Incidence of haemoglobinopathies in Sicily: the impact of screening and prenatal diagnosis

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SUMMARY

Background: Haemoglobinopathies are a major public health problem in Sicily; it was estimated a frequency of 1/245 couples are at risk of haemoglobinopathies. This paper reviews legislative actions, prevention activities, carrier screening, genetic counselling, foetal sampling and laboratory methodology analysis evolution reporting the results of 30 years of prevention actions to assess the efficiency of our preventative programme in the control of haemoglobinopathies in Sicily.

Methods: This programme consisted principally of five phases: legislative actions, public awareness campaign, carrier screening, genetic counselling and prenatal diagnosis. Results: These programmes have been very effective, which we can see from a greater public awareness of thalassaemia and its prevention in the target population furthermore by a marked decline in the incidence of thalassaemia major and sickle cell anaemia from 1 in 245 live births in the absence of prevention to 1 in 2000, with a reduction in about 85%. The residual cases were because of a conscious choice by expecting parents in relation to improved life expectancy as well as improved quality of life of the affected patients. Conclusion: The study suggests that public health authorities should act and invest in a similar programme for prevention of thalassaemia, as well as in relation to the increased survival of patients and the consequent organ complications.

Introduction

Haemoglobinopathies are autosomal recessive conditions affecting the expression or the structure of the globin gene products (1). They occur with high frequency in areas endemic for malaria where carriers of the disease are genetically protected and therefore selectively advantaged (2–4). Haemoglobinopathies are a major public health problem in Sicily (5). Our study on the Sicilian population permitted us to estimate a prevalence of 8% of α-thalassaemia (Passarello C., personal communication), 8% of β-thalassaemia (6), 2.5% of δ-globin gene defects (7) and 2% of β-globin gene variants (8,9) characterised by a heterogeneous distribution. The phenotypic and genetic heterogeneity of β-haemoglobinopathies in Sicily has been widely reported and more than 82 molecular defects were identified (Figure 1) (10), with the presence of eight defects (both major and mild mutations) occurring in 95.11% of beta globin thalassaemic mutations and the presence of HbS occurring in 72.1% of the beta variants (9). The same heterogeneity is also found in delta and alpha mutations, but in both cases a single mutation is the most frequent: the HbA2-Yialousa occurring in 81.4% of δ-mutations (7) and the deletion –α3.7 occurring in 73.2% of α-mutations (Passarello C., personal communication).

In the 1980s, 1/245 couples were at risk of β-thalassaemia, considering the number of newborns per year, about 50 new patients with Cooley’s anaemia (beta thalassaemia major) were observed (11). From 1983, in Sicily prenatal diagnosis programmes for the prevention of the most important haemoglobin disorders were developed. This paper reviews the characteristics, the evolution and the results of this ongoing programme of prevention of haemoglobinopathies in Sicily, through a retrospective analysis of data collected over 30 years of activities in our centre.

Methods

Haemoglobinopathies in our country meet the requirements necessary to undertake a targeted
genetic prevention programme to facilitate informed choices in screening, identify couples at risk of haemoglobinopathies, and to provide an appropriate medical report for prenatal diagnosis. This programme consisted principally of five phases: legislative actions, providing information to the population, carrier screening, genetic counselling and prenatal diagnosis (Figure 2).

We performed a retrospective analysis of data collected over 30 years of activities in our centre collecting information both in our database and in paper archive.

**Legislative actions**

For years, thalassaemia and sickle cell disease have been identified as pathologies of high social interest.
not only for the patients and their families but also for the Public Health System which had to take care of the ever increasing cost of the medical follow-up of affected subjects. Sicily is the Italian region with the highest number of surviving patients, 2508 at the end of 2012, of which there were 1762 with thalassaemia major, 126 patients homozygous for HbS, 492 compound βS/β-thalassaemia and 128 with compound haemoglobin variants and thalassaemia (RESTE, 2012). The Sicilian Department of Health (SDH) has invested human and financial resources through appropriate legislative measures or acts of health planning and guidance to establish a network of diagnostic care promoting prevention activities for the research of healthy carriers to identify couples at risk of having a child affected with this disorder (10). Moreover the SDH built a network of services that covers the entire region providing young people with thalassaemia with therapeutic standards of quality care moreover to ensure a financial commitment in favour of thalassaemia as well as associations promoting health education. These benefits were subsequently extended to sickle cell disease and to other forms of congenital anaemia (i.e. glucose-6-phosphate dehydrogenase deficiency, hereditary spherocytosis, dyserythropoietic congenital anaemias, etc.).

**Public awareness campaign**

Most of the couples were informed about β-thalassaemia and drepanocytosis (sickle cell anaemia) through the mass media, general practitioners, paediatricians, obstetricians, meetings with physicians, family planning associations, nurses, social workers and education campaigns. These programmes were directed either at couples with affected children (retrospective diagnosis) or at childless couples (prospective diagnosis). Before the large scale health education campaigns started, laboratories for screening and antenatal diagnosis were organised, a prerequisite of a successful programme. Successively, there was an increase in the number of laboratories for screening and prenatal diagnosis to meet the growing demand of patients.

**Carrier screening and molecular analysis**

In 1990, a regional law established a series of measures in the field of prevention and research for haemoglobinopathies. Free laboratory tests for all women of childbearing age between 13 and 50 years old are now available. If the woman results as a carrier, further investigation for her partner and families would be required. The screening programme was targeted at couples about to marry, conceive as well as in the early stages of pregnancy and was also trialed with students in their last year of high school with great success. The screening programme was designed to obtain a reliable, but essentially presumptive diagnosis of haemoglobinopathies carried out in public and private laboratories distributed throughout the island. Particular reference is given to the centralised laboratory of Palermo where definitive diagnosis is performed, especially in more complex diagnostic cases (Figure 3). All laboratories were strongly recommended to participate in a quality control test regarding the determination of the HbA2 value sponsored by the Department of Sicilian Health (12). To measure and identify abnormal haemoglobin (HbC, HbD, HbO Arab, HbE) and HbF, electrophoresis at pH 8.6 by cellulose acetate membrane was initially used (cut-off = 4.0%), successively anion-exchange microchromatography on column (cut-off = 3.5%) and finally the HPLC method (Dia- mat, and Variant II Bio-Rad Laboratories, Richmond, CA and G8 Tosoh Corporation Shiba, Minato-ku Tokyo, Japan) (cut-off = 3.4%) was a compulsory requirement of approved laboratories for the screening of haemoglobinopathies (13). These cut-off values of HbA2 have been used on the basis of our laboratory experience with reference to the Sicilian population.

![Figure 3](image-url) Organisation of the Regional Network in Sicily for the prevention of haemoglobinopathies
Basic laboratory analysis includes the complete blood count by an automatic machine, a measurement of ferritin to evaluate the iron status and a measurement of HbA2.

More than 20000 subjects were screened every year in Sicily for thalassaemia or abnormal haemoglobin using the flow chart adopted in the screening as reported in Figure 4. A molecular analysis of globin genes (α, β, δ and γ) was performed by one of the several available polymerase chain reaction (PCR)-based methods. Initially dot blot analysis, restriction enzymatic analysis, amplification refractory mutation system (ARMS), reverse dot blot analysis (RDB) and denaturant gradient gel electrophoresis were used (14–18). Actually our procedure involves the use of sequencing analysis of the individual globin gene in relation to the first level results (19). This method provides us with a more complete data of the gene sequence without the use of other methodologies. In our experience, direct sequencing of β-globin gene from position c.-180 in the 5′UTR to c.*266 in the 3′UTR allowed us to identify almost all of the thalassemic mutations and to have a definitive diagnosis for Hb variants in the population. In very rare cases showing an atypical phenotype, the β-globin gene resulted without any sequence alterations (20). GAP-PCR was used in case of suspected carriers for Sicilian δβ-thalassaemia (21), Hb-Lepore-Boston (22), β-globin gene deletion (23). For α-thalassaemia mutations GAP-PCR and gene sequencing analysis were performed (24). In the rare case of suspected large deletions in the globin clusters, including the deletion of the Locus Control Region, a multiple ligation-dependent probe was used as an alternative method of analysis (25).

Genetic counselling

All couples at risk of haemoglobinopathies have undergone genetic counselling prior to prenatal diagnosis. Genetic counselling was carried out in the presence of different specialists: a haematologist, a foetal medicine gynaecologist and a molecular geneticist. Each specialist provided information specific to his field of study: The haematologist provided information regarding the transmission of the disease as well as the prognosis and treatment of patients with thalassaemia, the gynaecologist highlighted the procedures for the sampling of foetal material as well as the risk of miscarriage while the molecular geneticist reported laboratory methods used for the analysis of foetal material and the risk of misdiagnosis. The information provided was clear, complete and unbiased. At the end of genetic counselling, a consent form was signed by the patients for both their DNA and that of their unborn child’s DNA to be tested, as well as for DNA storage and the use of remaining DNA for research purposes. Molecular techniques for DNA analysis and the risk of misdiagnosis were clearly reported.

Prenatal diagnosis: foetal material sampling and laboratory analysis

In 1983, fetoscopy was introduced to obtain foetal blood from the umbilical vein in the second trimester of pregnancy (26). This procedure, performed on 328 pregnancies, involved the insertion of a fibreoptic endoscope into the amniotic cavity.
and a little sample of foetal blood was taken under direct visualisation by passing a long 27-gauge needle through the sidearm of the fetoscopy cannula. The foetal blood aspirated was used for globin chain synthesis to measure the percentage of beta chain produced (27). Fetoscopy was replaced by cordocentesis that permitted us to obtain a better quality sample of foetal blood and to reduce the probability of foetal loss (28); the cordocentesis was performed on 642 pregnancies. In 1990’s the PCR method (29) was introduced to amplify regions of globin genes and in the same period villocentesis (CVS, chorionic villus sampling) (30) and amniocentesis (31) were used to obtain chorionic villi or amniotic fluid from XI to XVI weeks of gestation (2970 villocentesis and 652 amniocentesis). Successively, coelocentesis was applied to 222 pregnancies to obtain coelomatic fluid from coelomatic cavity at 7–8 week of gestation, in a period between the end of the 7 week and the beginning of the 9 week of pregnancy, representing the most early invasive prenatal diagnosis for haemoglobinopathies (Table 1) (32–34).

It should be noted that 4477 were prenatal diagnoses for β-thalassaemia, 241 for sickle beta thalassaemia and 96 for sickle cell disease.

During prenatal diagnosis, misdiagnosis may occur for several reasons: i.e. amplification failure of one DNA fragment (allele drop out) because of rare nucleotide variations that may prevent annealing of the PCR primers used in the protocol/method, mispaternity, maternal contamination and sample exchange (35,36). To limit the possibility of misdiagnosis, the foetal trophoblast was examined through an inverted microscope and two different pieces of chorionic villi were used to obtain the foetal DNA. At least two different PCR-based procedures were then used for each prenatal diagnosis (ARMS or RDB and direct sequencing were used for beta globin gene genotyping). Besides mutation analysis, we also carried out tests to detect maternal contamination and/or mispaternity by DNA polymorphism, using variable number tandem repeats (37), HLA-DQα analysis by RDB (38) and the successive analysis of 28 STR by QF-PCR (Figure 4) (39).

**Ethical approval**

Sample collection was started after a written informed consent was administered to the patients, and approved by the Hospital’s ethics committee.

**Results**

**Legislative actions and preventive programme**

Legislative actions were very productive, increasing the number of subjects who come to the centres for screening and the number of at-risk couples who have undergone prenatal diagnosis contributing to the reduction in the incidence of patients with Cooley’s anaemia and drepanocytosis. The effectiveness of this strategy is obvious, since it has been estimated that the treatment cost of 1–2 newborns affected each year is equal to the cost of prevention given that an affected patient would require specialist care for the rest of his/her life (40,41). The success of intensive education programmes has reversed the number of retrospective diagnosis with respect to the prospective diagnosis. Information and screening of high school students (at the end of secondary school) was also efficient prior to conception because awareness of thalassaemia was acquired and maintained at the time of pregnancy. In the last year, we observed an increase in testing for thalassaemia from legal and illegal immigrants living in our country. Most of the women were already pregnant and were of African or Asian descent. They were sent to our institute by their gynaecologists, or from reception and counseling centres for non-EU citizens (Cannata M., personal communication).

**Carrier screening**

Every year, in Sicily more than 20000 carrier screenings were conducted. The number of screenings has been on the rise since 1980 while in the last years the number of subjects undergoing the above test has varied between 16000 and 18000 per year. Basic analysis were performed at the satellite and reference laboratories distributed throughout Sicily. In Figure 5 a simple flow chart is shown used by our laboratory for molecular analysis relating to our experience.

Over 50% of the subjects that arrived at the Reference Laboratory in Palermo showed one or more altered haematologic or electrophoretic parameters or iron deficiency (42).

In the case of a pregnant female with iron deficiency, if possible, the screening was performed on the partner and in the case of a beta-carrier, molecu-

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**Table 1** Diagnostic procedures for prenatal diagnosis of haemoglobinopathies with reference to gestational age

| Procedure for prenatal diagnosis | Week of gestation |
|---------------------------------|-------------------|
| Fetoscopy (globin chain analysis) | 18–22             |
| Cordocentesis (globin chain analysis) | 16–22           |
| Amniocentesis (molecular analysis) | 16–18            |
| Villocentesis (molecular analysis) | 11–15            |
| Coelocentesis (molecular analysis) | 7–9              |

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lar analysis was conducted in both; in the case of the non-availability of the partner, molecular analysis was performed immediately on the woman.

For these reasons, an intensive molecular diagnosis of α, β, and δ-globin genes was performed in relation to the high heterogeneity of molecular defects that affects all globin genes in Sicily. Eighty-two β-globin gene defects have been identified so far of which the large majority are point mutations and two deletions that produce the Sicilian dβ-thalassaemia and Hb-Lepore Boston (9). A consistent number of mutations in alpha globin genes, included both triplicated arrangements of α-globin genes (Passarello C., personal communication) and delta globin gene defects were observed (7). A significant proportion of the population (20%), especially pregnant women, had ferritin levels below the normal range (25–250 mol/l) showing the same microcytic blood picture as α-thalassaemia, therefore the two conditions can easily be confused or coexist (43). A substantial number of subjects were observed with a normal or borderline level of HbA2 showing a triplication of β-globin genes (aaa anti 3.7 and aaa anti 4.2), silent promoter mutations (the -92 C>T and -101 C>T) (44) or mild β-thalassaemia defects co-inherited with δ-thalassaemia mutations which determine a decrease in δ-chain production. Careful observation of the blood and electrophoretic data allows us to recognise if one or more parameters are not normal or borderline. In the cases of subjects with mild mutations of the β-globin gene such as IVS1 nt 6 in association with a mutation of the δ-globin gene it is easy to observe a reduced value of MCV in the presence of a normal HbA2 level, while in the case of carriers of mutations in the promoter gene the haematological parameters are normal while the HbA2 value will be borderline. The presence of a δ-chain A value may also be associated with normal HbA2 levels (45). This condition may be recognised from the presence of an additional peak on the chromatogram in the region, where HbA2 also migrates (46).

**Genetic counselling**

A questionnaire administered to couples after genetic counselling showed that 96% had fully understood the information received while in 4% of cases subsequent counselling was required. It was estimated that couples during their first pregnancy had more difficulty in understanding as well as in their approach to prenatal diagnosis because of a particular emotional state.

**Prenatal diagnosis**

Between 1983 and 2013, 4814 prenatal diagnoses were performed of which 970 by globin gene synthesis after blood sampling and 3844 by molecular analysis after CVS, amniocentesis and, in the recent years, by coelomatic fluid sampling (Table 2). A progressive increase in the number of prospective diagnoses was observed compared with retrospective diagnoses, which nowadays represent the large majority (more than 80%). Failures of foetal material sampling were low, 6 cases (0.6%) for foetal blood sampling while in 8 cases (0.2%), it was necessary to repeat the sampling of chorionic villi. According to our experience, foetal loss after sampling was about 2.5% in fetoscopy, 1.5% in cordocentesis, 0.7% in villocentesis, 0.3% in amniocentesis and 0.7% in coelocentesis. Almost all pregnancies with affected foetuses were terminated within 1 week.
DNA sequencing of the complete β-globin gene helped us to exclude an eventually thalassaemic paternal allele. In three couples at risk of β-thalassaemia (0.07%), prenatal diagnosis was envisaged at 50% owing to the fact that one of the parents showed β-thalassaemia like haematological features, however, the β-globin gene was found to be structurally intact by sequence analysis from position −600 to +400 bp relating to the β-globin gene. The test failed to detect any disease-causing mutations. The analysis of the triplicated α globin gene rearrangements and the study of the linkage, both the silent and the typical β-like determinants, were found not to be linked to the β-globin cluster. Two couples were asked for prenatal diagnoses, while the third refused a prenatal diagnosis and the child was born unaffected. Four prenatal diagnoses were performed for α-thalassaemia because of the fact that the couples were at risk of foetal hydrops.

**Efficacy of screening and prenatal diagnosis**

A substantial decline in the birth rate of affected subjects has been observed since the programme of screening and prenatal diagnosis started in Sicily. The incidence of haemoglobinopathies has declined from 1:245 live births to 1:2000 because of this prevention programme, even if there was certain variability between different years.

**Discussion**

Sicily is a region located in southern Italy with a resident population of 5 million. 8% of the general population carries the β-thalassaemia trait thus there is an incidence of about 50 babies born each year with a severe form of β-thalassaemia and sickle cell disease. In Sicily, the management of thalassaemia and other haemoglobinopathies was achieved through population screening involving 220 public institutes and private laboratories spread all over the region, the counselling of carriers and prenatal diagnosis of high-risk couples. The primary effect of the programmes for preventing β-thalassaemia has been the acquisition by the couples at risk of the appropriate knowledge to make informed decisions regarding the available reproductive options. This awareness of the genetic risk and methods of prevention has led to a marked acceptance of foetal testing with a consistent decline in the birth rate of thalassaemia major. In our experience, CVS appears to be the safest and most reliable procedure owing to the fact that it is carried out in the first trimester of pregnancy for speed, feasibility and low risk of foetal loss. In recent years, there has been an increased request for coelocentesis because of the fact that it is

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**Table 2** Data from activity of prenatal diagnosis of haemoglobinopathies in Sicily from 1983 to 2013

| Prenatal diagnosis of haemoglobinopathies in Sicily (1983–2013) |  |
|---|---|
| Globin chain analysis | 970 |
| DNA analysis | 3844 |
| **Total** | **4814** |
| **Affected foetuses** | **1177 (24.45%)** |

| Diagnostic errors |  |
|---|---|
| Globin chain analysis | 4/970 |
| DNA analysis | 1/3844 |

8% of at risk couples consciously refused prenatal diagnosis or they did not want to terminate the pregnancy of the affected foetus after prenatal diagnosis. During the course of this study, 21 twin pregnancies were referred for prenatal diagnosis. CVS or amniocentesis was used to sample the twin foetuses at 12–18 weeks of gestation. In 13 of the twin pregnancies, both babies were either normal or carriers, in three cases both foetuses were affected and in five pregnancies one foetus was normal and the other was affected. Selective selection was performed in three of these pregnancies while in two other cases the couple decided to continue the pregnancy and the babies were born as diagnosed. Six of prenatal diagnoses by globin chain synthesis were incorrect. Errors were because of borderline results for β-thalassaemia and homozygous foetuses diagnosed as heterozygous. In all cases, DNA analysis confirmed a compound heterozygosity for the mild β+-thalassaemia mutation IVSI-6 (T>C), with a high frequency in Sicily (14.62%) that could not have been correctly diagnosed because of the limits of this technique. No human errors were recorded for misinterpretation of results or the crossing-over of samples from different pregnancies. In 1992, 1 of 3844 prenatal diagnoses were incorrect for maternal DNA contamination using PCR and RDB. In this case, both subjects of the couple were carriers of beta IVSI-1 mutation and the foetus showed one mutated allele (IVS1-1) while the other one was normal. At birth, it was discovered that the baby was affected and homozygous for IVS1-1 mutation. The cause of misdiagnosis was because of the presence of maternal tissue in the foetal samples that contaminated the DNA; the maternal contamination was not detected because of the fact that no contamination test was available in that period. The data in 3844 diagnoses showed fifteen cases of non-paternity (0.4%). In 10 cases, a definitive diagnosis of the foetal genotype could be given because it was possible to study the DNA of the biological father, while in the other five cases direct
the earliest procedure for invasive prenatal diagnosis (7–9 weeks of gestation) and the acceptance of this procedure was greater than for other procedures, especially for women who had undergone an abortion after the diagnosis of an affected foetus.

The incidence of thalassaemia major and sickle cell disease showed a decreasing trend of newborns with haemoglobinopathies from 1:246 to 1:2000 after the introduction of prenatal diagnosis with an effective reduction in about 63% of cases, even if there was a certain variability between different years (Figure 6). A slight increase in the incidence of cases after 2000 was observed, this phenomenon will be followed over time to distinguish whether it was an increase that occurred at random or whether it was in fact a real trend. From data extrapolated from The Sicilian Thalassaemia and Haemoglobinopathies Register (RE.S.TE), the apparent upward trend of recent 3 years may be largely because of a conscious choice of parents in relation as well as because of the improved quality of life of patients with thalassaemia and SCA. As shown in Figure 7, before 1984, in Sicily, the largest number of subjects living with thalassaemia major had an age between 5 and 15 years and they did not exceed 30 years of age. Reassessing the distribution of thalassaemia it is possible to observe an increase in life expectancy of patients with a median age that increased from 10 years in 1984 to 33 years by 2012. An increase in living thalassemic patients over 50 years was also noted while there was a noted reduction in affected subjects from 1 to 14 years of age. Regarding the affected newborns, 65% of births were because of a conscious choice of couples that refused foetal diagnosis or abortion, while 35% was because of two main causes: (i) the lack of information sharing between many health professionals like family physicians, gynaecologists and obstetricians (5%), (ii) laboratory errors (30%) in the carrier screening test. Regarding these last two points, it is necessary to expand the information campaign for health professionals and to improve quality controls, particularly for private laboratories.

Consequently, the effectiveness of the prevention programme was in fact higher than the percentage of 85% mentioned above.

Although α-thalassaemia is present at a similar frequency to β-thalassaemia, −α[3.7] deletion is the most common α-thalassaemic defect in Sicily (73.2%) therefore in these specific cases prenatal diagnosis is not required, except in the rare cases of haemoglobin H hydrops foetalis syndrome.

In the majority of cases, Hb H disease results from the double heterozygosity for α²-thalassaemia because of deletions that remove both linked α-globin genes on chromosome 16, and deletional α²-thalassaemia from single α-globin gene deletions (−/−α). How-
ever, Hb H disease may occur from interactions between $\alpha^2$-thalassaemia with non-deletional mutations ($\alpha^2$ or $\alpha^2$) (47). Two unstable haemoglobins are present in the blood: Haemoglobin Barts (tetrameric $\gamma$ chains) and Haemoglobin H (tetrameric $\beta$ chains). Both of these unstable haemoglobins have a higher affinity for oxygen than normal haemoglobin, resulting in poor oxygen delivery to the tissues. There is a microcytic hypochromic anaemia with target cells and Heinz bodies (precipitated HbH) on the peripheral blood smear, as well as hepatosplenomegaly. In a steady state, patients with Hb H diseases have haemoglobin levels around 9–10 g/dl; however, during haemolytic crisis, which frequently develops in or after acute infections with high fever, the haemoglobin level may drop significantly and patients can develop shock or renal shutdown. Although splenectomy leads to a significant elevation of haemoglobin levels, it is not recommended owing to the fact that the majority of patients do well with steady-state haemoglobin levels. Patients with non-deletional Hb H diseases are usually more anaemic with significant splenomegaly. Some may require regular blood transfusions and may present with symptoms as severe as ‘Hb H hydrops foetalis’. However, there is no clear genotype phenotype correlation associated with this severe clinical syndrome since patients with identical genotypes do not necessarily present with the same severity of symptoms (47).

In the last 5 years, the number of diagnostic errors at first level analysis was reduced. This is probably because of the obligatory introduction of quality assurance of the dosage of HbA2 (EQA-HbA2), the most important parameter to define the state of a healthy carrier of beta thalassaemia, by public and private laboratories involved in the diagnosis of haemoglobinopathies. This action has led to a higher standard of practice within laboratories throughout Sicily and a greater awareness of the importance of the screening for haemoglobinopathies. It reveals, however, the need to continue the promotion of public awareness campaigns and health education programmes in the community and the research of healthy carriers, to limit the births of new cases only to those resulting from choice. The increased life expectancy that seems to emerge, however, poses new care demands because of the high incidence of organ complications caused by the prolongation of exposure to risk factors, in relation to the increased survival of patients with severe forms of thalassaemia.

We must consider that care and prevention are not alternatives, they are complementary aspects of medical help for a family with a genetic problem. The data from this study suggest that clinical and public health authorities should act and invest in a similar programme for the prevention of thalassaemia based on health education programmes, and screening activities, which could change the pattern of this disease within a short number of years.

Programmes for the prevention of haemoglobin disorders have been applied and accepted in high prevalence areas for several decades. In fact, these programmes are still being adopted in many parts of the world, developed and adapted to fit the local culture.

The World Health Organisation has advocated and promoted the adoption of these programmes from the early 1970s (48).

Other examples of the successful balance between prevention and patient care are seen in another Italian region (Sardinia) (49) and in Greece (50).

The prevention of various genetic diseases, such as Tay-Sachs disease and haemophilia, had been initiated long before the haemoglobinopathy programmes (51). Programmes aiming at the prevention of new affected births of genetic diseases have been the cause of much controversy and debate since issues of bioethics, culture and religion as well as legal issues are involved. Therefore, the structure of our programme could be easily applied for the prevention of other genetic disorders, developed and adapted to the local genetic background.

**Author contributions**

AG, GD, MV, CJ, MC, FC, FP, GS, VC, DR, FL, CP, AM all contributed to the design of the study. AG, MV and CP drafted the paper. GD, CJ, FP, GS, VC contributed to gynaecologic genetic counselling and foetal material sampling. MV, MC, FC, FL and CP conducted molecular and statistical analysis. DR contributed to haematological genetic counselling and AM, head of the Department of Hematology for rare diseases of blood and blood-forming organs, coordinated the study and supervised the writing. All authors provided revisions of the paper and have approved the final manuscript. AG is the guarantor.

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