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Detection of Cystic Fibrosis Δ F508 Mutation in the Çukurova Region

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Received: January 1, 2000

Cystic fibrosis (CF) is a well-known inherited multi-system disorder characterised primarily by chronic obstructive lung disease and maldigestion. The frequency of the disease varies among ethnic groups, being the highest in northern Europe where about 1 in 2500 newborns is affected. The CF gene itself is quite large; the coding region is made up of 27 exons and the gene product is called cystic fibrosis transmembrane conductance regulator (CFTR). A 3 base pair deletion in exon 10 of the gene, which results in the loss of a single amino acid, phenylalanine, is identified in approximately 70% of CF patients and designated as Δ F508 (1,2,3). The frequency of this mutation varies among different populations, being higher in northern Europe with respect to southern Europe (4). The aim of this study was to set up a method in our laboratory for the detection of the Δ F508 mutation and to obtain an estimate of the frequency of this mutation in CF patients in the Çukurova region. The samples of blood were obtained from patients who were diagnosed as having cystic fibrosis by the Paediatric Metabolism Clinic according to their clinical symptoms and sweat chloride test results. DNA was prepared from leukocytes for the amplification reactions. Selected portions of DNA are amplified by a polymerase chain reaction. Heteroduplexes are generated by heat denaturation and reannealing of a mixture of wild type and mutant DNA molecules. A heteroduplex between the normal and mutant sequences is detected as extra bands of slower

mobility in a polyacrylamide gel system, providing a convenient test for identifying heterozygous individuals. However, since the 3 base pair deletion is relatively small, it is impossible to discriminate between a homozygous patient and one without the deletion. To differentiate between these two genotypes, DNA of a normal sequence is added in equal amounts to each of the test samples, and if a mutant sequence exists in the DNA mixture, as in the homozygous cases, then heteroduplexes form after the amplification. In 8% (w/v) polyacrylamide gel, homoduplexes and heteroduplexes exhibit distinct electrophoretic mobilities. The bands are visualised under UV light by ethidium bromide staining (5,6). A total of 51 patients (102 chromosomes) were included in our study. The paediatricians set the diagnostic criteria by the clinical assessment and a cut-off point of 60 meq/L for the sweat chloride test as the average of triplicate analysis. A total of 7 heterozygotes and only one homozygote for Δ F508 were detected and the frequency was calculated to be 8.8%. The homozygous patients' DNA showed a single band, just like the wild type, at the beginning, but the polymerase chain reaction of the mixture of normal DNA and the patient DNA sample in equal amounts revealed the characteristic heteroduplexes. The mutation was also detected in the parents of the homozygous and they were found to be the carriers of Δ F508 as expected, and the diagnosis was confirmed (Figure). Genetic counselling was given to this couple, and prenatal diagnosis is pro-

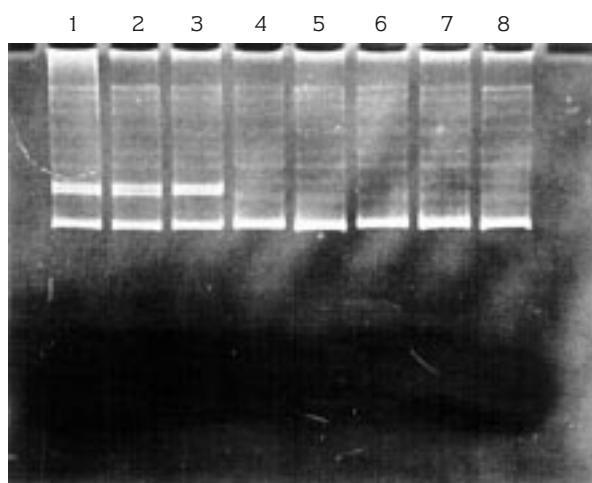


Figure The heteroduplicates of the parents of the homozygous patient. 1. Heterozygous Control; 2. Mother; 3. Father; 4-7 other patients (after the application of mixture of equal amounts of normal and Patient DNA, still giving a single band; excluding Δ F508 mutation; need to search for other mutations); 8. Homozygous Control.

posed for further pregnancies. The results of the mutation analyses from 35 centres of Europe in 1990 were published in a special issue of Human Genetics and the discussions on the variety of mutations have started

worldwide. Across Europe and neighbouring areas of Asia, there is a clear northwest to southeast gradient in the relative frequency of Δ F508 (7). Cystic fibrosis is known to be less common in the other groups, but significant numbers of affected individuals are found in southern Europe, in the Ashkenazi Jewish population, and in American blacks. Almost 90% of the cystic fibrosis mutations detected in the Danish population are Δ F508 (8). In contrast, while only 22% of the CF chromosomes in the Ashkenazi Jewish population in Jerusalem carry Δ F508, the frequency for W1282X is 60% (9,10). In 1990, the only results about Turkish population were detected by German scientists as 22% from the Turkish minority living in West Germany (11). Afterwards, Onay et al. from Istanbul and Yilmaz et al. from Ankara, published 15% and 28.4% frequencies of the Δ F508 mutation in Turkish CF patients, respectively (12,13). It was rather surprising to find out that our result of 8.8% is even lower than those from Istanbul and Ankara. We found that only eight of the fifty-one CF patients carried the Δ F508 mutation. Therefore, it would be very interesting to search for the unknown mutations and perhaps find a Turkish type of mutation just like the W1282X mutation in Israel.

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