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PRENATAL DIAGNOSIS AND SCREENING OF THE HAEMOGLOBINOPATHIES

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SUMMARY

The most important aspects of carrier detection procedures, genetic counselling, population screening and fetal diagnosis of the thalassaemias and sickle cell anaemia are reviewed. Carrier d etection can be made retrospectively, i.e. following the birth of an affected child, or prospectively. Most carrier detection and genetic counselling in population at risk for alpha-thalassaemia an sickle cell anaemia is retrospective. However, some prospective carrier screening programmes for sickle cel anaemia are ongoing in Cuba and Gu adeloupe and very limited screening for alpha-thalassaemia is in progress in some South East Asian populations. As regards beta-thalassaemia, several programmes, based on carrier screening and counselling of couples at marriage, preconception, or early pregnancy, have been operating with several pop ulations at risk in the Mediterranean. These programmes have been very effective, as is proved by the fact that the target population has improved its knowledge of thalassaemia and its prevention, and by the marked decline that has been observed in the incidence of thalassaemia major. Carrier dete ction is carried out by haematological methods, followed by mutation dete ction by DNA analysis. Prenatal diagn osis is accomplished by mutation analysis on PCR-amplified DNA from chorionic villi. Future prospects include automation of the process of mutation detection, simplification of preconce ption and preimplantation diagnosis, and fetal diagnosis by analysis of fetal cells in the maternal circulation.

In this article

- The most important aspect of carrier detection procedures, genetic counselling, population screening and fetal diagnosis of the thalassaemias and sickle cell anaemia are reviewed
- **Key words: beta**-thalassaemia; alpha-thalassaemia, sickle cell anaemia; carrier detection, population screening, prenatal diagnosis

INTRODUCTION

The inherited haemoglobinopathies are large groups of autosomal recessive disorders that include the thalassaemias and sickle cell anaemia. The thalassa emias are caused by the defective (+) or absent (0) production of one of the globin chains of the haemoglobin tetramer. According to the type of globin chain involved, we distinguish alpha-, beta- and delta-thalassaemias (Weatherall and Clegg, 1981). The sickle disorders most commonly resul from homozygosity for the S mutation, an A• T substitution at codon 6 of the beta-globin gene leading to the replac ement of valine for glutamic acid.

The incidence of beta-thalassaemias is high in the Mediterranean area, the Middle East, the In dian Subcontinent, and the Far East. The frequency of the alpha-thalassaemias is particularly high in the Far East, but the cond ition is not rare in the Mediterranean area, the Middle East, and the Indian Su b-continent. Sickle cell anaemia show the highest incidence in tropical Africa. However, due to the population flow and the slave trade, these disease have spread widely nowadays and occur also with relatively high frequency in northern Europe, North and South America, and in the Caribbean. The best available approximate estimate indicates that about 250 million people, 4.5% of the world population, are carr iers of a defective globin gene. Each year about 300,000 affected homoz ygotes are born, approximately equally

divided between sickle cell disorders and thalassaemia syndromes (WHO Scientific Group, 1996).

At present a limited proportion of affected homozygotes may be cured definitively by bone marrow transplantation from human leukocyte antigen (HLA) identical siblings. However, the majority of patients can only count on supportive management at present.

We herein review carrier detection and fetal diagnosis of inherited haemoglobinopathies, which are fundamental issues in the clinical management of these disorders nowadays.

CARRIER DETECTION

Heterozygous beta-thalassaemia, both the beta° and the beta+ type, is characterised by a high red blood cell count, microcytosis, hypochromia, increased haemoglobin A2 (HbA2) levels, and an unbalanced alpha-globin non-alpha-globin chain synthesis. However, several environmental or genetic factors may modify this haematological phenotype, causing carrier identification difficulties (Table 1).

Although iron deficiency may decrease the typical high HbA_2 levels of heterozygous beta-thalassaemia, in our experience they remain within the beta-thalassaemia carrier range except in the case of severe anaemia (Galanello et al, 1981). At any rate, iron studies may serve to rule out associated iron deficiency.

In many carrier detection procedures, the preliminary selection of individuals at risk of being heterozygous for a form of thalassaemia is based on the determination of mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values. It is worth noting, however, that since double heterozygotes for beta -thalassaemia and alpha-thalassaemia may have normal MCV and MCH values, they could be missed by this approach (Melis et al, 1983; Rosatelli et al, 1984). In carrier of beta°-thalassaemia, the alpha-thalassaemia determinants that can give this effect are deletion of two of the four $alpha_2$-globin structural gene and presence of non-deletion mutations affecting the major $alpha_2$-globin gene.

Elevation of HbA_2 is the most important feature in the identification of heterozygous beta-thalassaemia (Weatherall and Clegg, 1981). Nevertheless, a number of heterozygotes for beta-thalassaemia may have normal or borderline HbA_2 levels (Galanello et al, 1994) (Table 2). The first groups of these atypical carriers are heterozygotes for a few mild beta+-thalassaemia mutations, i.e. mutation associated with a consistent residual

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Table 1. Selected heterozygous b-thalassaemia: phenotype modifications

Phenotype	Genotype
Normal red cell indices	a and b thalassaemia interaction
Normal Hb A_2 level	▪ iron deficiency ▪ coinheritance of d and b thalassaemi ▪ some mild b thalassaemia mutation ▪ gdb thalassaemia
Normal red cell indices and HbA_2 level (silent)	▪ silent b thalassaemia mutations ▪ a globin gene triplication
Severe heterozygous b thalassaemia	▪ hyperunstable haemoglobin ▪ coinheritance of heterozygous b thalassaemia and triple a globin gene

output of beta-globin chains from the affected beta locus. A typical example in this category is the case of heterozygotes for the beta+ IVS I nt 6 T • C mutation. A normal HbA_2 level is also a characteristic feature of the beta - and delta-thalassaemia double heterozygotes, who still maintain low MCV an MCH values. These double heterozygotes should be differentiated from the alpha-thalassaemia carrier by globin chain synthesis analysis and/or gene analysis. delta-globin gene analysis may be carried out by the same methods described for beta -thalassaemia, using complementary primers or probes to the prevalent delta-globin gene mutation in each population at risk. Gamma-delta-beta- and delta-beta-thalassaemias also have normal HbA_2. However, delta-beta-thalassaemia is easily defined by the presence of thalassaemia-like haematological features and by a marked increase in HbF, which is heterogeneously distributed.

Another major problem in carrier screening is the identification of silent beta-thalassaemia and the triple alpha-globin gene arrangement, both of which may lead to intermediate forms of thalassaemia by interaction with typical heterozygous beta-thalassaemia. Silen beta-thalassaemias are characterised by normal MCV and MCH values and normal HbA_2 and HbF, and are defined only by a slight imbalance in the alpha - globin/non alpha-globin synthesis (Gonzales-Redondo et al, 1989; G alanello et al, 1994). However, on examining the haematological features of these carriers, it should at times be possible to find some borderline HbA_2 or MCV and MCH values, which may signal the presence of atypical beta-thalassaemia, thus calling for further studies. The most common silent beta - thalassaemia is the beta+ -101 G• T mutation, the other types being very rare (Gonzales-Redondo et al, 1989). Nevertheless, according to globin chain synthesis analysis, the phenotype resulting from the triple alpha-globin gene arrangement is at times co mpletely silent. It is worth noting, however, that compound heterozygotes for silent or typical beta-thalassaemia an double heterozygotes for typical beta - thalassaemia and the triple alpha-globin gene arrangement result in a tenuated forms of thalassaemia (Galanello et al, 1983; Thein et al, 1984; Kulozik et al, 1987).

Table 2. b-thalassaemia heterozygotes with normal or borderline Hb A

Reduced MCV	Normal MCV
IVS I-6 T• C	-101 C• T
delta-thalassaemia + beta thalassaemia	-92 C• T
	IVS II –844 C• G
	Triplicated a gen

An extreme though rare instance of the thalassaemia gene combination that may result in pitfalls of carrier diagnosis is the presence of alpha-, delta- and beta-thalassaemias together. This case may lead to a completely silent phenotype (Galanello et al, 1988).

The sickle cell trait is easily identified by haemoglobin electrophoresis or high-pressure liquid chromatography (HPLC). The phenotype of the sickle cell trait may be modified by co-inherited alpha-thalassaemia, which leads to reduced HbS levels in varying degrees, depending on the number of affected• alpha-globin genes. However, this does not lead to carrier identification problems (Higgs et al., 1982). Based on this analysis of carrier identification, we suggest to follow the flow chart outlined in Figure 1 (Cao and Rosatelli, 1993).

The first group of tests includes MCV and MCH determination and haemoglobin chromatography by HPLC. HPLC may lead to the detection of the most common, clinically relevant haemoglobin variants, such as HbS, HbC, HbD Punjab, HbO Arab and HbE, all of which may result in a sickle disorder in homozygosity or compound heterozygosity.

" In couples at risk, identified by the above carrier detection procedure, the specific mutation is define using one of the several polymerase chain reaction (PCR) based methods."

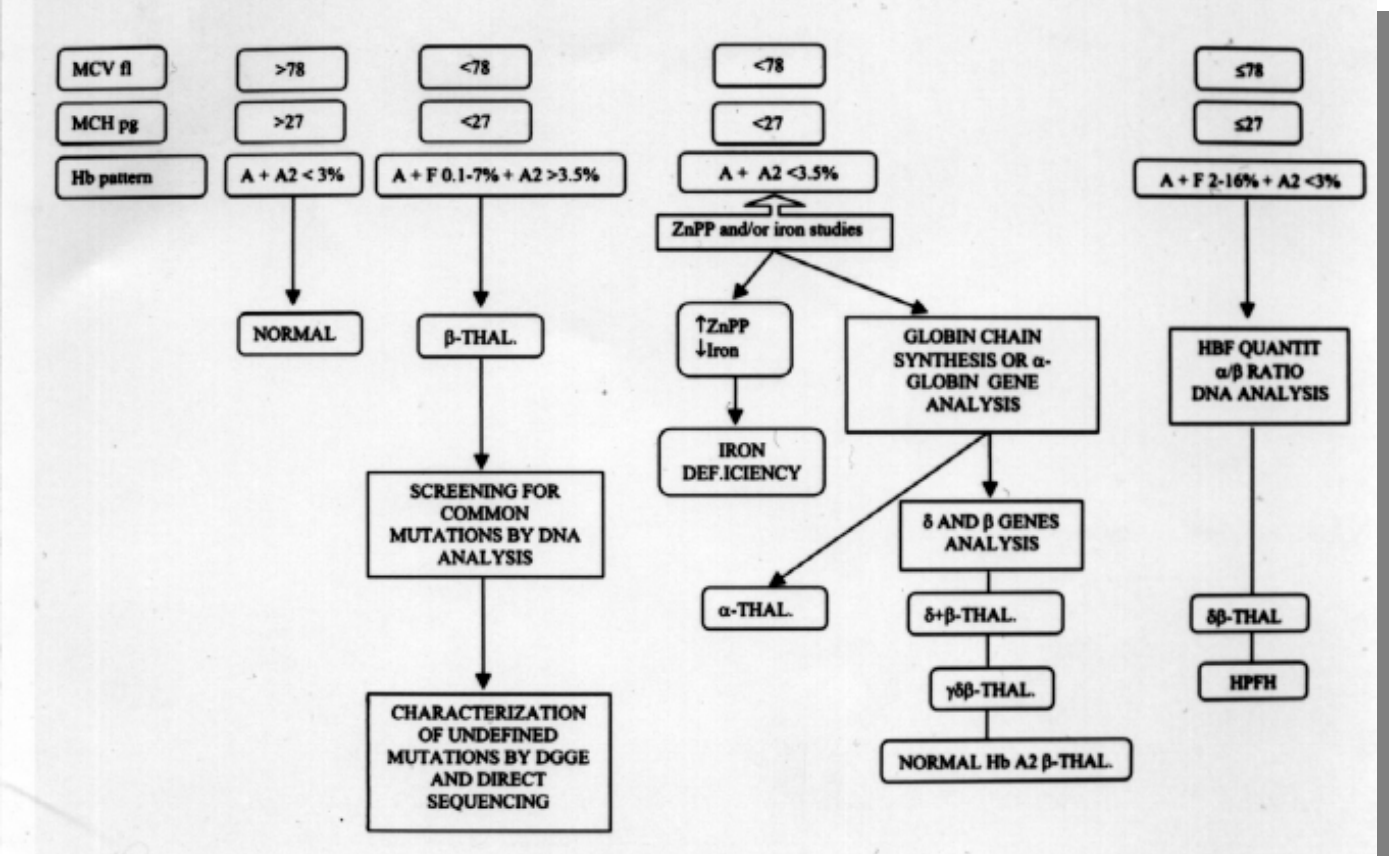

Figure 1: Flow Chart For β-Thalassaemia carrier screening

HPLC may also be used to quantitate HbA_2 and HbF (Galanello et al, 1995). It should be noted that HPL can also detect Hb Knossos, a mil beta-thalassaemia allele, which is no defined by commonly used electrophoretic procedures in haemoglobin analysis. With this flow chart, the only cases that could be missed are the silent beta-thalassaemia and the triple alpha-globin gene arrangement. In the presence of low MCH and MCV levels and high HbA_2 levels, a diagnosis of heterozygous beta-thalassaemia is made. A phenotype characterised by microcytosis, hypochromia, normal-borderline HbA_2 and normal HbF may result from iron deficiency, alpha-thalassaemia, gamma-delta-beta- thalassaemia, beta+delta-thalassaemia or mild beta - thalassaemia. After excluding iron deficiency by erythrocyte ZnPP determination and evaluation of transferrin saturation, the different thalassaemia determinants leading to this phenotype are discriminated by globin chain s nthesis analysis and eventually by alpha-,• delta- and beta-globin gene analysis. In the presence of normal MCV and borderline HbA_2 levels, we suspect the presence of a silent mut ation (for instance beta -101 C• T, beta+ -92 C• T, or beta+ IVS II nt 84 C• G) or the triple alpha-globin gene arrangement, and roceed directly to alpha- and beta-globin gene analysis, since in many of these cases the alpha/ beta ratio could be normal. Defi nition of the type of thalassaemia in these carriers is recommended only in the event of mating with a person with a typical, high HbA_2-beta-thalassaemia, or with a carrier of an undetermine type of thalassaemia. In those rare cases that show normal or low MCH and MCV, normal or reduced HbA_2 levels and high HbF, we suspect the presence of delta-beta-thalassaemia, which should be differentiated from HPFH. We distinguish between delta-beta-thalassaemia and HPFH by analysing the red blood cell distribution of HbF, which is heterogeneous in delta-beta-thalassaemia and homogeneous in

> *"Reverse dot-blot hybridisation uses membrane-bound allele-specific oligonucleotide probes that hybridise to the complementary sequence of the PCR product prepare using patient DNA as the starting template"*

Table. 3 Population distribution of the most common b-thalassemia mutations

ITALY		TURKEY		CHINA	
Mutatio CD	%	Mutation IVS1-110	% 38.7	Mutation CD41/42	% 42.1
IVS1-110	23	IVS1-6	17	CD 17	27.8
IVS1-1	10.5	IVS2-1	11.9	-28	9.5
IVS-6	9.8	CD 8	6.5	IVS-654	7.9
Others	16.7	Others	24.4	Others	12.7

GREECE		INDIA		THAI	
Mutatio IVS1-110	% 42	Mutation -619 bp	% 21.5	Mutation CD 41/42	% 50.9
CD 39	17	IVS1-5	22.4	IVS2-654	11.2
IVS1-1	13.2	CD 8/9	19.5	-28	10.3
IVS-6	7.2	IVS1-1 (G-T)	13.6	CD 17	10.3
Others	20.1	CD 41/42	11.7	Others	17.3

HPFH, by globin chain synthesis analysis (normal in HPFH and unbalance in delta-beta-thalassaemia) and/or bet cluster gene analysis

In populations with a relatively low incidence of both beta- and alpha-thalassaemia, screening by MCV and MCH or osmotic fragility could be acceptable, because in this condition the number of false negatives resulting from double heterozygosity for alpha- and beta-thalassaemia may be very low.

MOLECULAR DIAGNOSIS

In couples at risk, identified by the above carrier detection procedure, the specific mutation is defined using one of the several polymerase chain reaction (PCR) based methods.

Beta-Thalassemia

Beta-Thalassemia is very heterozygou at the molecular level. To date, at least 150 different molecular defects have defined (Huisman and Carver, 1998). The majority of mutations affecting the beta-globin gene are point mutations or oligonucleotide additions or deletions.

Very rarely beta-thalassemia result from the globin gene deletion mechanism. In spite of this marked heterogenity a limited number of molecular defects are prevalent in every population at risk (Table 3). This may be very useful in practice, because the most appropriate probes or primes can be selected according to the carrier's ethnic origin. Mutation detection is carried out on PCR-amplified-beta-globin genes. The most commonly use screening procedures for known mutations today are reverse oligonucleotide hybridisation (RDB) with oligonucleotide probes or primer specific amplification (ARMS).

Reverse dot-blot hybridisation (RDB)

Reverse dot-blot hybridisation use membrane-bound allele-specific oligonucleotide probes that hybridise to the complementary sequence of the PCR product prepared using patient DNA as the starting template (SAIKI et al, 1989) (Figure 2). In this format, multiple pairs of normal and mutant allele-specific oligonucleotides can be placed

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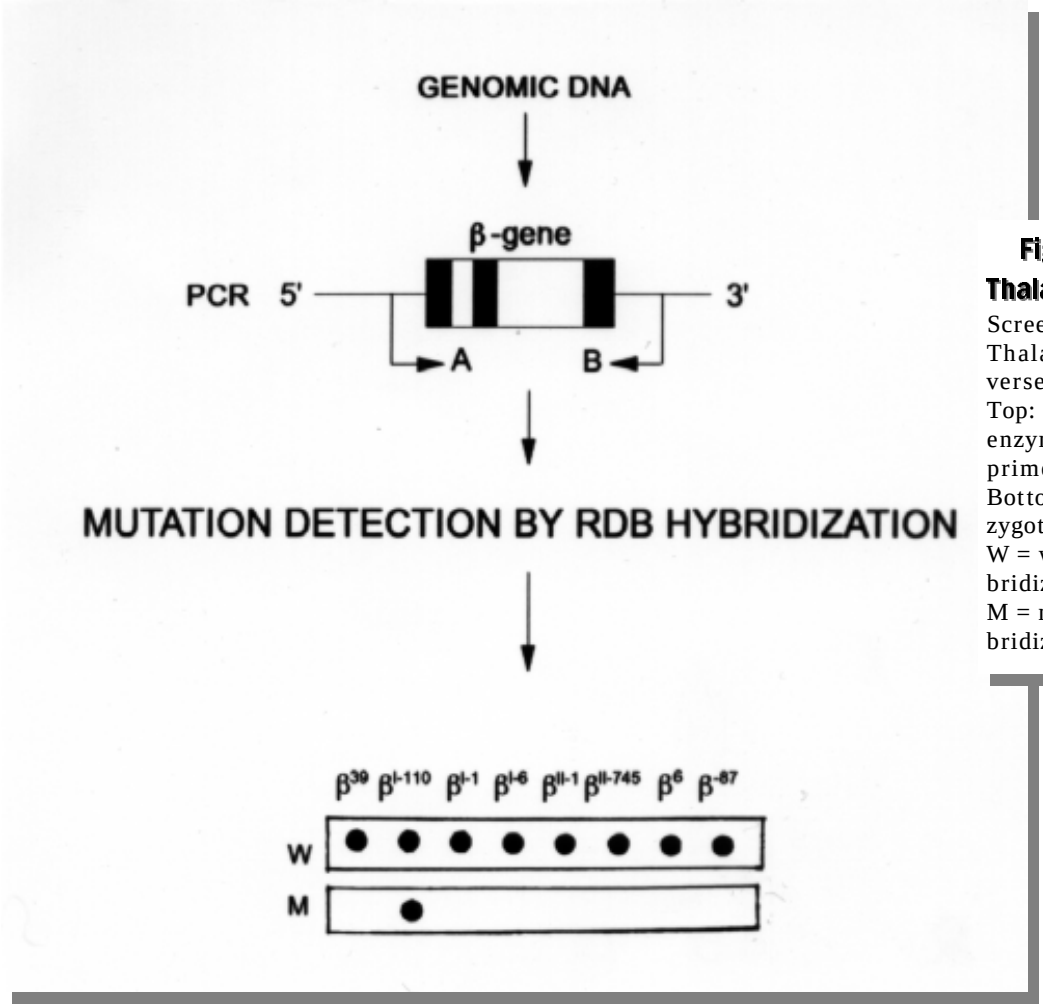

Figure 2: Flow Chart For β-Thalassaemia carrier screening
Screening for the most common ß-Thalassaemia mutations by reverse dot blot analysis.
Top: the whole ß-globin gene is enzymatically amplified usin primer A and B.
Bottom: ß-thalassaemia heterozygote for ß IVS I-110 mutation.
W = wild type oligonucleotide hybridizatio
M = mutated oligonucleotide hybridizatio

on a small strip of membrane. Hybridisation with PCR-amplified beta -globin gene DNA will detect any of the mutations screened in a single procedure. Up to 20-30 mutations have indee been screened in one single step.

Primer-specific amplification (ARMS)

With this method, the target DNA fra gment is amplified using a common primer and either of two primers: a primer complementary to the mutation to be detected (beta-thalassemia primer), or a primer complementary to the normal DNA at the same position (normal primer). Another beta-globin gene fragment is simultaneously co - amplified to control the amplification step of the procedure (Newton et al, 1989). Normal DNA is am plified only by the normal primer DNA from hom o-zygotes only by the beta -thalassemia primer and DNA from heterozygotes by both primers.

Other known mutation-detection procedures

Other methodologies which could be used for mutation detection in beta-thalassemia carriers are oligonucle o-tide ligation assay (Nickerson et al, 1990), restriction enzyme digestion (Pirastu et al, 1989), denaturing gradient gel electrophoresis (Myer et al, 1985, Cai and Kan, 1990; Rosatelli e al, 1992), and primer specific restriction map modification (Gasparini et al 1992).

Unknown mutations

For parents in whom the definition of the beta-thalassemia mutation is not made by one the procedures above de-

scribed, characterisation of beta-thalassemia is obtained by denaturing gradient gel electrophoresis, chemical mismatch cleavage analysis (Orita e al, 1989) followed by direct sequencing (Sanger et al, 1977) on amplified single-strand DNA (Gyllesten and Erlich, 1988).

The most widely used among these methods is DGGE, a gel system tha separates DNA fragments as a function of melting temperature. The beta -globin gene is amplified by using five to seven pairs of primers, one pair of each with an added GC clamp. Normal DNA and DNA from homozygous beta -thalassemia produce a single band with a typical migration pattern, that depend on the globin gene sequences contained in the amplified fragments (Rosatelli et al, 1992a). DNA from heterozygous beta -thalassemia results in the formation of four bands, two of which are homoduplexes of the normal and mutated allele, while the other two are heteroduplexes resulting from a nnealing the strands of the normal allele to those of the mutated allele (Figure 3).

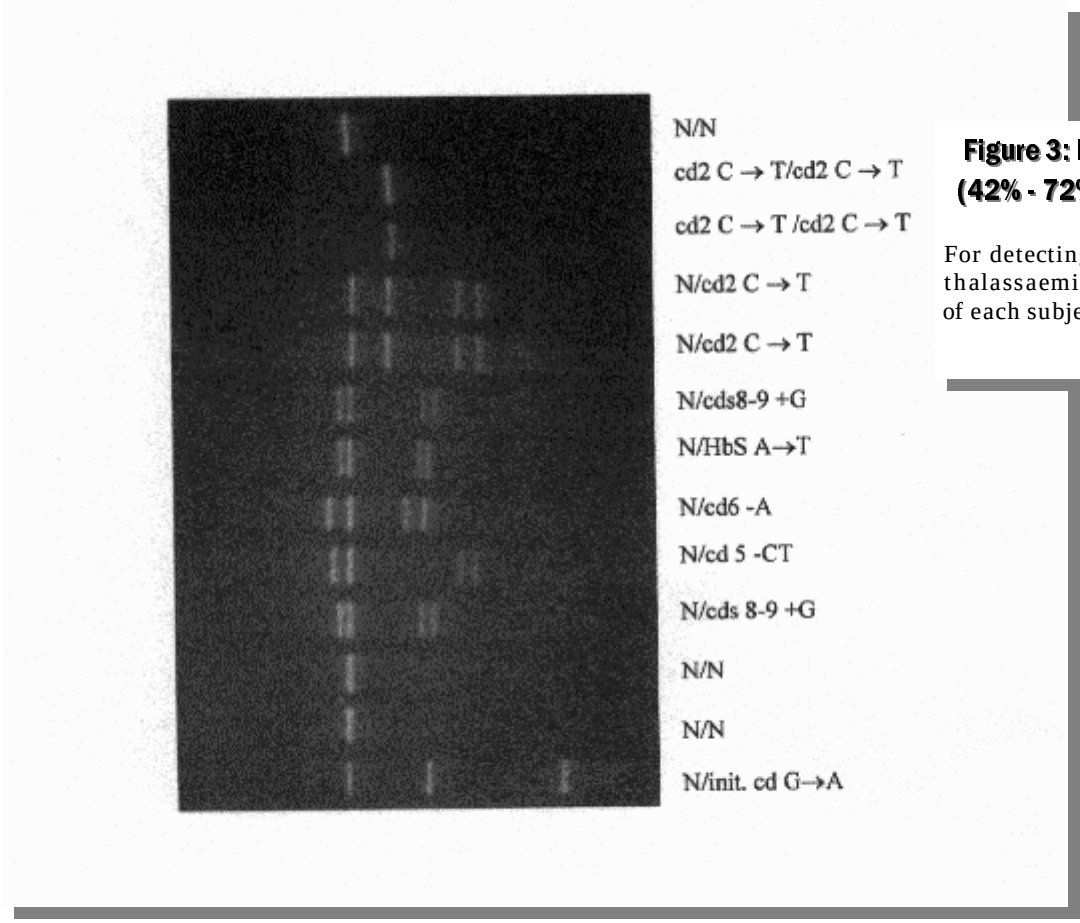

Figure 3: Denaturating Gradient (42% - 72%) Gel Electrophoresis.

For detecting heterozygotes for ß-thalassaemia. The ß-globin genotype of each subject is indicated on top

After localisation by DGGE, the mutation is defined by direct sequencing of the DNA contained in the fragment. Direct beta-globin gene sequencing may be carried out manually or automatically. Alternatively, the unknown mutation may be detected by a fully automated integrated system for DNA fragment analysis (AHPLC).
If a mutation is not detected by DGGE analysis, we search for the presence of small deletions by polyacrylamide ge electrophoresis of the PCR-amplifie products prepared for ARMS or RDB analysis. This may lead to the detection of small deleti ons of the beta-globin gene, suspected from very high HbA $_2$

levels. Larger deletions of the cluster may be identified with restriction fragment length polymorphism analysis by Southern blot or PCR-based procedures.

In a very limited number of cases, direct sequencing from position –600 t 60 bp downstream from the beta-globin gene failed to detect a mutation causing beta-thalassemia (Murru et al, 1990, 1992; Rosatelli et al, 1992b). In these cases, the molecular defect may reside either in the Locus Control Region at the beta-globin gene cluster, or in one of the genes outside the beta-globin gene region encoding for DNA-binding protein that regulates the function of the beta-globin gene (for a review see Orkin , 1990 and Townes an Behriger 1990).

Prediction of a mild phenotype

Homozygosity or compound heterozygosity for beta-thalassaemia usually results in the clinical phenotype of transfusion-dependent thalassaemi major. However, a substantial propo rtion of these homozygotes develo milder forms that range in severity from the asymptomatic carrier state to thalassaemia major (thalassaemia intermedia).

The main pathophysiological determinant of the severity of the thalassaemia syndrome is the extent of alpha/nonalpha chain imbalance. In other words any factor capable of reducing this alpha/nonalpha imbalance may have an ameliorating effect on the clinical picture. The most clinically relevant mechanism that consistently results in thalassaemia intermedia is co-inheritance of homozygosity or compound heterozygosity for a silent or mild beta-thalassaemia allele, namely a beta-thalassaemia defect associate with a consistent residual output of beta chains from the affected beta-globin locus (Gonzales – Redondo et al, 1989, Rosatelli et al, 1994, 19 95). By contrast, compound heterozygotes for a mild or silent and a severe mutation may result in a spectrum of phenotypes including severe and mild forms.

Other mechanisms capable of ameliorating the phenotype of homozygou beta-thalassaemia are the co-inheritance of alpha-thalassaemia (Wainscoat et al, 1983; Galanello et al, 1989) or genetic determinants capable of sustaining the continuous production of gamma-chains in adult life, thereby reducing the extent of the alpha/non-alpha chain unbalance (Rochette et al, 1994).

However, neither mechanism can be used to predict a mild phenotype prospectively, because neither results in a consistent effect. It is worth noting that thalassaemia intermedia may also result from heterozygosity for hyper-unstable Hb or a compound heterozygous state for typical heterozygous beta-thalassaemia and the triple alpha-globin arrangement.

Nevertheless, in many cases the determinant for the mild phenotype has not been defined so far.

alpha-Thalassaemias

Deletion alpha^{o} or alpha+-thalassaemias are detected by PCR sing two primers flanking the deletion breakpoints that amplify a DNA segment only in presence of the specific deletions (reviewed in Kattamis et al, 1996). As a control, DNA from a norma chromosome is simultaneously amplified using one of the primers flanking the breakpoint and a primer homologous to a DNA region deleted by the mutation. In addition, non-deletion alpha-thalassaemias are detected by restriction enzyme analysis on selectively amplified alpha$_1$- and alpha$_2$-globin genes or, when restriction is not applicable, by dot blot analysis with allele-specific oligonucleotide probes. Beside defining the molecular defect in carriers of alpha-thalassaemia, alpha-globin gene analysis can also discriminate between heterozygous alpha-thalassaemia and double heterozygosity for delta- and beta-thalassaemia or gamma-delta-beta-thalassaemia. Definition of the alpha-globin gene arrangement could also be useful to pr e-

dict the clinical phenotype of homoz y-gous beta-thalassaemia.

Sickle cell anaemia

Sickle cell anaemia most commonly results from homozygosity for the HbS mutation. Nevertheless, it may also be caused by compound heterozygosity for the HbS mutation and HbC, beta+, or beta°-thalassemia, or other rare ha e-moglobin variants such as HbO Arab. Molecular diagnosis for carriers of these conditions may be carried out with the same procedur e described for beta-thalassaemia, and especially by dot blot analysis with allele-specific probes or primer-specific amplification.

The severity of sickle cell ana e-mia may be modulated by a number of co-inherited modifying genes, alpha -thalassaemia and HPHF being the most noteworthy (Steinberg, 1996). The only determinant consistently associ-ated with a milder phenotype is pres-ence of high HbF resulting from the group of heterogeneous conditions di s-cussed previously under genetic coun-selling for couples at risk for beta-thalassaemia.

In order to predict the clinical phe-notype appropriately, during genetic counselling to couples at risk, these modifying factors should be defined by appropriate procedures (see section on beta-thalassaemia).

Prenatal diagnosis

Prenatal diagnosis of both alpha- and beta-thalassaemia was accom-plished for the first time in the 1970 with globin chain synthesis analysis of fetal blood obtained by fetoscopy or pla-cental aspiration (Kan et al, 1975). Mo-lecular definition of the thalassaemias, the development of procedures for their detection by DNA analysis, and the i n-troduction of chorionic villus sampling in the last decade have dramatically improved prenatal detection of these disorders. For a short period the diag-nosis of thalassaemia was obtained e i-ther indirectly by polymorphism anal y-sis (Kan et al, 1980) or directly by oligo-nucleotide hybridisation on electroph o-retically separated DNA fragments (Pirastu et al, 1983). Today thalassae-mias are detected directly by the anal y-sis of amplified DNA from fetal troph o-blast or amniotic fluid cells.

Fetal DNA sampling

Fetal DNA for analysis can be ob-tained from amniocytes or from chori-onic villi. At present the most widely used procedure is chorionic villi sam-pling, mainly because of the clear a d-vantage of being carried out during the first trimester of pregnancy, generally at the 10th -12th week of gestation (Hogge et al, 1986; Cao et al, 1987; Brambati et al, 1988). The risk of feta mortality associated with this metho is of the order of 1%. Chorionic vill may be obtained transcervically or transabdominally. We prefer the trans-abdominal route for several reasons, mainly because it has a low infection rate, a lower incidence of amniotic fluid leakage, because it is a simple proc e-dure, and also because it is largely pr e-ferred by pregnant women.

Fetal DNA analysis

Fetal DNA is analysed using the methods described earlier for the dete c-tion of known mutations during carrier definition. To limit the possibility of misdiagnosis, we analyse chorionic vil-lous DNA with two different proce-dures: i.e. RDB hybridisation and primer-specific amplification (ARMS).

Misdiagnosis may occur for several reasons: failure to amplify the target DNA fragment, mispaternity, maternal contamination, and sample exchange. Misdiagnosis for failure of DNA ampli-fication is obviously limited by the do u-ble approach described above. To avoi misdiagnosis resulting from mispater-nity or maternal contamination, we carry out DNA polymorphism analysis parallelly with mutation analysis. In addition to this, he presence or effec of maternal contamination could also be limited by careful dissection of the maternal decidua from the fetal troph o-blast under the inverted microscope, and by the fact that a minimal amount

(about 3 microg) of chorionic villi are requested to reduce the chances of co-amplifying the DNA from the maternal decidua.

The advent of DNA amplification has made it possible to analyse the genotype of a single cell. This has paved the way for pre-implantation or even pre-conceptional diagnosis (Monk and Holding, 1990; Handyside et al, 1992).

Pre-implantation may be carried out by a biopsy of the blastula, obtained by washing the uterine cavity after in vivo fertilisation, or by analysis of a single blastomere from an eight-cell embryo after in vitro fertilisation. Pre-conception diagnosis is based on the analysis of the first polar body of unfertilised eggs, and may lead to distinguish between unfertilised eggs tha carry the defective gene and those without the defect. By fertilising in vitro only the eggs without the defect an replacing them in the mother, a successful pregnancy with a normal fetus can be obtained. Of course, the genotype of the fetus will be checked further by chorionic villus biopsy.

Successful pregnancies following the transfer of human embryos in which a single gene defect has been excluded, have been reported.

Pre-implantation and pre-conception gamete diagnoses are very useful for couples against pregnancy termination for ethical reasons, and especially for those who have already ha therapeutic abortions due to genetic risks. At present, however, its use in routine monitoring of pregnancies at risk is precluded by the technical demand for these procedures, the difficulty organising the service, and the high costs.

POPULATION SCREENING AND COUNSELLING FOR HAEMOGLOBIN DISORDERS

Couples at risk for haemoglobin disorders may be identified retrospectively, i.e. following the birth of an affected child, or prospectively by analysing childless spouses. The benefit fro prospective identification is obviously greater, because it gives parents the opportunity of planning a family wit hout disease, and it alleviates the health burden to society. Prospective identification of couples at risk is carried out by population screening. To date programmes aimed at prospective identification and counselling of couples at risk for inherited haemoglobinopathie have been carried out significantly only for beta-thalassaemia. These programmes are ongoing in several areas at risk in the Mediterranean basin, such as Cyprus, Greece, several regions of continental Italy, and Sardinia (Angastiniotis et al, 1995; Cao et al, 1996; Loukopoulos, 1996).

All these programmes are characterised by intensive education and involvement of the population, screening of prospective couples and non-directive counselling. They have been very successful, because the population was well informed about thalassaemi and the methodology for its prevention, and there were no consistent adverse effects on those found to be carriers. Furthermore, in populations in the Mediterranean area where screening and counselling have been introduced, a marked decline has been observed in the incidence of thalassaemia major, the homozygous state of beta-thalassaemia (Angastiniotis et al, 1995; Cao et al, 1996; Loukopoulos, 1996).

Very limited prospective screening for alpha-thalassaemia is carried out in a few areas of South East Asia (Hong-Kong, Southern China, Thailand, Taiwan), and for sickle cell anaemia in the Caribbean, the United Kingdom and the USA.

FUTURE PROSPECTS

Technically, in carrier screening and prenatal diagnosis we can realistically predict further simplification and full automation of the procedures for the detection of the beta-thalassaemia mutation. Primer-specific amplification and reverse oligonucleotide hybridisation, for instance, could easily become

fully automated. An oligonucleotide microchip assay has been proposed recently for the large-scale detection of mutations in genetic diseases, including beta-thalassaemia. Given the alternative features of high throughput, automation and modest cost, the DNA chip has the potential to become a valuable method in future applications of mutation detection in medicine (Yershov et al, 1996).

The foreseeable progressive reduction in the cost of DNA analysis may lead to the use of mutation detection as a future screening method, thus skipping all carrier detection steps based on haematological analysis.

Simplification of pre-implantation and pre-conception gamete diagnosis may lead to a more extensive use of the procedure in the future, especially by couples against pregnancy termination. However, the most relevant advance would be fetal diagnosis by analysis of fetal cells in maternal circulation.

So far, many methods have been proposed for analysis, but none has given reliable results (Bianchi et al, 1990; Ganshirt-Ahlert et al, 1993).

Point mutations responsible for beta-thalassaemia or sickle cell anaemia have recently been identified successfully on fetal cells with a procedure based on the density gradient separation of mononuclear cells from maternal blood, the enrichment of fetal cell by magnetically activated cell sorting using the anti-transferrin receptor antibody, the identification of fetal cell by immunostaining with anti-fetal or embryonic haemoglobin antibodies, the isolation of nucleated red cells by micro-dissection under light microscopy, and non-radioactive PCR analysis (Cheung et al, 1996). The simplification and partial automation of this procedure may lead to the introduction of prenatal diagnosis by analysis of fetal cells in the maternal circulation in clinical practice.

Nevertheless, the most important challenge for the future is the organisation of similar genetic preventive programmes to those ongoing in the Mediterranean area, in parts of the world where beta-thalassaemia is prevalent, namely the Middle East, the Indian subcontinent, and the Far East. Nonetheless, the resources for population education and the present state of technical development seem to preclude the realisation of such a programme.

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