

## ORIGINAL ARTICLE

# CARRIER SCREENING AND PRENATAL DIAGNOSIS OF HEMOGLOBINOPATHIES. A STUDY OF INDIGENOUS AND IMMIGRANT COUPLES IN NORTHERN GREECE, OVER THE LAST 5 YEARS

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□ *Hemoglobinopathies constitute the most frequent monogenic disorders worldwide and thalassemias are the most frequent genetic disorders in Greece. Over a 5-year period (2002–2006), 1,375 couples were screened for hemoglobinopathies and counseled at our Thalassaemia Prevention Unit, Hippokration Hospital, Thessaloniki, Greece. In 148 cases (10.7%), both partners carried an abnormal hemoglobin (Hb) gene and genetic counseling was offered. One hundred out of 116 pregnancies were at-risk of giving birth to an offspring carrying either the homozygous or double heterozygous forms of the mutations under discussion. The remaining 16 pregnancies involved couples who were heterozygous for mutations that did not cause severe clinical disease, and were exempted from prenatal diagnosis. Twenty-six fetuses were found to be homozygotes or double heterozygotes for clinically significant mutations. These couples were informed of the danger of having an affected child but the termination or continuation of the pregnancy was left to the couples to decide. Nevertheless, all the couples preferred to terminate the pregnancies. The National Thalassaemia Prevention Programme has effectively decreased the incidence of thalassemia major and sickle cell syndromes in Greece.*

**Keywords** Thalassaemia, Hemoglobinopathy, Gene mutations, Genetic counseling, Carrier screening, Prenatal diagnosis

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## INTRODUCTION

Hemoglobinopathies constitute the most frequent monogenic disorders worldwide. They are due to mutations that affect the synthesis of the normal hemoglobin (Hb) chains or cause structural changes to the Hb molecule.

The thalassemias are the most frequent genetic disorders in Greece.  $\beta$ -Thalassemia ( $\beta$ -thal) carrier frequency is approximately 8%, while 1.5% of the population are carriers of the Hb S [ $\beta$ 6(A3)Glu $\rightarrow$ Val] mutation. The rate of  $\beta$ -thal carriers could be as high as 15–20% in some areas (1). The risk of giving birth to a thalassemic child depends on the incidence of the thalassemic gene in the population under study. This may vary from 1/24 to 1/150 in married couples. It has been reported that Greece shares the same set of thalassemia mutations and a large number of rare defects similar to other Mediterranean countries (2). Currently, this incidence is influenced by the immigration to our country of people of Albanian and Southeast Asian origin (Kurds, Iranians, Iraqi, Pakistani and Philippines). In both areas, the thalassemia carrier rate and the incidence of various hemoglobinopathies are high. Currently, it is estimated that 10% of the population in Greece are immigrants, and of these, most (58%) are Albanians who have immigrated and settled in Greece in the last two decades (3).

Different investigators claim that DNA-based prenatal diagnosis was the most important contribution to thalassemia research in the three decades that followed the mid 1970s (4). Indeed, since the introduction of amniocentesis, chorionic villus sampling (CVS) and DNA analysis, thousands of couples have pregnancies without the fear of giving birth to thalassemic offspring.

The National Programme for Prevention of Thalassaemia was established in 1973. Through population screening and prenatal diagnosis programs that are performed by the National Centre for Thalassaemia in Athens (Greece) and the various Prevention Units throughout the country, ethnic Greeks and immigrants are screened and counseled free of charge. Carrier identification is not obligatory. Nevertheless, one of the main aims of the preventive program is that the whole population, and especially those at risk, are continually informed and made aware of the genetically inherited anemias that affect the Greek population, by implementing methods such as educational programs at schools, in the Armed Forces and through mass media. Maternity clinics also play a big role in directing pregnant women to the Thalassaemia Prevention Units for carrier identification. We report our findings on prenatal diagnosis of thalassemias and hemoglobinopathies in Northern Greece over a 5-year period (2002–2006).

## MATERIALS AND METHODS

The hematological analyzer, Coulter ONYX (Beckman Coulter, Fullerton, CA, USA), was used to determine red cell indices (RBC, Hb, PCV, MCV, MCH, RDW). The VARIANT™ Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA, USA), the  $\beta$ -Thalassemia Short program, an automated cation exchange high performance liquid chromatography (HPLC) instrument, was used for the quantification of Hbs A, F, A<sub>2</sub>, S, C, and other variants. Hb A<sub>2</sub> levels were also quantified by column microchromatography (Helena BioSciences Europe, Gateshead, Tyne and Wear, UK). Alkaline pH and acid electrophoresis was 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-ELISA technique (Abbott Laboratories, Longford, County Westmeath, Ireland).

Individuals found to be heterozygous carriers were informed as to the nature of the genetic disorder, and they were also provided with an informative leaflet. Couples identified as being at-risk were also given genetic counseling and prenatal diagnosis was offered. The methods used for fetal sampling (i.e., CVS, amniocentesis and fetal blood sampling) were explained to the couple. Characterization of the type of mutation in the parents was carried out before or at the same time with fetal DNA analysis. Both DNA analysis and Hb biosynthesis were performed at the National Thalassaemia Centre, Laikon General Hospital, Athens, Greece.

## RESULTS

During the 5-year period, a total of 21,372 subjects were screened individually or as couples at our Thalassaemia Prevention Unit at the Hippokration Hospital in Thessaloniki, Greece. We found that 9.9% were heterozygotes for  $\beta$ -thal, 3.8% for  $\alpha$ -thal and 2.8% had Hb S [ $\beta$ 6(A3) Glu→Val], Hb D-Punjab [ $\beta$ 121(GH4)Glu→Gln], Hb O-Arab [ $\beta$ 121(GH4) Glu→Lys] and Hb C [ $\beta$ 6(A3)Glu→Lys] mutations.

There were 1,375 couples screened for hemoglobinopathies. This represents 6.5% of the total number of births in our hospital. Ninety-one percent of those screened were ethnic Greeks, while 9% were immigrants from the Balkans, Eastern Europe, Asia and Africa. In 811 couples (58.9%), both partners were not carriers of any abnormal Hb gene, while in 416 couples (30.2%), only one partner was heterozygous for thalassemia or another hemoglobinopathy. In 148 couples (10.7%), both partners carried an abnormal Hb gene and genetic counseling was offered. The proportion of at-risk pregnancies and the percentage of carriers in the population examined was found to be relatively high because our Thalassaemia Prevention

Unit, being a reference center for northern Greece, examined selected cases that came from different peripheral hospitals and private clinics.

One hundred out of 116 pregnancies were at-risk of giving birth to an affected child. The remaining 16 pregnancies involved couples who were double heterozygotes for mutations that did not cause severe clinical disease, and were exempted from prenatal diagnosis. A few had a positive family history, while the rest were identified through preconceptional and prenatal carrier screening.

Prenatal diagnosis was mainly carried out by CVS at 11–12 weeks of gestation ( $n = 87$ ), and in a few cases by amniotic fluid sampling ( $n = 11$ ) collected at 16–18 weeks. Two late-comers were tested by fetal blood sampling at 20–24 weeks of gestation. The gene interactions are as follows:  $\beta^0$ -thal/ $\beta^0$ -thal or  $\beta^0$ -thal/ $\beta^+$ -thal (75 cases);  $\beta^0$ -thal/ $\delta\beta$ -thal (four cases);  $\beta^0$ -thal/Hb Lepore (four cases);  $\beta^0$ -thal/ $\beta^0$ -thal +  $\alpha$ -thal (one case);  $\beta^0$ -thal/Hb O-Arab (two cases);  $\beta^0$ -thal/Hb Osu Christiansborg [ $\beta 52(D3)Asp \rightarrow Asn$ ] (one case);  $\beta^0$ -thal/Hb Knossos [ $\beta 27(B9)Ala \rightarrow Ser$ ] (one case);  $\beta^S/\beta^0$ -thal or  $\beta^S/\beta^+$ -thal (six cases);  $\beta^S/\beta^S$  (one case);  $\beta^S$ /Hb D-Punjab (one case);  $\beta^S/\delta\beta$ -thal (one case);  $\beta^S$ /Hb Lepore (three cases).

The results of DNA analyses of the samples were as follows: 26 fetuses were found to be homozygotes or double heterozygotes for clinically significant mutations. These couples were informed of the danger of having an affected child but the termination or continuation of the pregnancy was left to the couples to decide. Nevertheless, all the couples preferred to terminate the pregnancies. Forty-three fetuses were heterozygous carriers and 31 fetuses were completely healthy. In the last two groups, the pregnancies continued to term as expected. Selective abortion of the affected fetus was performed in one of the three cases of twin pregnancies. There have been no reported cases of misdiagnosed pregnancies.

Concerning the couple with  $\beta^0$ -thal/Hb Osu Christianborg (both  $\beta$  gene mutations), prenatal diagnosis was performed because no data regarding the nature and outcome of the interaction of this combination was available. Fortunately, the result of the prenatal diagnosis was a fetus heterozygous for Hb Osu Christianborg.

As far as the two cases of  $\beta^0$ -thal/Hb O-Arab are concerned, there was uncertainty regarding the severity of the clinical condition in the affected proband, which may vary from mild to severe disease (5,6). Therefore, it was decided to proceed with prenatal diagnosis and leave the decision of whether to continue or terminate the pregnancy to the couples themselves after appropriate genetic counseling.

As mentioned previously, prenatal diagnosis was not carried out in 16 pregnancies, because it was not indicated (7–11). The gene interactions in these 16 cases were as follows:  $\beta$ -thal/ $\alpha$ -thal (globin chain biosynthesis)

(10 cases);  $\beta^E/\beta^E$  [Hb E or  $\beta 26(B8)Gly\rightarrow Asp$ ] (one case); Hb E-Saskatoon [ $\beta 22(B4)Glu\rightarrow Lys$ ]/ $\beta^S$  (one case); Hb E-Saskatoon/ $\beta$ -thal (one case); Hb O-Arab/Hb O-Arab (one case); Hb D-Punjab/ $\alpha$ -thal (one case); Hb D-Punjab/ $\beta$ -thal (one case).

## DISCUSSION

It is universally accepted that thalassemia prevention programs are successful in countries with a high frequency of Hb mutations, and prenatal diagnosis is mandatory in all at-risk couples (10). The National Thalassemia Prevention Programme has effectively decreased the incidence of thalassemia major and sickle cell syndromes in our country. Today, the very rare cases of children born with severe thalassemia major or sickle cell disease are due to couples failing to use the Thalassemia Prevention Programme for one reason or another. Recently, the spectrum of mutations we observed was influenced by the number of immigrants from different geographic areas. Due to this, unexpected mutations can be discovered, as was the case of a new  $\beta^0$ -thal mutation at codon 7 (G>T) that was reported for the first time in a male from Albania (2,11). Interesting combinations appear and genetic counseling is based on previously reported data concerning such rare cases.

As is the case for all genetic diseases, counseling of parents with the potential danger of bearing an affected child requires extreme sensitivity. Although invasive methods of prenatal diagnosis of thalassemias and hemoglobinopathies have proved successful in decreasing the birth rate of thalassaemic children, the aim in the future is the implementation of less invasive methods, such as prenatal diagnosis through fetal DNA in the maternal plasma or fetal cells in maternal circulation and preimplantation genetic diagnosis in cases of in vitro fertilization. Increased awareness among at-risk population groups and their health care providers is also essential.

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