Hemoglobin
international journal for hemoglobin research

ISSN: 0363-0269 (Print) 1532-432X (Online) Journal homepage: http://www.tandfonline.com/loi/ihem20

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To cite this article: Stamatia Theodoridou, Nikolaos Prapas, Angeliki Balassopoulou, Effrossyni Boutou, Timoleon-Achilleas Vyzantiadis, Despoina Adamidou, Evangelia-Eleni Delaki, Eleni Yfanti, Marina Economou, Aikaterini Teli, Olga Karakasidou, Evangelia Skatharoudi, Theodoros Theodoridis & Ersi Voskaridou (2018): Efficacy of the National Thalassaemia and Sickle Cell Disease Prevention Programme in Northern Greece: 15-Year Experience, Practice and Policy Gaps for Natives and Migrants, Hemoglobin

To link to this article: https://doi.org/10.1080/03630269.2018.1528986

Published online: 03 Dec 2018.

HEMOGLOBIN
https://doi.org/10.1080/03630269.2018.1528986

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RESEARCH-ARTICLE

Efficacy of the National Thalassaemia and Sickle Cell Disease Prevention Programme in Northern Greece: 15-Year Experience, Practice and Policy Gaps for Natives and Migrants

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ABSTRACT

Hemoglobinopathies constitute the most frequent monogenic disorders worldwide and in Greece. In Greece, carrier frequency is estimated at about 8.0%, resulting in a heavy disease burden in the past. Therefore, the implementation of a national prevention program of the disease was an urgent necessity. Moreover, due to migration flow from different geographic areas in the last two decades, the observed spectrum of underlying mutations was expanded, leading to the adaptation of diagnostic approaches. We report the results of the National Thalassaemia Prevention Programme in Northern Greece, over a 15-year period (2001-2015). In total 33,837 healthy at-risk individuals (individuals or couples, 91.0% Greeks) were screened. We have screened 1598 pregnancies in 371 (23.0%) (10.0% non Greeks), of whom both parents carried gene defects and were offered genetic counseling. Seventy-six fetuses (23.0%) were predicted to be affected by severe forms of the disease. Following informed parental choices, 73 of the above pregnancies were terminated. Meanwhile, within the study period, 58 new thalassemic babies (five non Greeks) were referred to the Thalassaemia and Sickle Cell Disease Care Unit of Northern Greece, reflecting mostly parental unawareness, choice or the program failure. Based on the region's population, the birth rate and the prevalence of the disease, the anticipated number of new cases is about 45 annually. According to our data, four thalassemic newborns were registered annually at a stable rate in the last 15 years, reaching a reduction of 90.0% of new affected births. Overall, the National Thalassaemia Prevention Programme effectively decreased the incidence of affected newborns in our region.

ARTICLE HISTORY
Received 9 July 2018
Revised 7 September 2018
Accepted 10 September 2018

KEYWORDS
Carrier screening; gene mutations; genetic counseling; hemoglobinopathy; prenatal diagnosis (PND); thalassemia

Introduction

Hemoglobinopathies, mainly thalassemia and sickle cell disease, are caused by gene mutations that affect the synthesis of the normal hemoglobin (Hb) chains or cause structural changes to the Hb molecule and constitute the most frequent monogenic disorders worldwide [1,2]. In Greece, a country of approximately 11 million people, hemoglobinopathies are the most frequent genetic diseases [3].

β-Thalassemia (β-thal) carrier frequency is about 8.0%, while 1.5% of the population are carriers of the β^S gene [β6(A3)Glu→Val, *HBB*: c.20A>T]. The rate of β-thal carriers could be as high as 15.0-20.0% in some areas, showing a totally uneven distribution throughout Greece. The low altitude areas of Thessaly, Western Epirus and Western Peloponnesus display frequencies of up to 15.0%, whereas in high altitude areas and Macedonia (Northern Greece), the incidence is significantly lower [3–5].

The risk of giving birth to a thalassemic child varies from one in 24 to one in 150 couples. Moreover, the number of patients with hemoglobinopathies has increased in many European countries but accurate data is missing in the majority of European Union (EU) countries [6–14].

Greece is a pioneering Mediterranean country in the implementation of both a national hemoglobinopathy prevention program together with a structured patient management plan. The National Programme for Prevention of Thalassaemia was established in 1973 and prenatal diagnosis (PND) has been provided since 1977 [5,15,16].

It was designed with a holistic view in order to diminish the affected newborn cases. Through population screening and PND programs, performed at certain prevention units throughout the country and coordinated by the Thalassaemia National Centre at Laiko General Hospital of Athens, Athens, Greece, indigenous Greeks and immigrants were screened and counseled using their

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national insurance number. When such a number was not available, the required low cost was passed on to the screened individual.

Given the fact that carrier identification is not obligatory, the main aim of the preventive program is to efficiently inform and screen the whole population for the genetically inherited hemoglobinopathies and for the risk of giving birth to a child affected by a severe disease. Many methods have been implemented such as educational programs at schools, in the Armed Forces and through mass media. Moreover the maternity clinics play a crucial role in directing pregnant women to the Thalassaemia Prevention Units for carrier identification. The clinical management of patients is made at dedicated centers (Thalassaemia and Sickle Cell Disease Departments) by specialized physicians all over the country. Currently, the number of registered patients at the care units in Northern Greece is increasing and this is parallel to the immigrants flooding our region. They are mainly people of Albanian, South and East Asian (Kurdistan, Iran, Iraq, Pakistan, Philippines) and African origin [15]. In all these areas, the thalassemia carrier rate and incidence of various hemoglobinopathies are high. Currently, it is estimated that 10.0% of the population in Greece are immigrants, and of these, most (58.0%) are of Albanian origin, who have migrated and settled in Greece in the last two decades [7,9]. The aim of this study was to evaluate the efficacy of the Thalassaemia Prevention Programme in Northern Greece over a 15-year period (2001–2015) and to identify the practice and policy gaps with special attention to the immigration role.

Patients and methods

All referred individuals and couples were screened at the Thalassaemia Prevention Unit of Hippokration Hospital of Thessaloniki, Thessaloniki, Greece, from 2001 to 2015. The hospital acts as a referral center for all the region of Macedonia and Thrace, and is affiliated with the Thalassaemia National Centre at Laiko General Hospital of Athens, Athens, Greece.

Before performing the laboratory tests, a full personal and family medical history was taken by specialized physicians, while the individuals to be tested were fully informed beforehand on the nature of the examinations and the clinical importance of any possible (or negative) findings on an individual basis. The first line screening panel for identifying the carriers of abnormal globin genes and couples at-risk is simple, low cost and similar throughout the prevention network of the country. A complete blood count (CBC), together with the peripheral smear microscopy, ferritin levels, Hb HPLC (high performance liquid chromatography) and electrophoresis were carried out for all subjects of this study, following standard laboratory operating procedures. The laboratory quality assurance was provided by the use of internal and external controls for all the assays at all times, with acceptable intra and inter coefficient of variability.

The hematological analyzer, Coulter ONYX (Beckman Coulter Inc., Fullerton, CA, USA), was used to determine the red cell indices [red blood cell (RBC) count, Hb, hematocrit or packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), RBC distribution width (RDW)]. The VARIANT^{TM} Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA), the VARIANT II^{TM} program, an automated cation exchange HPLC instrument was used for the quantification of Hbs A, F, A_2, S, C [β6(A3)Glu→Lys, *HBB*: c.19G>A] and other Hbs. Hb A_2 levels were further quantified by column microchromatography (Hb A_2; Helena Biosciences Europe, Gateshead, Tyne and Wear, UK). Alkaline and acid pH electrophoresis were carried out whenever abnormal Hbs were detected. Hb H inclusion bodies were identified by incubating the peripheral blood for 30 min. at 37 °C with brilliant cresyl blue. Serum ferritin levels were measured by a micro-enzyme-linked immuno-sorbent assay (ELISA) technique (Abbott Laboratories, Longford, County Westmeath, Ireland) to exclude iron deficiency and the NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) test was performed in all samples.

Heterozygotes were informed about the genetic disorder, and were also provided with an informative leaflet by a specialized physician. It is noteworthy that no special leaflets were available for the counseling of immigrants. Both partners of identified at-risk couples were given genetic counseling in private meetings and PND was offered after explaining the available diagnostic procedures: chorionic villus sampling (CVS) transabdominally or transcervically, or amniocentesis and fetal blood sampling, depending mainly on the gestation age at the time of the procedure. In order to avoid red cell alloimmunization and the risk of hemolytic newborn disease due to the procedure, all pregnant women were fully ABO/Rhesus phenotyped beforehand and screened by the indirect Coombs test. Should the need arise, Rhesus D negative pregnant women were given anti D prophylaxis immediately after the procedure. Characterization of the type of mutation in the parents was carried out regularly before, or in a few cases, simultaneously with fetal DNA analysis.

The screening methods used for DNA analysis were denaturating gradient gel electrophoresis (DGGE), allele-specific oligonucleotide (ASO) analysis, high resolution melting analysis (HRMA) and other polymerase chain reaction (PCR) directed methods [amplification refractory mutation system (ARMS), gap-PCR (PCR amplification across breakpoints)] and DNA sequencing. In selected cases, Hb biosynthesis was performed at the Molecular Genetics Laboratory of the National Thalassaemia Centre, Laiko General Hospital of Athens, Athens, Greece.

The psychological support of indigenous Greek and immigrant couples was made by the same doctor, while, unfortunately, no special social worker was available. Genetic counseling was also offered by the responsible physician of the Northern Greece Unit in close collaboration, where necessary, with the scientific staff of the National Thalassaemia Centre in Athens.

Table 1. Hemoglobinopathy screening, numbers and percentage results for 15 years.

Hemoglobin Screening	*n*	%
β-thal trait	3993	11.80
sickle cell trait	541	1.60
α-thal trait	1557	4.60
δβ-thal trait/Hb Lepore-Boston-Washington	379	1.12
Hb H	34	0.10
Hb C, Hb O-Arab, Hb D-Punjab, and others	805	2.38
iron deficiency	1353	4.00
normal	25,175	74.40
total	33,837	100.00

Results

During the 15-year study period, a total of 33,837 at-risk individuals were screened, either as individuals (30,641) based on the family history or the investigation of RBC indices alterations on the CBC count or in couples (3196) mostly for pre or postnatal purposes at our Thalassaemia Prevention Unit. As shown (Table 1), 9.9% were found to be heterozygotes for β-thalassemia (β-thal), 3.8% for α-thalassemia (α-thal) and 2.8% carried Hb S, Hb D-Punjab (*HBB*: c.364G>C), Hb O-Arab (*HBB*: c.364G>A), and Hb C. Tested individuals were mainly female (63.0%) and the median age of the study group was 34 (14-80) years. In terms of origin, 91.0% were Greeks, while the others were mainly Albanians (3.0%), Africans (3.0%) and Middle East Asians (3.0%).

Within the study population, 1598 couples with ongoing pregnancies were screened. Of those couples, 91.0% were indigenous Greeks, while 9.0% were immigrants from Albania, the Former Yugoslav Republic of Macedonia, Somalia, Nigeria, Republic of Rwanda and Thailand.

In most of the tested pregnancies (97.0%), screening was of a prospective nature. At-risk couples were identified on time, early in pregnancy, showing good adherence to the national prevention program. However, in 13 pregnancies there was a positive family history and the prevention procedure was retrospective, performed only after the birth of a first affected child (eight cases of Greek origin and five immigrant cases). This approach of prevention covered 2.95% of the total indigenous couples at-risk and 18.5% of the total immigrant couples. In 371 (23.2%) couples, both partners (10.0% non Greeks) carried an abnormal globin gene and genetic counseling was offered.

The gene interactions in 247 pregnancies were related with risk of β-thal major (β-TM) for the offpring, in 84 newborns with sickle cell disease and in one for α-thal (Hb Bart's hydrops fetalis) [Table 2(A)]. The remaining 42 pregnancies involved couples who were doubly heterozygous for mutations that do not cause severe clinical disease and were exempted from PND [Table 2(B)].

In the study group, fetal samples were obtained by CVS, performed between 11th and 13th week of gestation ($n = 298$), or by amniocentesis between the 15th and 18th week of gestation ($n = 21$). A very few late-comers were tested by fetal blood sampling ($n = 5$) at 20 weeks of gestation. There were six twin pregnancies (two monogeneic) and only four vaginal samplings (Figure 1). The mean time for results was 5.5 days.

Table 2. (A) Combinations of β-globin gene mutations in couples who underwent prenatal diagnosis.

Combinations of β-Globin Gene Mutations	Number of Cases
β^0-thal/β^0-thal[a] or β^0-thal/β^+-thal	210
β^0-thal/δβ-thal	8
β^0-thal/$\delta\beta^{LBW}$ (NG_000007.3: g.63632_71046del)	22
β^0-thal/β^0-thal + α-thal[a]	1
β^0-thal/$\beta^{O\text{-}Arab}$ (*HBB*: c.364G>A)	3
β^0-thal/$\beta^{Osu\ Christiansborg}$ (*HBB*: c.157G>A)	1
β^0-thal/$\beta^{Knossos}$ (*HBB*: c.82G>T)	1
β^0-thal/β^E (*HBB*: c.79G>A)	1
β^S (*HBB*: c.20A>T)/β^0-thal or β^+-thal	57
β^S (*HBB*: c.20A>T)/β^S (*HBB*: c.20A>T)	20
β^S (*HBB*: c.20A>T) + α-thal/β^S (*HBB*: c.20A>T) + α-thal	2
β^S (*HBB*: c.20A>T)/$\beta^{D\text{-}Punjab}$ (*HBB*: c.364G>C)	1
β^S (*HBB*: c.20A>T)/δβ-thal	1
β^S (*HBB*: c.20A>T)/$\delta\beta^{LBW}$ (NG_000007.3: g.63632_71046del)	3
α-thal/α-thal	1

LBW: Hb Lepore-Boston-Washington.

Table 2. (B) Combinations of β- and α-globin gene mutations for which prenatal diagnosis is not recommended.

Combinations of Globin Gene Mutations	Number of Cases
β-thal/α-thal (globin chain biosynthesis or DNA)	30
β^E (*HBB*: c.79G>A)/β^E (*HBB*: c.79G>A)	1
$\beta^{E\text{-}Saskatoon}$ (*HBB*: c.67G>A)/β^S (*HBB*: c.20A>T)	1
$\beta^{E\text{-}Saskatoon}$ (*HBB*: c.67G>A)/β-thal	1[a]
$\beta^{O\text{-}Arab}$ (*HBB*: c.364G>A)/$\beta^{O\text{-}Arab}$ (*HBB*: c.364G>A)	1
$\beta^{D\text{-}Punjab}$ (*HBB*: c.364G>C)/α-thal	1
$\beta^{D\text{-}Punjab}$ (*HBB*: c.364G>C)/β-thal	1
α^{Setif} (*HBA2*: c.283G>T)/β^S (*HBB*: c.20A>T)	1
α^{Setif} (*HBA2*: c.283G>T)/β-thal	2
−101(C>T) (*HBB*: c.-151C>T)/−101(C>T) (*HBB*: c.-151C>T)	1
−101(C>T) (*HBB*: c.-151C>T)/−87 (*HBB*: c.-137C>G)	1
β-thal/α^{Brugg} (*HBA1*: c.63C>A)	1

[a]Three pregnancies.

The DNA analysis of the fetal samples revealed that only 23.0% of the examined fetuses were positive for a clinically significant hemoglobinopathy. In more detail (Figure 2): 76 (23.0%) fetuses were homozygotes or compound heterozygotes for clinically significant mutations. The couples were thoroughly informed of all the currently available therapeutic approaches for β-TM or sickle cell disease, but the decision for the termination or continuation of the pregnancy was left to parental choice. All the couples except three, preferred the termination of such a pregnancy. One hundred and seventy-five (52.0%) fetuses were heterozygotes (carriers) and, 84 (25.0%) fetuses were normal. In the last two groups, the pregnancies continued to term as expected. In two of the six cases of twin pregnancies, selective abortion of the affected fetus was successfully performed. No cases of misdiagnosed pregnancies with affected newborns were reported in our study population. Throughout the study period, although CVS and amniocentesis are both invasive procedures with approximately 1.0% risk of a miscarriage [17], there was only one obstetric complication (rupture of membranes) that led to a miscarriage in the studied group. No cases of red cell alloimmunization in pregnancy attributed to the prenatal invasive procedure were detected, or a case of induced hemolytic disease of newborn.

Overall, during the study period, every year, on average 15-32 couples underwent PND. It is noteworthy that some couples had several pregnancies and the pregnant women willingly underwent the prenatal screening procedure from

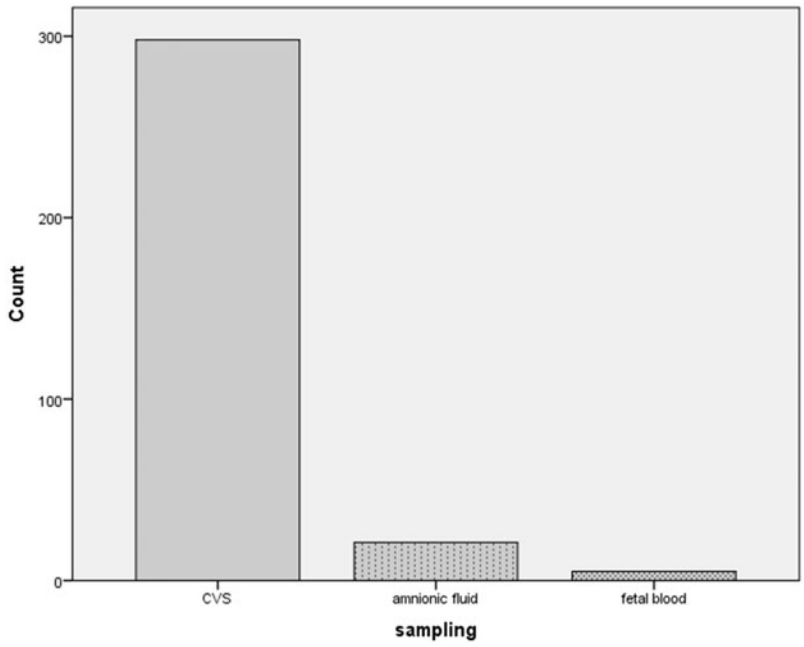

Figure 1. Way of obtaining fetal samples.

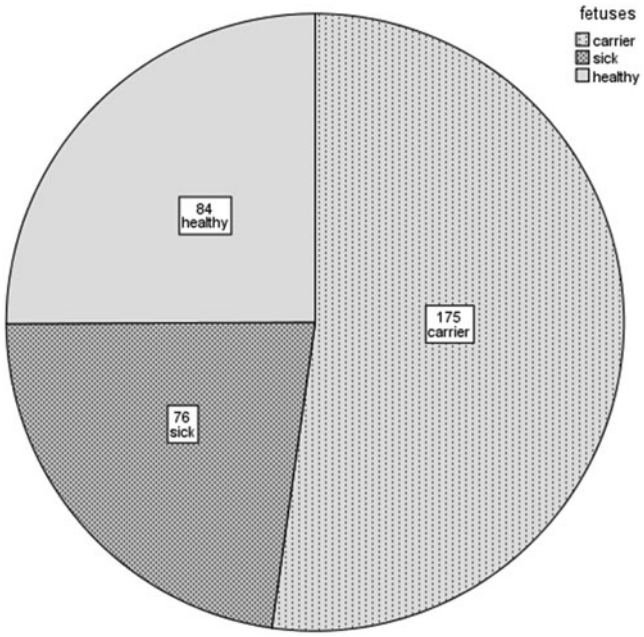

Figure 2. Results of prenatal diagnosis.

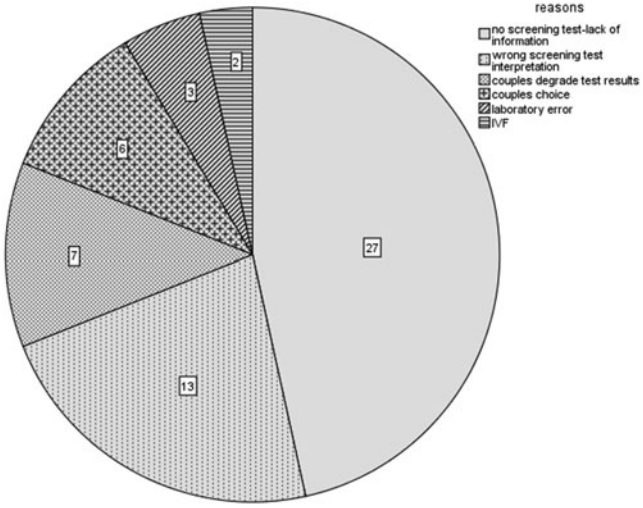

Figure 3. Reasons for new affected births.

two to seven times, in each index pregnancy, in order to give birth to unaffected children.

Meanwhile, within the study period, 58 affected newborns were registered at the local Thalassaemia and Sickle Cell Department, First Department of Paediatrics, Aristotle University of Thessaloniki, Thessaloniki, Greece. Apart from the three cases mentioned above, in which the parents chose not to have an abortion, there were 28 new cases with β-TM, 14 cases with β-thal intermedia (β-TI), and 16 cases with sickle cell disease (compound heterozygotes for Hb S-β-thal). All but five newborns were of Greek origin. The reason for the majority of these births (46.0%) was the inadequate information provided to the couples who did not undergo any prenatal tests for hemoglobinopathies. For the remaining cases, in 22.0%, the test was wrongly evaluated due to obstetrician malpractice, in 15.0%, the couples chose not to consider cautiously the information given, in 10.0% it was a matter of personal way of thinking or religious beliefs. In 5.0%, a laboratory error was identified, either in first line screening or through molecular PND, the analysis and coordination of which, interestingly enough, took place in the private sector, whereas in 2.0%, the reason for the failure to detect an affected fetus was the lack of gamete donors' screening, in cases of *in vitro* fertilization (Figure 3).

Based on our region's population, the birth rate and the local prevalence of carriers of hemoblobinopathies, the anticipated rate of new cases in Northern Greece is around 45 per year [18,19]. In our registries, a stable rate of about four thalassemic newborns was registered annually, the last 15 years reaching a reduction of 90.0% in new affected births.

Discussion

Population carrier screening programs (voluntary or mandatory) are conducted throughout the world and universally have successfully reduced the incidence of β-TM and sickle cell disease birth rates, especially in countries with a high frequency of thalassemia [16,20]. The Greek National Thalassaemia Prevention Programme started as (and still is) a nationwide government-sponsored program [6]. It offers a voluntary and prospective prevention of hemoglobinopathies, before a first affected offspring is born, mainly preconception or early in pregnancy. In our series prenatal counseling and diagnosis was entirely performed always at the same sites Thalassaemia Prevention Unit at Hippokration Hospital of Thessaloniki, Aristotle University Departments of Obstetrics and the Genetic Laboratory of the National Thalassaemia Centre) for 15 years and all couples had at least one healthy offspring. Of utmost importance, as in all genetic diseases, it is the fact that counseling parents at-risk of an affected birth requires extreme sensitivity and involves both parents in the whole process [21]. Counseling should be offered before the first pregnancy in order to give the couple time to make informed reproductive choice. The availability of prenatal genetic diagnostic facilities together with consistency of all component parts involved, had significantly influenced, in our opinion, good compliance.

With regard to specific genotype-phenotype correlations, there were revealed a few uncertainties that were managed on an individualized basis. In detail, concerning the genotype β^0-thal/Hb Osu Christiansborg (*HBB*: c.157G>A) [22] (both β-globin gene mutations), PND was performed, as parents decided to reconsider their choices following the PND result due to unavailability of data about the predicted clinical phenotype of this combination. Fortunately, the result of the PND was compatible with a heterozygous fetus for Hb Osu Christiansborg. As far as the three cases of β^0-thal/Hb O-Arab (*HBB*: c.364G>A), there is uncertainty regarding the phenotypic severity in the affected proband, which may vary from mild to severe disease [23]). As mentioned previously, PND was not carried out in 42 pregnancies, because it was not indicated [24–27].

According to the data of this study, it appears that most couples requesting PND opted for medical abortion when the fetus was predicted to be affected. The fact that all but three couples decided on medical abortion, shows that pregnancy termination in such cases has been ethically accepted in Greece, and more specifically, in the geographical provinces of Macedonia, Thessaly and Thrace, that are covered by our Unit.

Despite the fact that Greece for many decades in the past has suffered from a population drain toward countries with increased demand in work force, lately, the country is experiencing a new era in terms of migration flows. On the one hand, Greece has become a reception country for thousands of immigrants as well as refugees, from at least 120 different countries of the world, and on the other hand, the 'brain drain' of highly trained Greeks is increasing due to the financial recession. In view of all these recent geopolitical and economical changes, which resulted in migration alterations, it is probable that both the incidences of hemoglobinopathies as well as the profile of the screened population would be significantly affected.

The spectrum of mutations was influenced by the number of immigrants from different geographic areas and mutations not previously reported, as is the case of a new β^0-thal mutation codon 7 (G>T) [GAG(Glu)-*T*AG(stop codon)], which was reported for the first time in a male from Albania [28]. Interesting combinations appear to be due to migration, while genetic counseling is based on previously reported data concerning such rare cases. Although there are no official minorities in Greece, there are certain populations such as the Muslim community of Western Thrace and the Romanies who form special groups in the Greek state. In our data within the last 15 years, apart from the Greek couples who participated in the PND procedure, there were also three couples from the Muslin community of Western Thrace, one Romany couple permanently situated in our region, 14 immigrant couples who, in total, had taken part in the PND procedure 27 times (8.3%). It is remarkable that in general terms, the immigrants efficiently used the public health services, even though most of them did not speak the Greek language, were not informed about the prevention of hemoglobinopathies by translated leaflets, and no special policy for the prevention of hemoglobinopathies in immigrants exists. This is mainly accomplished by the Greek National Health Service outpatient maternity clinics that ask all pregnant women to have the hemoglobinopathy screening test. All the salaried immigrants, the asylum seekers, the Romanies and the Thrace Muslim citizens had insurance coverage except for one couple from Nigeria. There were also two couples from the Former Yugoslav Republic of Macedonia and two couples from Albania who came to Greece in order to benefit from the medical prevention program that was not available in their home countries.

Another interesting finding was that out of the 58 affected newborns registered in our region, only five were immigrants. As shown in Figure 4, the main cause of failure to predict the fetus status was that the screening test was not performed at the Public Prevention Unit. Immigrants, probably due to economical and other reasons, showed better compliance and preferred the National Prevention Program as a unique alternative for them. It is of extreme importance for each couple to seek advice and medical care from accredited centers (public or private) and highly specialized professionals in the field, as PND is a complex process involving more than one specialist [29].

In terms of national and personal cost, it is generally accepted that the prevention programs have a significant financial impact and the cost to benefit analysis is in favor of the prevention programs in both developing and developed countries [30,31]. Moreover, the cost of treatment including blood transfusions, iron chelation, treatment of complications and stem cell transplantation, could not be affordable for a large number of patients and mainly for the government.

In conclusion, it is evident that the National Prevention Programme has effectively decreased the incidence of β-TM and sickle cell disease in our region within the last 15 years

(2001–2015), yet there is still room for further improvement. This comprises both the informed attitudes of the Greek population as well as the practices followed by physicians, scientists and other health professionals involved in the field. Although the forced financial migration and the refugee situation pose a large challenge for many countries and require rapid adaptations by the Health Services, immigrants had a low impact on the new cases affected by hemoglobinopathies in our region for the study period. However, the tremendous increase of refugee numbers in our country for the years 2014 and 2015 and on, are some totally new situations that have to be thoroughly studied through the scope of the National Prevention Programme for Haemoglobinopathies. Finally, the constant evaluation of the National Prevention Programme is extremely useful in order to diminish possible new events of severely diseased people with implementation of improvements and adaptations to current needs.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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