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SHORT COMMUNICATION

# PRENATAL DIAGNOSIS OF β-THALASSAEMIA AND SICKLE CELL ANAEMIA IN TURKEY

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## SUMMARY

This paper reports our experience of molecular analysis and diagnosis of β-thalassaemia and sickle cell anaemia (HbS) in 70 prospective parents of Turkish descent and their fetuses. Molecular screening was carried out by allele-specific oligonucleotide (ASO) hybridization of amplified DNA to the 12 most common mutations in the Turkish population. By using this approach, we were able to define the mutation in 95 per cent of chromosomes investigated. Genomic sequencing led to the additional detection of three rare mutations: Cd 44 (−C), IVS-I-5 (G–C), and IVS-I-116 (T–G). All diagnoses were successfully accomplished and no misdiagnosis occurred. Consanguineous marriage appears to contribute significantly to the frequency of affected births in Turkey. Out of the 14 homozygous fetuses, six were the result of close consanguinity. This study indicates that fetal diagnosis of β-thalassaemia and HbS may be obtained in practically all cases, even in a heterogeneous population like the Turkish population, when early methods of fetal sampling are combined with polymerase chain reaction (PCR)-based techniques. Until gene therapy becomes a reality, the only approaches to the control of haemoglobinopathies are prevention and avoidance. The most relevant and common aspects of the programmes, which have been very effective in reducing the birth rate of β-thalassaemia major in several at-risk areas of the Mediterranean basin, are the continuous educational campaigns directed at the population at large, the voluntary basis, and non-directive counselling. The most important challenge for the eradication of the haemoglobinopathies in Turkey is the organization of a nation-wide and comprehensive genetic preventive programme based on DNA technology.

KEY WORDS: β-thalassaemia; sickle cell anaemia; prenatal diagnosis; DNA analysis

## INTRODUCTION

The genetic disorders of haemoglobin, particularly sickle cell anaemia (HbS) and the α- and β-thalassaemias, were the first conditions to which fetal DNA analysis was applied for prenatal detection. This was first achieved in the 1970s by the study of globin synthesis in fetal blood, following the development of the technique of fetal blood sampling (Kan *et al.*, 1975). The first successful prenatal diagnosis using DNA, derived from chorionic villus sampling (CVS), was reported in 1982 (Old *et al.*, 1982).

β-Thalassaemia results from a variety of molecular defects that reduce or abolish the synthesis of the β-globin chains of haemoglobin. Patients with β-thalassaemia suffer from severe transfusion-dependent anaemia. In spite of the optimal treatment available today, they have a short life-span, usually succumbing by early adulthood; thus, at present, prenatal diagnosis is the best option for couples at risk.

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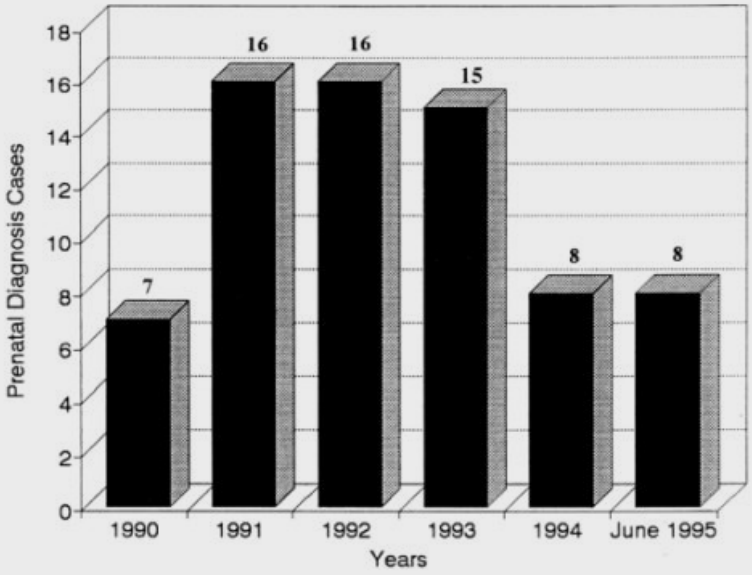


Fig. 1—Fetal diagnoses performed in the Department of Molecular Biology and Genetics (Boğaziçi University) between 1990 and 1995

The overall frequency of β-thalassaemia in Turkey is 2 per cent, and regions with higher figures are known to exist (Aksoy *et al.*, 1985). Unlike β-thalassaemia, HbS is not widely distributed in Turkey and is almost limited to one group of people (Eti-Turks) living along the country's south-eastern coast, where its frequency varies from 0·3 to 37 per cent (Altay and Başak, 1995). In addition to the elevated rate of consanguineous marriages (21 per cent) within certain communities having a high incidence of β-thalassaemia (Başaran *et al.*, 1988), Turkey has one of the highest rates of population increase in the world (36:1000; Census, 1994). Both figures appear to contribute drastically to the frequency of affected births. The expected number of infants born annually with β-thalassaemia and sickle cell anaemia in Turkey has been calculated to be around 150 and 40, respectively. Hence approximately 800 pregnant women are estimated to require prenatal diagnosis for both disorders each year (Altay and Başak, 1995).

Advances in the molecular understanding of β-thalassaemia have facilitated the implementation of several prevention programmes based on carrier detection and early prenatal diagnosis. In a number of at-risk populations in the Mediterranean area (e.g. Greeks, Cypriots, Continental Italians, and Sardinians; Angastiniotis and Hadjiminas, 1980; Loukopoulos *et al.*, 1985; Cao *et al.*, 1989), such a strategy has been highly effective in reducing the incidence of β-thalassaemia major. The efficient application of prenatal diagnosis in Sardinia, for example, has led to a decline in the annual birth rate of β-thalassaemia major cases from 1:250 to 1:1000 live births (Cao *et al.*, 1990).

In the present study, we report on the establishment of a prenatal diagnosis programme in Turkey and its successful application to 70 Turkish families at risk for β-thalassaemia and sickle cell anaemia.

## MATERIAL AND METHODS

### *Subjects*

Seventy couples of Turkish descent at risk for β-thalassaemia or sickle cell anaemia, having an affected child in most instances, were the subjects of this study. Their blood samples were obtained either separately or together with the fetal material.

### *Methods*

DNA was extracted from the peripheral blood cells of the prospective parents and, when present, from their homozygously affected children (Miller *et al.*, 1988). Fetal DNA for analysis was obtained by transcervical or transabdominal chorionic villus biopsy (Hogge *et al.*, 1986; Brambati *et al.*, 1988), or in late referrals, from amniotic fluid cells. The chorionic villi were separated from the maternal decidua under an inverted microscope.

The β-thalassaemia mutations were defined by dot-blot analysis of amplified DNA with $^{32}$P-labelled ASO probes (Saiki *et al.*, 1988); simultaneously, ARMS and, if applicable, restriction enzyme analysis were used. If the nature of the mutations occurring in a family under investigation was not known at the moment of biopsy, the family was screened for the 12 most common mutations predominating in Turkey by using the above-mentioned procedures. By way of this approach, prenatal diagnosis was always available within 24 h to 10 days. If the mutation(s) could not be defined by these techniques, the DNA samples were subjected to DGGE combined with genomic sequencing (Losekoot *et al.*, 1992; Sanger *et al.*, 1977).

## RESULTS

### *Fetal diagnosis*

Figure 1 shows the distribution of 70 fetal diagnoses carried out in our laboratory in the last 5 years. In most cases, the sampling procedure was a first-trimester CV biopsy. In addition, there were four second-trimester CVS procedures, two amniocenteses, and one fetal blood sampling. Sufficient fetal material and DNA (5–30 μg) for analysis were obtained in all sampling procedures at the first attempt. The fetal loss rate in the 70 biopsies performed was calculated to be 4·3 per cent.

A successful diagnosis was obtained in all 70 cases investigated; 13 fetuses were normal (18·56 per cent, healthy), 36 were heterozygous for either β-thalassaemia or HbS (51·43 per cent, carrier), and 14 were homozygous or compound heterozygous (20·01 per cent, affected). In the latter case, most of the inherited mutations were severe $\beta^+$-(IVS-I-110, IVS-II-745, IVS-I-5 G–C) or $\beta^0$-types (Cd 39, FSC 44, IVS-II-1, FSC 8, FSC 8/9, IVS-I-116) resulting in the phenotype of β-thalassaemia major. Out of the 14 fetuses shown to be affected in the framework of this study, 13 were chosen to be electively aborted and only one pregnancy was allowed to continue to term due to the co-presence of two mild mutations (IVS-I-6/-30) in the fetus. Seven fetuses were diagnosed as normal or heterozygous; complete diagnosis could not be given to these families since one of the parental mutations could not be defined during the fetal analysis. However, in all seven cases the pregnancy was allowed to continue. Since luckily all seven fetuses turned out not to carry the known mutation of the family, they were diagnosed as worst-case carriers (Table I). All results were confirmed after the birth of the child. No misdiagnosis occurred.

Table I—Results of DNA analysis in 70 fetuses at risk for β-thalassaemia and HbS

| Fetal genotype | No. of cases | % |
|---|---|---|
| Homozygous cases | | |
| IVS-I-110/IVS-I-110 | 1 | 1·43 |
| FSC 8/FSC 8 | 2 | 2·86 |
| IVS-I-110/IVS-I-6 | 4 | 5·71 |
| IVS-I-110/FSC 8 | 1 | 1·43 |
| IVS-I-110/IVS-I-5 (G–C) | 1 | 1·43 |
| IVS-I-6/-30 | 1† | 1·43 |
| Cd39/IVS-II-745 | 1* | 1·43 |
| IVS-II-1/FSC 44 | 1 | 1·43 |
| HbS/HbS | 2 | 2·86 |
| Subtotal | 14 | 20·01 |
| Heterozygous cases (carriers) | | |
| IVS-I-110/N* | 16+1* | 24·28 |
| IVS-I-6/N | 1 | 1·43 |
| IVS-I-1/N | 4 | 5·71 |
| Cd 39/N | 2 | 2·86 |
| FSC 8/N | 1 | 1·43 |
| IVS-II-1/N | 3 | 4·29 |
| IVS-II-745/N | 2 | 2·86 |
| FSC 5/N | 1 | 1·43 |
| FSC 44/N | 1 | 1·43 |
| HbS/N | 4 | 5·71 |
| Subtotal | 36 | 51·43 |
| Normal cases | | |
| N/N | 12+1* | 18·56 |
| Subtotal | 13 | 18·56 |
| Heterozygous/normal cases | | |
| N/? | 7 | 10·00 |
| Subtotal | 7 | 10·00 |
| Total | 70 | 100·00 |

*Spontaneous abortion.
†Pregnancy not interrupted.

### *Distribution of the β-thalassaemia and HbS mutations*

The frequencies of the 16 mutations, including the HbS mutation, observed among the 140

Table II—Frequencies and types of mutations observed among the 140 chromosomes studied for prenatal diagnosis

| Mutations | Type | No. of chromosomes | Frequency (%) |
|---|---|---|---|
| IVS-I-110 (G–A) | $\beta^+$ | 52 | 37·15 |
| IVS-I-6 (T–C) | $\beta^+$ | 10 | 7·15 |
| IVS-I-1 (G–A) | $\beta^0$ | 10 | 7·15 |
| Cd 39 (C–T) | $\beta^0$ | 9 | 6·43 |
| FSC-8 ( − AA) | $\beta^0$ | 8 | 5·71 |
| IVS-II-1 (G–A) | $\beta^0$ | 8 | 5·71 |
| IVS-II-745 (C–G) | $\beta^+$ | 5 | 3·57 |
| FSC-5 ( − CT) | $\beta^0$ | 4 | 2·86 |
| FSC 44 ( − C) | $\beta^0$ | 2 | 1·43 |
| IVS-I-5 (G–C) | $\beta^+$ | 2 | 1·43 |
| IVS-I-116 (T–G) | $\beta^0$ | 2 | 1·43 |
| -30 (T–A) | $\beta^+$ | 1 | 0·71 |
| -87 (C–G) | $\beta^+$ | 1 | 0·71 |
| FSC 6 ( − A) | $\beta^0$ | 1 | 0·71 |
| FSC-8/9 (+G) | $\beta^0$ | 1 | 0·71 Σ 82·86 |
| HbS | $\beta^S$ | 17 | 12·14 Σ 95·00 |
| Unknown | | 7 | 5·00 |
| Total | | 140 | 100·00 |

chromosomes investigated in the framework of this study are given in Table II. The seven diagnoses (5 per cent) in which one of the parental mutations remained uncharacterized are now being investigated by DGGE and genomic sequencing.

In 70 prospective parents at risk for β-thalassaemia and HbS, as many as 29 combinations of 16 different mutations were found to be present (Table III). The rate of consanguinity in all couples investigated was 34·3 per cent. One-third of the consanguineous couples had two different β-thalassaemia mutations, whereas two-thirds carried the same mutations.

## DISCUSSION

Although there is no definitive cure for β-thalassaemia at present, the methods of clinical management have improved considerably over the past few years and the life expectancy of affected individuals has been significantly increased. However, the treatment required is very expensive and is not a realistic means of controlling the disorder for many developing countries. Therefore, several countries are applying an alternative method of control, which involves screening the population for carriers, identifying the couples at risk, and providing a prenatal diagnosis service (Angastiniotis and Hadjiminas, 1980; Loukopoulos *et al.*, 1985; Cao *et al.*, 1990). The identification of nearly 180 β-thalassaemia mutations (Baysal, 1995) has revolutionized prenatal diagnosis in many countries by facilitating rapid first-trimester testing using DNA analysis from chorionic villi. However, in some countries there is a great mutational diversity. For example, in Turkey, situated at the meeting point of three continents and having been a melting pot for various ethnic groups and cultures for several centuries, at least 30 β-thalassaemia mutations have been discovered thus far (Altay and Başak, 1995). The marked heterogeneity poses great difficulty in performing diagnosis by mutational analysis.

Seventy pregnancies for β-thalassaemia and HbS have been investigated in the framework of this study. The distribution of the diagnoses over the years 1990–1995 shows a peak in the years 1991–1993, which gradually decreases in 1994 (Fig. 1). This may be explained by the recent establishment of two additional prenatal diagnosis centres in Ankara and Adana. Since the number of pregnancies at risk for β-thalassaemia and HbS in

Table III—Parental genotypes encountered in 70 families at risk

| Parental genotypes | No. of cases | % | |
|---|---|---|---|
| IVS-I-110/IVS-I-110 | 8+7* | 21·42 | |
| IVS-I-1/IVS-I-1 | 3* | 4·28 | |
| FSC 8/FSC 8 | 1+1* | 2·86 | |
| Cd 39/Cd 39 | 1+1* | 2·86 | |
| IVS-II-1/IVS-II-1 | 1+1* | 2·86 | |
| IVS-II-745/IVS-II-745 | 1* | 1·43 | |
| FSC 5/FSC 5 | 1* | 1·43 | |
| IVS-I-116/IVS-I-116 | 1* | 1·43 | |
| HbS/HbS | 6+1* | 9·99 | Σ 48·56 |
| IVS-I-110/IVS-I-6 | 2+4* | 8·56 | |
| IVS-I-110/IVS-I-1 | 2 | 2·86 | |
| IVS-I-110/Cd 39 | 2 | 2·86 | |
| IVS-I-110/FSC 8 | 3 | 4·28 | |
| IVS-I-110/IVS-II-1 | 1 | 1·43 | |
| IVS-I-110/IVS-I-5 (G–C) | 2* | 2·86 | |
| IVS-I-110/FSC 6 | 1 | 1·43 | |
| IVS-I-110/Hbs | 2 | 2·86 | |
| IVS-I-110/? | 3 | 4·28 | |
| IVS-I-6/FSC 5 | 1 | 1·43 | |
| IVS-I-6/-30 | 1 | 1·43 | |
| IVS-I-6/? | 2 | 2·86 | |
| IVS-I-1/FSC 8 | 1 | 1·43 | |
| IVS-I-1/FSC 5 | 1 | 1·43 | |
| Cd 39/IVS-II-745 | 2 | 2·86 | |
| Cd 39/? | 1 | 1·43 | |
| IVS-II-1/FSC 44 | 1+1* | 2·86 | |
| IVS-II-1/FSC 8/9 | 1 | 1·43 | |
| IVS-II-745/-87 | 1 | 1·43 | |
| HbS/? | 1 | 1·43 | Σ 51·44 |
| Total | 70 | 100·00 | |

*Consanguineous couple(s).

Turkey is calculated to be around 800 each year, and since the total number of pregnancies monitored collectively in the three centres did not exceed 100 in the last 2 years (Altay and Başak, 1995), the urgent necessity of educating health workers and the population at large in the field of preventive genetics becomes apparent.

As previously reported (Diaz-Chico *et al.*, 1988; Aulehla-Scholz *et al.*, 1990; Öner *et al.*, 1990; Başak *et al.*, 1992), Turkey is very heterogeneous with respect to β-thalassaemia mutations. In the 140 chromosomes investigated in the framework of this study, 15 different β-thalassaemia mutations and the HbS mutation have been shown to be present. According to the distribution in Table II, the ratio of $\beta^0$- to $\beta^+$-thalassaemia is 3:2, and since the majority of the $\beta^+$-thalassaemia mutations occurring in Turkey are severe, the phenotype of β-thalassaemia major is considerably favoured versus β-thalassaemia intermedia (1:10). In particular parts of the country, where HbS is also relatively common, compound heterozygosity for these two disorders (β-thal/HbS) is frequently encountered which is also expressed in a β-thalassaemia major type of disease (Altay and Başak, 1995).

The presence of an affected child in the family may provide critical information for predicting as accurately as possible the expected clinical phenotype of the fetus. Such a case was encountered in this study, in which fetal diagnosis was requested by the parents; termination of the pregnancy was not chosen despite the fetus being affected, as compound heterozygosity for IVS-I-6 and -30 was known to result in a mild expression of the disease in the previous child of the family. In fact, the question now arises of whether the invasive biopsy and the expensive DNA diagnosis should be completely avoided in such cases in the future. Surprisingly, true homozygosity for the mild IVS-I-6 T–C allele, which is the second most common mutation in Turkey (Başak *et al.*, 1992), was not encountered in the framework of this study.

Consanguineous marriage appears to contribute significantly to the frequency of affected births in Turkey. The overall consanguinity in Turkey is 21 per cent, but individual rates may increase up to 46 per cent. Generally, first-cousin marriage is the single most frequent type of liaison (Başaran *et al.*, 1988). Thus, the high numbers of homozygosity for the same mutation can be partly explained by the close relationship among some parents (Table III). In the framework of this study, consanguineous marriage was associated with 34 per cent of the cases; six out of 14 fetuses were the result of close consanguinity. The interesting finding that a considerable proportion of consanguineous couples (30 per cent) carry two different β-thalassaemia mutations confirms, on the other hand, the high frequency and diversity of mutations in Turkey. However, since the majority of consanguineous couples still seem to carry the same mutation (70 per cent), we can state that the marital habits of the Turkish population do simplify the complexity of prenatal diagnosis to a certain extent.

Another factor simplifying prenatal diagnosis services includes the rapidly developing technology involved in sample collection and analysis. The most promising procedures, which are also amenable to automation, are non-radioactive and reverse dot-blot hybridizations and ARMS (Chehab, 1993). The isolation of fetal cells from the maternal circulation, which is one of the most recent innovations in the field of sample collection, will certainly have a great impact on prenatal diagnosis (Bianchi and Klinger, 1992).

Although the great heterogeneity of mutations present in Turkey was expected to create difficulties in DNA analysis, this study demonstrates that prenatal diagnosis is feasible in Turkey when early methods of fetal sampling are combined with the advent of PCR-based techniques. The most important challenge for the future is to organize a comprehensive genetic preventive programme in Turkey like those going on in many Mediterranean countries. For this prospective control of β-thalassaemia major, intense involvement of the population, e.g. community education and informed genetic counselling, is an important prerequisite. The materialization and realization of such a programme rely greatly on the resources for education of the population and the nation-wide transfer of know-how and technical facilities, present in research centres, to routine clinical laboratories.

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