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Multiplex PCR for Rapid Detection of Three Mutations in the Cystic Fibrosis Gene

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ΔF508, the major mutation causing cystic fibrosis (CF),[1] occurs on 55.6% (297/534) of CF chromosomes in the Italian population. Screening for the additional 28 mutations carried out to estimate the frequency of more rare molecular defects revealed that 1717-1G→A[2] and G542X[3] are also relatively abundant, as they account for 11.6% (21/181) and 9.7% (20/207) of non-ΔF508 CF chromosomes, respectively. To simplify the analysis of these mutations, we have developed a multiplex PCR using allele-specific primers for the detection of ΔF508 and a PCR-mediated site-directed mutagenesis (PSDM)[4] for the analysis of 1717-1G→A, already described elsewhere,[5] and G542X (Fig. 1).

Modified reverse primers (5′-CTC-TGCAAACTTGGAGAGGTC-3′ for 1717-1G→A and 5′-CAGTGTGATTCC-ACCTTCAC-3′ for G542X) containing a single-base mismatch were designed to create an artificial restriction site (*Ava*II for 1717-1G→A and *Hph*I for

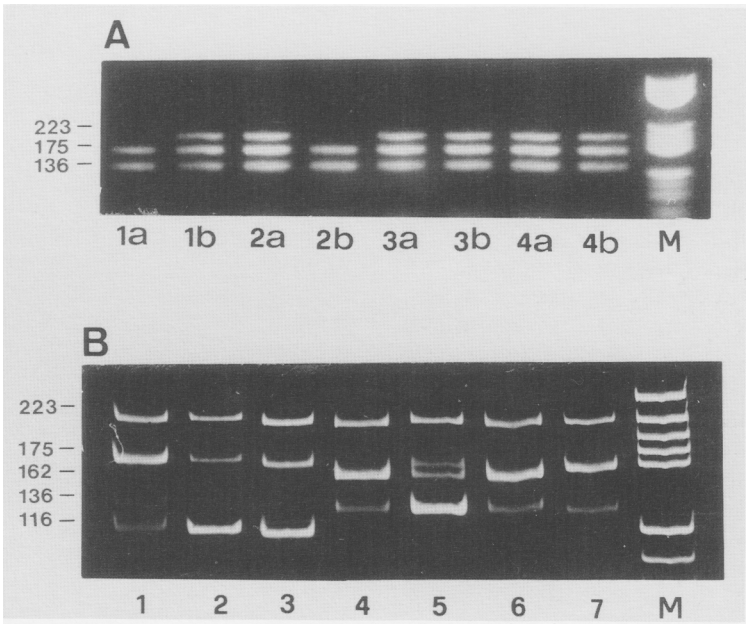

FIGURE 1 Multiplex PCR for the analysis of ΔF508, 1717-1G→A and G542X mutations. (*A*) Ethidium bromide-stained NuSieve-agarose gel electrophoresis of PCR products for the identification of the ΔF508 mutation. PCR for ΔF508 generates a 223-bp fragment, for G542X a 175-bp fragment, and for 1717-1G→A a 136-bp fragment. (Lanes *1a*) Samples amplified with the allele-specific primer corresponding to the wild-type allele for ΔF508; (lanes *1b*) same samples, amplified with the allele-specific primer corresponding to the mutated allele for ΔF508. Patient 1, ΔF508/ΔF508; Patient 2, control N/N; Patient 3, ΔF508/1717-1G→A; Patient 4, ΔF508/G542X. (Lane *M*) pBR322/*Hae*III molecular weight marker. (*B*) Ethidium bromide-stained PAGE electrophoresis of digested PCR products for the detection of the 1717-1G→A and G542X mutations. The amplified 175-bp fragment for G542X, after *Hph*I digestion, gives rise to 162- and 13-bp fragments for wild-type alleles and remains undigested for mutated alleles. The amplified 136-bp fragment for 1717-1G→A, after *Ava*II digestion, gives rise to 116- and 20-bp fragments for wild-type alleles and remains undigested for mutated alleles. (Lanes *1–3*) *Ava*II digestion; (lanes *4–6*) *Hph*I digestion. (Lane *1*) Patient 3 (ΔF508/1717-1G→A): *Ava*II digestion produces both a 116-bp fragment, due to the presence of the site in the wild-type allele for 1717-1G→A, and the 136-bp undigested fragment, due to the presence of 1717-1G→A on the mutated allele. (Lane *2*) Patient 4 (ΔF508/G542X): *Ava*II digestion generates the 116-bp fragment corresponding to both wild-type alleles for 1717-1G→A. (Lane *3*) Patient 2 (control N/N): *Ava*II digestion generates the 116-bp fragment corresponding to both wild-type alleles. (Lane *4*) Patient 3 (ΔF508/1717-1G→A). *Hph*I digestion generates the 162-bp fragment corresponding to both wild-type alleles for G542X. (Lane *5*) Patient 4 (ΔF508/G542X): *Hph*I digestion generates the 162-bp fragment corresponding to the wild-type allele for G542X and leaves undigested the 175-bp fragment, corresponding to the G542X mutated allele. (Lane *6*) Patient 2 (control N/N): *Hph*I digestion produces the 162-bp fragment corresponding to both wild-type alleles. (Lane *7*) Undigested control sample. Sizes on the left are in base pairs.

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G542X) which is present in the PCR product of a wild-type allele but is lacking in an amplified mutated allele. Both PSDM reactions employ the forward primer made available by the CF Genetic Analysis Consortium to amplify exon 11 (5′-CAACTGTGGTTAAAGCAATAGTGT-3′)[3] For the detection of ΔF508, the allele-specific forward primer (either 5′-GGCACCATTAAAGAAAATATCATCTT-3′ for the wild-type allele or 5′-GGCACCATTAAAGAAAATATCATTGG-3′ for the ΔF508 deleted allele)[6] was employed in combination with the reverse primer (5′-CATTCACAGTAGCTTACCCA-3′) commonly used to amplify exon 10.[3]

PCR was performed on 1 μg of genomic purified DNA at the following conditions: 10 mM Tris•HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM each dNTP, *Taq* polymerase 1.25 units (Perkin-Elmer Cetus), 50 pmoles of exon 11 forward primer, 20 pmoles of exon 10 reverse primer and each allele-specific primer for ΔF508, 22.5 pmoles of PSDM reverse primer for 1717-1G→A and 35 picomoles of PSDM reverse primer for G542X in a final volume of 50 μl. Denaturation was 1 min at 94°C, annealing 45 sec at 55°C, and extension 1 min at 72°C for a total of 30 cycles. The last cycle was followed by a 7-min step at 72°C. Alternatively, PCR can be directly performed on the 1.5-mm spots on Guthrie cards, after 30 min boiling extraction in water at 95–97°C of the card previously cut into very little slices. Amplification of Guthrie cards is better achieved at the above-mentioned conditions, adjusting the concentration of the reagents to a final volume of 100 μl.

For the detection of ΔF508, an aliquot (10 μl) of the PCR reaction was electrophoresed on an ethidium bromide-stained NuSieve 1%–agarose 3% minigel at 100 volts for 30 min. For the detection of 1717-1G→A and G542X in those samples that had not been diagnosed as ΔF508 homozygous by the previous gel, the amplified products were separately digested after ethanol precipitation, with either *Ava*II or *Hph*I, under the conditions recommended by the manufacturers. Electrophoresis was carried out on a 15% PAGE minigel at 150 volts for 2 hr. The reliability of the method was assessed on CF chromosomes carrying either the ΔF508 (40 chromosomes), or the 1717-1G→A or the G542X mutation (10 chromosomes each), which had already been characterized by different techniques such as direct sequencing or radiolabeled allele-specific oligonucleotide hybridization.

More than 150 different samples were analyzed using this method without any problem in multiplex amplification. The only difficulty in setting up the multiplex PCR condition was in determining the right proportion in picomoles of each set of primer to obtain fragments amplified with the correct efficiency for generating bands of comparable intensity. The possibility of employing mismatched primers to introduce base substitutions creating a novel restriction site in the wild-type and not in the mutated allelic form, coupled with the use of allele-specifc primers, allows a multiplex amplification for the detection of mutations that are predominant in our population. Because the combined analysis of the ΔF508, 1717-1G→A, and G542X mutations would allow the detection of about 65% of the molecular defects present in Italy, this test may constitute a fast and easy approach for adult and neonatal carrier screening.

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REFERENCES

1. Kerem, B., J.M. Rommens, J.A. Buchanan, D. Markiewicz, T.K. Cox, A. Chakravarti, M. Buchwald, and L.-C. Tsui. 1989. Identification of the cystic fibrosis gene: Genetic analysis. *Science* **245:** 1073–1080.
2. Guillermit, H., P. Fanen, and C. Ferec. 1990. A 3′ splice site consensus sequence mutation in the cystic fibrosis gene. *Hum. Genet.* **85:** 450–453.
3. Kerem, B., J. Zielenski, D. Markiewicz, D. Bozon, E. Gazit, J. Yahav, D. Kennedy, J.R. Riordan, F.S. Collins, J.M. Rommens, and L.-C. Tsui. 1990. Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. *Proc. Natl. Acad. Sci.* **87:** 8447–8451.
4. Haliassos, A., J.C. Chomel, M. Baudis, J. Kruh, J.C. Kaplan, and A. Kitzis. 1989. Modification of enzymatically amplified DNA for the detection of point mutations. *Nucleic Acid Res.* **17:** 3606.
5. Cremonesi, L., M. Seia, C. Magnani, and M. Ferrari. 1991. Rapid detection of 1717-1G→A mutation in CFTR gene by PCR-mediated site-directed mutagenesis. *Clin. Chem. 37:* 1447.
6. Ballabio, A., R.A. Gibbs, and C.T. Caskey. 1990. PCR test for cystic fibrosis deletion. *Nature* **343:** 220.

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