

CARE FOR HAEMOGLOBINOPATHY PATIENTS IN SLOVAKIA

Viera Fábryová¹, Peter Božek¹, Monika Drakulová², Andrea Kollárová³, Zuzana Lалуhoová Striežencová⁴, Michaela Macichová⁵, Adriana Sakalová⁴

¹Department of Haematology, Hospital St. Michael, Bratislava, Slovakia

²Laboratory of Haematology, Synlab Slovakia, Bratislava, Slovakia

³Laboratory of Molecular Genetic, Faculty Hospital, Nitra, Slovakia

⁴Department of Haematology, Paediatric Faculty Hospital, Bratislava, Slovakia

⁵Department of Haematology, L. Pasteur Faculty Hospital, Košice, Slovakia

SUMMARY

Background: The paper presents the results of 22-year study of screening and follow-up of haemoglobinopathies in Slovakia, an overview of genetic mutations, the coincidence with hereditary haemochromatosis mutations, and the procedure in genetic counselling.

Methods: Between 1993–2015, in three centres in Bratislava and in one centre in Kosice, carriers of beta-thalassaemic genes or other haemoglobinopathies were searched for. Diagnosis was performed by haematologists, whereby the family history was evaluated, together with the overall clinical condition, blood count and blood smear, iron and haemolysis parameters, mutations of hereditary haemochromatosis, and haemoglobin electrophoresis testing. In the last years the haemoglobin division also examined by high performance liquid chromatography (HPLC).

Results: A clinical suspicion of the heterozygous form of beta-thalassaemia or other haemoglobinopathies was documented in 554 patients. Of them 32 (5.8%) were foreigners. 213 (38.45%) patients were genetically examined. In 190 (33.93%) of them heterozygote beta-thalassaemia was confirmed. The most frequent mutations were IVS 1.110 (33.15%), IVS 2.1 (33.15%), and IVS 1.6 (14.7%). Evidence of haemoglobin S (heterozygote sickle cell anaemia) was also notable in two non-relative children, whose fathers were of African origin, and one patient from Ghana. One female patient was followed up for haemoglobin Santa Ana (non-stable haemoglobin previously diagnosed as mutation de novo). In our group, we took care of pregnant patients with haemoglobinopathies.

Conclusions: The study showed that there is a higher number of heterozygotes for beta-thalassaemia and rarely haemoglobinopathies in Slovakia. Over the past years, we have recorded an increase number of foreigners coming to our country. It is necessary to continue in search of pathological gene carriers to avoid serious forms of haemoglobinopathies.

Key words: haemoglobinopathies, thalassaemias, sickle cell anaemia, epidemiological study, Slovakia, ENERCA

Address for correspondence: V. Fábryová, St. Michael Hospital, Satinského 1, 81108 Bratislava, Slovakia. E-mail: viera.fabryova@nsmas.sk

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INTRODUCTION

Congenital diseases of haemoglobin, haemoglobinopathies, are the most common monogenic diseases worldwide. They arise by mutations of the genes for globin chains of haemoglobin. Basically, they are divided into two groups: thalassaemias arising as quantitative defects of normal globin genes with a decreased production of globin chains and structural haemoglobinopathies, which arise as qualitative structural defects of globin genes with the production of pathological globin chains.

Haemoglobinopathies existed originally mostly in the areas with high incidence of malaria, such as Mediterranean regions, some regions of Africa and South-East Asia. Since plasmodia of malaria were unable to survive in the afflicted erythrocytes, the haemoglobin mutations carriers had selective advantage survival and they were able to transfer the mutated genes to future generations.

However, over the past decades the situation has radically changed. Owing to migration of the population from the afore-

mentioned areas to Europe, Northern America and Australia, haemoglobinopathies started to appear in the countries in which they were previously practically unknown (1). Moreover, haemoglobinopathies often appear in serious clinical forms that require exacting diagnostics and treatment leading to economic burden on healthcare systems of the countries, which often cannot cope with the situation. Except for this, the gene pool of European countries also changes (not only in haematology), which may have consequences in the future (2). Furthermore, in Central Europe possible combination of silent alleles should not be underestimated (mostly in heterozygote form) in the original inhabitants as they may outlive in the population for long centuries after the Tartarian and Turkish invasions or in migrants from Southern Europe with new alleles and with the onset of serious homozygotic forms of diseases.

The World Health Organization (WHO) pinpointed this problem as early as in the nineties of the past century and initiated the foundation of the European Network for Rare and Congenital Anaemias (ENERCA), whose role is to map the incidence of rare

anaemias in the European states, to assist in epidemiological studies, diagnostics and treatment of rare anaemias, and finally numerically express the financial impact on healthcare systems (3). ENERCA along with other organizations dealing with the relevant problems, such as the Thalassaemia International Federation (TIF), proposed the creation of the directives for follow-up and screening of haemoglobinopathies in the countries with sporadic incidence and screening programmes for countries with high incidence of these diseases (2, 4, 5).

Slovakia belongs to the first group of countries in which the issue of haemoglobinopathies has not drawn much professional interest so far. However, the efforts to map haemoglobinopathies both in Slovakia and the Czech Republic have already been observed (6). With respect to the already mentioned changing geopolitical situation, in 1993, we decided to establish a study group for beta-thalassaemia and other haemoglobinopathies and began with the search of haemoglobinopathies in Slovakia. The centre of the study included 3 haematology centres in Bratislava (University Hospital Kramáre, Synlab Company; Paediatric Faculty Hospital, Department of Haematology; and Hospital St. Michael, Department of Haematology) and L. Pasteur Faculty Hospital, Department of Haematology in Kosice. Besides, 70 doctors from various workplaces participated in the study, who referred the patients or sent samples for examination to the above centres. The ongoing results of the study were published (7). The study lasted up to the year 2015 and the obtained results are currently presented.

MATERIALS AND METHODS

Patients with suspected haemoglobinopathies were addressed in the local haematological surgeries or were sent directly to the aforementioned haematological centres.

Criteria for participating in the study were as follows:

- positive family history (e.g. originating from certain ethnic areas);
- objective finding (e.g. icterus, splenomegaly);
- microcytosis of erythrocytes in the blood count; MCV (medium volume of erythrocytes) below 78 fl, possibly MCH (medium size of haemoglobin in the erythrocyte) below 25 pg, anaemia was not necessarily present;
- finding in the peripheral blood smear: microcytosis, target erythrocytes, possibly further morphological changes;
- normal or elevated levels of serum iron, particularly ferritin or persistent microcytosis of erythrocytes following treatment by iron preparations;
- positive tests for haemolysis (increased reticulocyte count, reduced levels of haptoglobin, elevated indirect bilirubin, etc.).

Methods:

- parameters of blood counts (reticulocyte count, MCV, MCH) were obtained from normal high-parametre counters of blood cells;
- peripheral blood smears were evaluated microscopically (Papenheim's staining);
- examination of parameters of iron, bilirubin and other biochemical parameters was conducted through standard biochemical analysers; haptoglobin test was performed by laser nephelometry;

- electrophoresis of haemoglobin tests was carried out by the acetate cellulose sheets method in the aforementioned haematological centres. The values of haemoglobin HbA2 below 3.5 % and foetal haemoglobin HbF below 1.1% (adults) were considered normal; higher values for HbA2, possibly for HbF were considered pathological.

- Over the last year, the haemoglobin division was also examined by high performance liquid chromatography (HPLC) method. Only patients meeting the above mentioned criteria were accepted to participate in the study.

If the results came back positive, we would ask the proband's relatives to be referred to us; this included ascending, descending and horizontal line of family.

The genetic diagnostics was conducted by the reverse hybridisation method in the Laboratory of Clinical Genetic, Faculty Hospital, Nitra. In rare cases, we consulted the Department of Biology, Faculty of Medicine, Palacký University Olomouc.

Whereas haemoglobinopathies tend to store iron we wanted to exclude another factor negatively affecting the metabolism of iron. Since 2013 we have also examined the mutations of hereditary haemochromatosis protein (HFE), C282Y, H63D and S65C in patients with the confirmed mutation for haemoglobinopathy.

RESULTS

Altogether 554 patients were diagnosed with suspected beta-thalassaemia or other haemoglobinopathy. Of them 32 (5.8%) were foreigners. 213 (38.45%) patients were genetically examined. In 190 of them (33.93%) heterozygote beta-thalassaemia was confirmed. The distribution of beta globin mutations is illustrated in Table 1. In 25 patients the mutation was unknown. Two non-relative children were diagnosed as heterozygotes for HbS (A→T), whose fathers were of African origin, and one patient from Ghana. One female patient was followed up for haemoglobin

Table 1. Distribution of the study group to the mutations type for beta-thalassaemias

Types of mutations	Patients/Family n	Percentage
IVS 1.110 (G→A)	63/19	33.15
IVS 2.1 (G→A)	63/15	33.15
IVS 1.6 (T→C)	28/9	14.70
Codon 39 (G→A)	21/6	11.05
Codon 8 (-AA)	12/3	6.31
IVS 1.1 (G→A)	10/4	5.26
Codon 121 (G→T)	8/3	4.21
IVS 2.745 (C→G)	6/3	3.16
Codon 27 (G→T), Knossos	4/1	2.23
Codon 5 (-CT)	3/1	1.58
IVS 1.5 (G→C)	3/1	1.58
Codon 17 (AAG TAG, LysSTOP)	1/1	0.53
-87 (C→G)	1/1	0.53
Total	190/67	100.00

A – adenine, C – cytosine, G – guanine, T – thymine, IVS – introne

Santa Ana (non-stabile haemoglobin previously diagnosed as mutation de novo). The group consisted of 44 families, the rest were individuals.

Results of Examinations of Hereditary Haemochromatosis Mutations

Out of 34 patients with the confirmed mutation for beta-thalassaemia, heterozygote mutation for hereditary haemochromatosis (HFE gen) was confirmed in 24 (70.59%): C282Y mutation in 12 patients, H63D mutation in 12 patients.

The coincidences:

IVS 2.745 + C282Y	4 patients
IVS 1.110 + C282Y	7 patients
Codon 39 + C282Y	1 patient
Codon 8 (-AA) + H63D	6 patients
Hb Knossos + H63D	1 patient
IVS 2.1 + H63D	1 patient
Codon 39 + H63D	1 patient
IVS 1.6 + H63D	1 patient
IVS 1.1 + H63D	1 patient
IVS 1.110 + H63D	1 patient

In patients in whom combination of mutation for beta-thalassaemia and for hereditary haemochromatosis was found, magnetic resonance of liver (SIR method) for iron content in dry liver tissue was performed. In neither case of heterozygote combination of beta-thalassaemia and hereditary haemochromatosis, the increased iron content (the normal value is up to 30 µmol iron/g dry liver tissue) was found. Just in one case in menopausal female patient with beta-thalassaemia minor, who is heterozygote for the mutation IVS 1.110 and homozygote for hereditary hemochromatosis with C282Y mutation, an increased value of 66 µmol of iron/g dry liver tissue was found. The female patient had long term treatment with deferasirox (Exjade) per os, (5–10 mg/kg of body weight). She also showed concurrent vitamin B12 deficiency, which was supplemented in injection form.

During 2012–2015, six pregnant patients with haemoglobinopathies were followed up (in 5 patients heterozygote mutations for beta-thalassaemia were found, 1 non-identified haemoglobinopathy was ascertained).

The diagnosis was carried out by ELFO haemoglobin, HPLC and method of molecular genetic diagnostic. Haematological parameters were followed up from the first trimester of pregnancy up to the delivery. The treatment consisted of follic acid and/or vitamin B12 injections intramuscularly. Four of them had normal delivery course, in one case Caesarean section was performed (due to the pathological pelvic anatomy), and in one case complication of non-haematological origin was observed towards the end of

pregnancy. The pregnancy was terminated by Caesarean section, there was postoperative bleeding, allergy to oxytocine and revision of uterus with haemotherapy was required. At control examination after puerperium all patients showed the same values of blood parameters as prior to pregnancy.

We examined the partners of all patients. None of them showed any pathological finding giving evidence of haemoglobinopathy.

In all patients with haemoglobinopathies we drew our attention to pre-conceptional and premarital counselling especially in couples in whom one of the partners came from the area with high incidence of haemoglobinopathies. We also informed all patients with confirmed heterozygote mutations about the nature of their disease, possible ways of transmission to further generations and preventive measures to avoid giving birth to babies with severe forms of the disease.

All patients in our study group showed only heterozygote forms of mutations clinically manifesting as haemoglobinopathy minor or intermedia. The treatment included vitamins, only the aforementioned case of female patient with combined heterozygote beta-thalassaemia and homozygote hereditary haemochromatosis was administered deferasirox (Exjade) tablets. In general, we avoided the administration of iron preparations, with the exception of women in fertile age with metrorrhagias in whom short-term ferrotherapy was used for extreme iron deficiency.

DISCUSSION

There is a great number of various screening programmes for the follow-up of haemoglobinopathies (8–10). The health-care systems of Southern European countries have a long-term practice in neonatal and prenatal screening. These countries (Italy, Greece, Spain) as the only EU countries, have the precise numbers of the incidence of single forms of diseases (11). As these countries have been recently confronted with the waves of migration from Africa and Asia, they have to re-evaluate their screening strategy (12).

Owing to good prenatal screening the countries of North and Western Europe with high percentage of migrants succeed in avoiding the most severe forms of haemoglobinopathies, preventing serious infections, splenic sequestration, acute haemolytic anaemia, and cerebrovascular insult (13). However, sickle cell anaemia, for example, has recently been the most common reason of patients' hospitalization (14).

In Germany, it is supposed that out of 9 million migrants from countries with high incidence of haemoglobinopathies, 4.5% are the carriers of pathological genes. From 1970 to 2010, 5,381 cases have been recorded at just one workplace (15).

Table 2. The incidence of the most frequent mutations in various parts of the world (23)

Europe	West Africa	South-East Asia	South Africa
Codon 39 C→T	29 A→G	Codon 41/45 TTCT	IVS 1.5 G→C
IVS 1.110 G→A	-88 C→T	IVS 1.5→C	IVS 1.1 G→T
IVS 1.6 T→C		Codon 17 A→T	Codon 8/9 + G
IVS 1.1 G→A		-28 A→G	Codon 41/42 TTCT
		IVS 2. 654 C→T	619 bp del

A – adenine, C – cytosine, G – guanine, T – thymine, IVS – introne

ENERCA suggests that the healthcare systems of Central and Eastern Europe should be informed about the emerging problem so that they could create prenatal and neonatal screening programme that should be incorporated into the existing screening of other congenital diseases (12, 16).

Early diagnostics and treatment prolong the life of patients with severe forms of haemoglobinopathies but they also present a certain burden on healthcare system. The individuals with mild heterozygote forms of haemoglobinopathies who transfer mutated genes represent a great risk for prospective generations. The only way to manage this situation and prevent birth of babies with severe homozygote forms of hemoglobinopathies is active screening and follow up of the affected individuals, the proper knowledge on haemoglobinopathy incidence, their types and severity in individual countries. These efforts must be connected with continuous flow of information to the inhabitants, media, patient organizations, and healthcare workers as well as the governments' willingness to support screening programmes (17).

Within preventive programmes the cooperation with selected individuals (couples), i.e. genetic counselling is of great importance (18). According to the type and combinations of mutation we may partially judge the risk of birth of affected baby, the severity of his/her state and treatment modes. The information gives the couples a possibility of prospective option, from preventing the conception to interruption of pregnancy in early stages in case of positive genetic finding of chorionic villi samples.

In our conditions, suspected haemoglobinopathy is tested in clinical or laboratory examination of the patient (mostly microcytosis of erythrocytes with normal values of iron, manifestations of haemolysis, etc.). The method that can confirm the above diagnosis embraces various types of ELFO haemoglobin and HPLC; it is conducted also in our country (19). The methods such as capillary electrophoresis, isoelectric focusing and mass spectrometry dominate the methods worldwide. The final diagnosis is confirmed by genetic tests (20).

In the treatment of haemoglobinopathies independent of transfusions, the follow-up and vitamin administration is recommended (21). This also applies to our study group consisting only of patients with transfusion independent forms of haemoglobinopathies. However, in the past there were also homozygote forms requiring haemotherapy (descendants of Croatian migrants in Western Slovakia) described by Hrubíško and Sakalová (22).

In our study group, still the issue of patients with highly increased value of haemoglobin F remains to be solved. In these patients genetic mutation of globin chain was not confirmed. This finding applies to both families and individuals. We can consider either delta-beta-thalassaemia, or hereditary persistence of foetal hemoglobine of various type.

Another problem to be solved is the diagnostics of 23 patients in whom, despite suspected finding, hemoglobinopathy was not confirmed by any available examination. Since these patients are foreigners and their children originate from marriages with our citizens, we consider the possibility of haemoglobinopathies, that will require the extended genetic examinations for Asian and African mutations. The incidence of the most frequent mutations in various parts of the world is given in Table 2 (23). The finding of mutation IVS 1.5 (G→C) in a Slovak family is peculiar because this type does not belong to common European mutations.

Furthermore, the coincidence of mutations of beta-globin chain and hereditary haemochromatosis also present an interesting problem. Both disease units have a potentiating effect upon the organism overloaded by iron. In our examinations, out of 34 patients more than 70% showed the coincidence of mutation for beta-thalassemia and hereditary haemochromatosis (part II). Due to a low number of the patients examined, unequivocal conclusions cannot be made yet.

CONCLUSION

The global change of geopolitical situation brings about various social and healthcare problems. The expansion of haemoglobinopathies in Europe with the growth of severe clinical forms may be one of them (24).

The WHO formulated new challenges in global epidemiology of haemoglobinopathies:

- Sickle cell anaemias and thalassaemias have widespread distributions throughout malaria endemic areas due to natural selection and globally due to migration;
- Complex interactions exist between different disorders, which influence their severity and geographic distribution;
- Treatments to improve the pathophysiology of these severe disorders are available but mostly only in high-income countries;
- Reliable spatial and temporal epidemiological data are required in order to define adequate and sustainable health policies;
- Primary prevention is necessary to reduce the incidence of affected progeny in healthy individuals (25).

The WHO, ENERCA and TIF conduct various activities to improve chances of couples at risk and the management of haemoglobinopathies. As every year, TIF celebrates 8th May as International Thalassaemia Day focusing on different topics. 2015 was the year of "Enhancing partnership towards patient-centred health systems: good health adds life to years!" aiming to achieve a change in healthcare system by becoming more patient-centred (26).

Over the past years, we have recorded an increased number of foreigners coming to our country, although Slovakia is not one of the target countries for Asian and African migrants. Moreover, our inhabitants work in different parts of the world, our doctors are active in developing countries with high incidence of haemoglobinopathies, there are a lot of mixed married couples who have children with hereditary mutations for haemoglobinopathies. As one of the EU countries we are obligated to take part in the European programmes dealing with different problems. The problem of high flow of migrants from the areas with high incidence of unusual genetic mutations is of greatest significance. It is very important to pay attention to these issues and healthcare institutions should be continuously informed to tackle the problem of haemoglobinopathies in the future.

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REFERENCES

1. Gulbis B, Eleftheriou A, Angastiniotis M, Ball S, Surrallés J, Castella M, et al. Epidemiology of rare anaemias in Europe. *Adv Exp Med Biol*. 2010;686:375-96.
2. Aguilar Martinez P, Angastiniotis M, Eleftheriou A, Gulbis B, Mañú Pereira M del Mar, Petrova-Benedict R, et al. Haemoglobinopathies in Europe: health & migration policy perspectives. *Orphanet J Rare Dis*. 2014 Jul 1;9:97. doi: 10.1186/1750-1172-9-97.
3. de Montalembert M, Ferster A, Colombatti R, Rees DC, Gulbis B; European Network for Rare and Congenital Anaemias. ENERCA clinical recommendations for disease management and prevention of complications of sickle cell disease in children. *Am J Hematol*. 2011 Jan;86(1):72-5.
4. European Union Committee of Experts on Rare Disease (EUCERD). EUCERD Core recommendations on rare disease patient registration and data collection [Internet]. EUCERD; 2013 [cited 2016 Dec 20]. Available from: http://www.eucerd.eu/wp-content/uploads/2013/06/EUCERD_Recommendations_RDRRegistryDataCollection_adopted.pdf.
5. Vives Corrons JL, Mañú Pereira M, Olaya L. New e-Health services for the European network for rare and congenital anaemias (e-ENERCA). *Orphanet J Rare Dis*. 2014 Jul 1;9(1):861. doi: 10.1186/1750-1172-9-S1-P9.
6. Indrák K, Divoký V, Brabec V, Chrobák L, Mociková K, Sakalová A, et al. Dominant beta-thalassaemia alleles in the Czech and Slovak population (beta-thalassaemia mutations in the codons 112 (TA) and 121 (GT) and unstable hemoglobin variants Hradec Králové or alpha,2,beta,2,115 (G17Ala-Asp). *Vnitr Lek*. 1994;40(4):223-30. (In Czech.)
7. Fábryová V, Babusík P, Láluhová-Striežencová Z, Drakulová M, Oslancová M, Macichová M, Sakalová A. Nineteen years study of beta-thalassaemia in Slovakia. *Cent Eur J Public Health*. 2012 Dec;20(4):239-43.
8. Ghosh K, Colah R, Mangani M, Choudhry VP, Verma I, Madan N, et al. Guidelines for screening, diagnosis and management of hemoglobinopathies. *Indian J Hum Genet*. 2014 Apr;20(2):101-19.
9. Thalassaemia International Federation. The future of hemoglobin disorders and rare anaemias in Europe: observations, key findings and recommendations. Consensus document Thalassaemia international federation (TIF). 4th Pan-European conference 7-9 November 2014, Athens, Greece. Nicosia: Thalassaemia International Federation; 2014.
10. Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis*. 2010 May 21;5:11. doi: 10.1186/1750-1172-5-11.
11. Mañú Pereira M, Corrons JL. Neonatal haemoglobinopathy screening in Spain. *J Clin Pathol*. 2009 Jan;62(1):22-5. doi: 10.1136/jcp.2008.058834.
12. ENERCA, TIF, IOM. Haemoglobinopathies on the Move: Is Europe ready? [Internet]. ENERCA; 2013 [cited 2016 Dec 20]. Available from: https://www.enerca.org/media/upload/arxiu/Training/Novartis_OncologyReport_2013.pdf.
13. Dyson SM, Culley L, Gill C, Hubbard S, Kennefick A, Morris P, et al. Ethnicity questions and antenatal screening for sickle cell/thalassaemia [EQUANS] in England: a randomised controlled trial of two questionnaires. *Ethn Health*. 2006 May;11(2):169-89.
14. Colombatti R, Dalla Pozza LV, Mazzucato M, Sainati L, Pierobon M, Facchin P. Hospitalization of children with sickle cell disease in a region with increasing immigration rates. *Haematologica*. 2008 Mar;93(3):463-4.
15. Kohne E. Hemoglobinopathies: clinical manifestations, diagnosis, and treatment. *Dtsch Arztebl Int*. 2011 Aug;108(31-32):532-40.
16. Ryan K, Bain BJ, Worthington D, James J, Plews D, Mason A, et al.; British Committee for Standards in Haematology. Significant haemoglobinopathies: guidelines for screening and diagnosis. *Br J Haematol*. 2010 Apr;149(1):35-49.
17. Loukopoulou D. Haemoglobinopathies in Greece: prevention programme over the past 35 years. *Indian J Med Res*. 2011 Oct;134:572-6.
18. Cao A. Carrier screening and genetic counselling in beta-thalassemia. *Int J Hematol*. 2002 Aug;76 Suppl 2:105-13.
19. Fábryová V, Božek P, Kollárová A. Haemoglobin diseases. Bratislava: A-medi management; 2015. (In Slovak.)
20. Divoká M, Partschova M, Kucerova J, Mojzíkova R, Cermak J, Pospisilova D, et al. Molecular Characterization of β -Thalassemia in the Czech and Slovak Populations: Mediterranean, Asian and Unique Mutations. *Hemoglobin*. 2016 Jun;40(3):156-62.
21. Taher A, Vichinsky E, Musallam K, Cappellini MD, Viprakasit V, Weatherall DJ. Guidelines for the management of non-transfusion dependent thalassaemia (NTDT). Nicosia, Cyprus: Thalassaemia international federation; 2013.
22. Sakalova A. Current problems of diagnosis and treatment of congenital haemolytic anaemia. *Slov Lek*. 1992;2/16(8-9):39-45. (In Slovak.)
23. Wood B, Higgs D. Molecular basis of thalassaemia syndromes. In: Beaumont C, Beris P, Beuzard Y, Brugnara C, editors. Disorders of erythropoiesis, erythrocytes and iron metabolism. Paris: ESH; 2009. p. 251-63.
24. Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. *Blood*. 2010 Jun 3;115(22):4331-6.
25. Piel F. Global epidemiology of haemoglobinopathies: new management challenges. European haematology association learning centre. 2015 Jun 12:103588.
26. Vives Corrons JL, Mañú Pereira M. ENERCA: The European network for patients with rare anaemias. *J Rare Dis Diagn Ther*. 2015; 1(1):1-9.

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