes in two patients with familial erythrocytosis: a novel molecular-genetic diagnostic test for *JAK2* negative erythrocytosis

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Introduction

Erythrocytosis is heterogeneous group of disorders characterized by the expansion of the erythrocyte compartment including elevated red blood cell (RBC) number, haematocrit, and haemoglobin content in the peripheral blood. Familial erythrocytosis (FE) is a group of rare congenital disorders with various genetic background. Erythropoietin receptor gene (*EPOR*) mutations are the indicator for primary familial erythrocytosis. Secondary erythrocytosis syndromes are typically associated with a defect in various genes included in oxygen sensing pathway that leads to the increased erythropoietin production [1]. The hormone erythropoietin (EPO) and its receptor (EPOR) are the main regulator of RBC production in the bone marrow. Current diagnostic procedure in Slovenia enables exclusion of *JAK2* gene mutations, the cause of polycythaemia vera (PV). **The aim of our study** was the introduction of new molecular-genetic test for *EPO* and *EPOR* genes in *JAK2* negative erythrocytosis.

Methods

We performed the search of the Ensembl database and literature (PubMed) to find genomic locations in *EPO* and *EPOR* genes previously connected with erythrocytosis. Findings were complemented with data from other databases: erythrocytosis.org, Leiden Open Variation Database (LOVD), Online Mendelian Inheritance in Man (OMIM), The Human Gene Mutation Database (HGMD), HUGO Gene Nomenclature Committee (HGNC), SNPedia, UniProt, ScienceDirect.
Second phase included PCR amplification and sequencing of promoter and 3' enhancer of *EPO* gene and exon 8 of *EPOR* gene (**Figure 1**).

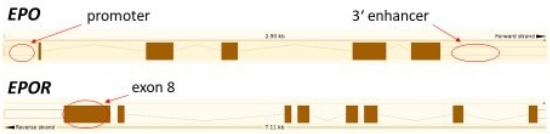

Figure 1: Sequenced regions of *EPO* (promoter and 3' enhancer) and *EPOR* (exon 8) genes.

Results

Two related patients with erythrocytosis were negative for *JAK2* V617F and *JAK2* exon 12 mutations, indicating exclusion of PV. The level of serum erythropoietin was in the reference intervals (**Table 1**). Secondary reasons for increased RBC mass (cardiac, pulmonary and endocrine) were excluded. Ensembl database and literature search was performed to detect the most common mutations in *EPO* and *EPOR* linked with previously described clinical cases of familial erythrocytosis. Sequence analysis of *EPO* promoter and 3' enhancer and *EPOR* exon 8 was performed (**Figure 2**).
Primary familial erythrocytosis due to *EPOR* mutation was excluded by sequence analysis in both patients. So far, 24 mutations in *EPOR*, located in exon 8, have been associated with erythrocytosis. Exon 8 encodes the C-terminal negative regulatory domain of the protein. Mutations are leading to cytoplasmic truncation of the receptor and loss of the C-terminal negative regulatory domain [2].
However sequence analysis revealed polymorphism rs551238 in 3' enhancer region of the *EPO* gene in both patients, previously described in blood donors with upper limit haematocrit (**Figure 3**) [3]. The role of erythropoietin in erythrocytosis is indirect and previously had not been linked to the disease.

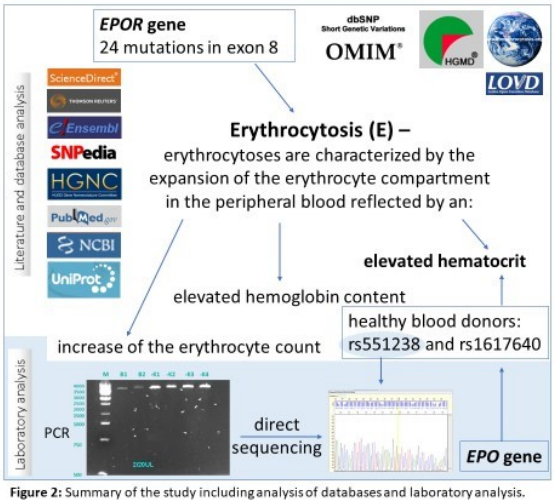

Figure 2: Summary of the study including analysis of databases and laboratory analysis.

	P1	P2	C1	C2	C3	C4	Reference	Unit
gender	M	M	F	F	M	F		
S-Epo	9.2	8.3	/	/	/	/	3.3-16.6	E/L
Hb	**221**	**192**	129	129	146	137	133-167	g/L
Ht	**0.650**	**0.552**	0.369	0.385	0.438	0.400	0.39-0.50	
RBC	**6.36**	**6.29**	4.17	4.26	4.81	4.46	4.32-5.66	10^{12} /L

Table 1: Haematological analysis of two patients (P1 and P2) and four negative controls (C1-C4), showing elevated values (bold) of haemoglobin (Hb), haematocrit (Ht) and total red blood cells (RBC) in both patients.

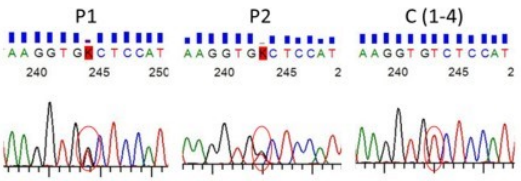

Figure 3: Result of the sequence analysis of the polymorphism rs551238, located within 3'UTR region (enhancer) of the *EPO* gene. Both patients (P1, P2) have heterozygous (GT) genotype and all negative controls (C1-C4) have homozygous (TT) genotype.

Conclusions

We have successfully introduced new molecular-genetic test for analysis of the *EPO* (promoter and 3' enhancer) and *EPOR* (exon 8) mutations and implemented it in clinical use. *EPO* polymorphism rs551238 might be involved in disease development, however, the study on a larger population of subjects in the Slovenian population is in progress. Both patients (P1, P2) have heterozygous (GT) genotype and all negative controls (C1-C4) have homozygous (TT) genotype for the analyzed polymorphism. Future recommendation is to complement the diagnostic algorithm with mutational analysis of other genes involved in disease development.

Acknowledgements
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References
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