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PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS IN A TURKISH FAMILY*

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Prenatal diagnosis of cystic fibrosis (CF) was made in a Turkish family whose first born child was diagnosed at necropsy as having CF. Two consecutive pregnancies followed. The fetus of the second pregnancy was diagnosed as having CF by the microvillar enzyme assay and was aborted. The diagnosis was verified by the DNA polymerase chain reaction analysis using chorionic villi from the abortus. In the third pregnancy, amniocentesis was performed in the 17th week, and KM19 polymorphism linked to CF was used to assess the status of the fetus. Since the fetus was determined to be a carrier, the family was advised to continue with the pregnancy.
Key words: cystic fibrosis, DNA amplification, KM19 polymorphism, prenatal diagnosis.

Cystic fibrosis (CF) is a common recessive genetic disorder affecting approximately 1 in 2500 Caucasians. The disease causes defective regulation of chloride ion transport in exocrine gland epithelia and is linked to a locus on chromosome 7q31 coding for cystic fibrosis transmembrane regulator (CFTR) protein[1].

Clinically, CF is characterized by recurrent respiratory tract infections and malabsorption. Although the survival rate of the disease has improved in recent years, the therapy is still symptomatic, being directed to the good control of pulmonary infections and provision of an optimal nutritional support[2]. Lack of

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radical therapy warrants that families affected by the disease seek prenatal diagnosis.

In the mid 1980s, decreased levels of microvillar enzymes in the amniotic fluid of women carrying fetuses affected by CF were demonstrated, providing a good, but not an absolute method, of prenatal diagnosis due to false positive and false negative results[3]. The discovery of tightly linked informative flanking markers made possible a DNA-based carrier test for the disorder, and prenatal diagnosis of CF was established by chorionic villus sampling in the first trimester for families in which there was at least one affected child.

In this report, we describe the use of the polymerase chain reaction (PCR) to analyse the KM19 polymorphism in a family affected by CF with regard to the prenatal diagnosis of the disease.

Material and Methods

The proband (Fig. 1), a three-month-old male, was the first-born child of nonconsanguineous parents. He suffered from chronic, progressive lung disease

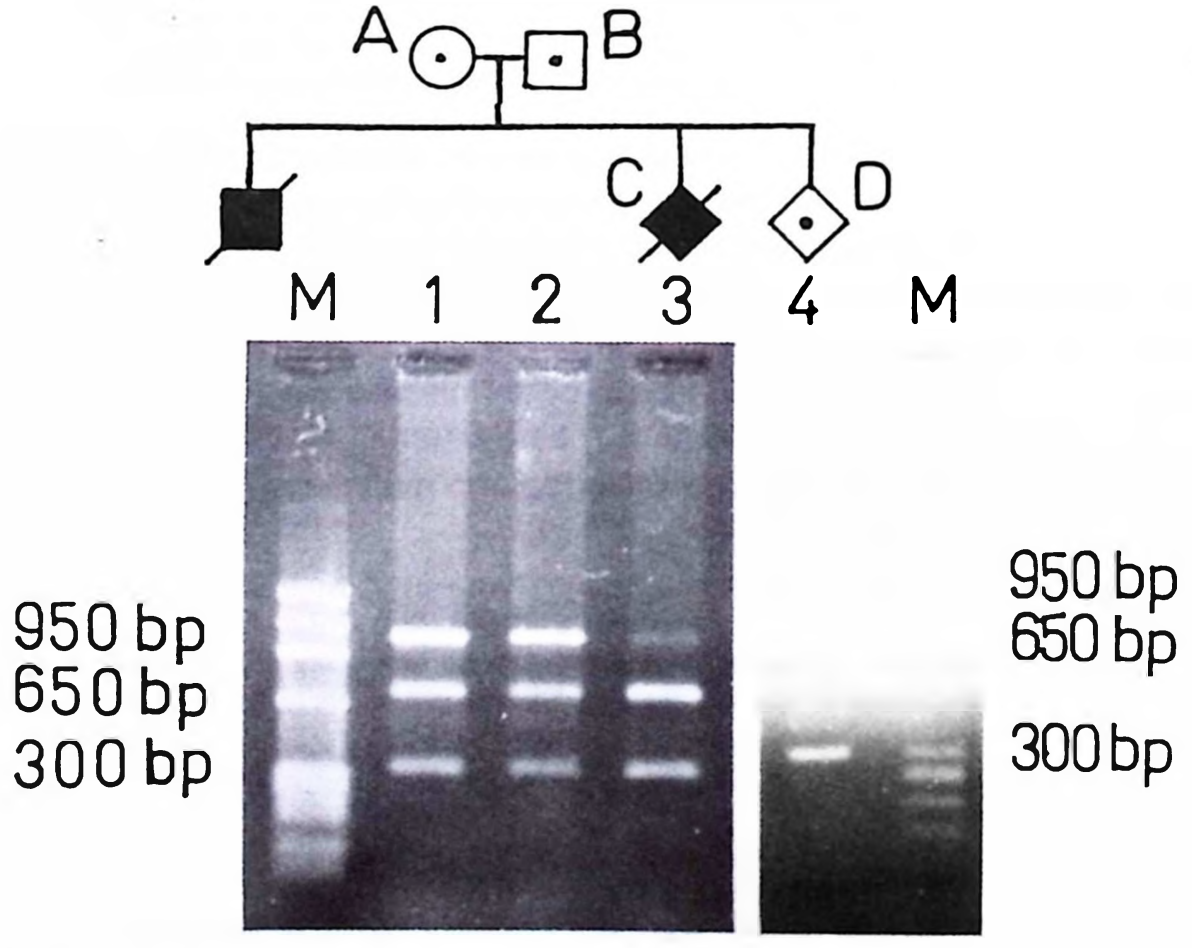

Fig. 1: Results of Pst I restriction digestion of amplified DNA samples. (A1): Mother; obligate carrier, B(2): Father; obligate carrier, D(3): Chorionic villi from abortus D(4): Prenatal diagnosis of fetus, M: øx174 Hae III digest size marker DNA.

and impaired growth. He subsequently died due to severe pulmonary infection. A diagnosis of CF was made at necropsy, and the parents were given thorough genetic counselling.

Prenatal diagnosis was offered to the parents in two subsequent pregnancies. In the second pregnancy, microvillar enzyme analyses of the amniotic fluid sample demonstrated that the fetus was affected by this genetic disorder (The analyses were performed by Prof. DJH Brock, Edinburgh, U.K).

The pregnancy was terminated. DNA was isolated from the chorionic villi of the abortus and analyzed by PCR to confirm the result of the enzyme analysis. In the present pregnancy, amniocentesis was performed during the 17th week of gestation, and DNA was isolated from the amniotic cells.

Preparation of genomic DNA[4]*:* The DNA was extracted from isolated lymphocytes, amniotic fluid cells or chorionic villi and cleared of maternal decidua by proteinase K (200 µg/ml) and sodium dodecyl sulphate (0.2 %) treatment for a 16 hour period at 37 °C, followed by phenol-chloroform isoamyl alcohol (25:24:1) extraction and precipitation with ethanol. DNA was redissolved in a TE buffer (10 mM Tris-HCl, 0.1 mM ethylenediamine tetra acetic acid, EDTA pH 7.0) and kept frozen until analysis.

DNA amplification: KM19 oligonucleotide primer sequences were (50 pmol/L) 5' (AAGGTACATGTTAATTTT) 3' (forward) and 5' (GCTGCATCATATAGTTGCC) 3' (reverse). The reaction mixture consisted of genomic DNA (1 µg), 1xTaq polymerase buffer (25 mM Tris-HCl pH 9.5, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mg/ml bovine serum albumin, 300 µg/ml activated calf thymus DNA), 0.2 mM of each of the deoxyribonucleotides (dATP, dGTP, dCTP, dTTP) and the thermostable DNA polymerase (Amersham Int.), (2.25 U per reaction) in a total volume of 0.1 ml. The initial template denaturation step was performed by adding 100 ml mineral oil to the reaction mixture present in 0.5 ml microcentrifuge tubes and heating it for ten minutes at 92 °C. The temperature cycling conditions for KM19 oligonucleotide primers were: 1 minute at 92 °C (denaturation), 1.2 minutes at 55 °C (annealing) and 2 minutes at 72 °C (extension) for a total of 30 cycles with a final extension step of 10 minutes instead of two minutes to fully extend any remaining single-stranded DNA[5]. The procedure was performed using a household manual system consisting of water baths that could be adjusted to the above temperatures.

PCR product analysis: PCR products were checked by 1.5 % agarose gel electrophoresis and the amplified fragments were visualized with ethidium bromide under ultraviolet light. Thirty microliters of the amplified product were digested by PstI restriction enzyme (25 U of the enzyme incubated at 37 °C for a period of 16 hours). Restriction fragments were observed using ethidium bromide stained 1.5% agarose gel[6].

Results

PCR products resulting from amplification when digested with PstI enzyme yielded electrophoretically separable fragments of different lengths which represented different alleles. (Fig. 1 a - d).

A: Obligate carrier mother; displayed three bands of 950 base pairs (bp), 650 bp and 300 bp each.

B: Obligate carrier father; displayed three bands of 950 bp, 650 bp and 300 bp each.

C: Chorionic villus material from the second pregnancy in which the fetus was found to be affected by microvillar enzyme analyses displayed only two bands of 650 bp and 300 bp each.

D: Amniocytes from present pregnancy displayed three bands of 950 bp, 650 bp and 300 bp each.

Discussion

As in any other recessively inherited autosomal disease, the risk of occurrence of CF in affected families is one in four. If the high risk of occurrence of CF in affected families and the limited impact of the present supportive approaches on the long-term prognosis of the disease is considered, there is a need for the use of methods for in-utero diagnosis of the disease, particularly in Turkey, where the frequency of the disease seems to be high[7].

Although rare (5%), diagnostic errors in the prenatal diagnosis of CF may occur when the microvillar enzyme assay of amniotic fluid is used. Recent developments in the field of DNA technology make it possible to diagnose CF, and many other genetic disorders more accurately. During the search for the CF gene KM19, XV2C and C57, probes have been identified that define the locus D7S23 which lies close to the CF locus[8].

A linkage disequilibrium between particular D7S23 haplotypes and CF has been shown[9]. Therefore, these markers can be used in clinical applications should prenatal testing and carrier detection be offered to families where there is an index CF case. Besides conventional Southern blotting, for haplotyping, sequences flanking these polymorphic regions can be used as oligonucleotide primers to amplify the region and check for polymorphism by use of restriction enzymes (KM19/Pst I, XV2C/Taq I, CS7/Hha I)[9,10]. In this case, the use of PCR technology for DNA amplification obviates the use of radioactivity for probe labeling and shortens the diagnostic procedure considerably. However, since this is not a direct determination of the mutation causing CF, the markers should be tested initially in the parental DNA samples. To make a decision concerning the fetus in regard to prenatal diagnosis, there should be data about the parents regarding the

markers and the linkage phase of the disease should be confirmed by the results of the index case.

In our study, we used KM19 polymorphism in the in-utero evaluation of the present pregnancy in this family. The longer DNA fragment demonstrated by agarose gel electrophoresis after Pst I digestion of the amplified sample was termed "code 1" and the shorter DNA fragment, "code 2", corresponding to the absence (1), or presence (2) of the restriction site. There are two copies of chromosome 7, thus there are three possible allelic combinations:

1/1 Normal: homozygous for the absence of the restriction site.

1/2 Carrier: one chromosome bears the restriction site, the other does not.

2/2 Affected: the restriction site is present on both alleles (homozygote for the disease).

A normal individual (1/1) carries only the 950 bp fragment whereas the individual affected by CF (2/2) carries 650 bp and 300 bp fragments. Any individual who is a carrier of the disease (1/2) exhibits three bands 950 bp, 650 bp and 300 bp.

Analysis of the DNA sample isolated from the chorionic villi of the abortus of the second pregnancy revealed two bands of 650 bp and 300 bp. This pattern was dissimilar to the pattern seen in the obligate carrier parents, who had 950 bp, 650 bp and 300 bp bands. Therefore, the diagnosis that the fetus was affected, was confirmed. The last pregnancy was also evaluated in the same manner, and the fetus was found to be heterozygote (1/2) for CF since an electrophoretic pattern similar to that of the parents was found. The mother was advised to continue with the present pregnancy.

A reliable prenatal diagnosis is now available for CF by using PCR technology if there is data with regard to the marker polymorphism of the parents of the index case. The Δ F508 mutation, which is linked to about 70 percent of the CF alleles of Northern Europeans, is observed in only 30 percent of the alleles of Turks Therefore, this mutation frequency can not be used as a direct test for CF in Turkey. The same situation also applies to other genetic diseases such as phenylketonuria where the alleles in the Turkish and European populations are linked to different haplotypes[11]. Studies are continuing in our laboratory to determine the major mutation in association with CF alleles in the Turkish population. Depending on these results, a direct determination of the mutation will be applied both for prenatal diagnosis and carrier detection.

Note Added in Proof:

Subsequent to the prenatal diagnosis of the index case as a CF carrier by KM-19 polymorphism, the status of the newborn was determined. KM-19 polymorphism was checked by PCR amplification of blood leukocyte DNA obtained from the newborn, and the diagnosis of CF carrier was confirmed.

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