

Abstract

Rapid Detection of Genomic Rearrangements in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene by Quantitative Multiplex PCR Amplification of Short Fluorescent Fragments: Extensive Allelic Heterogeneity and Diverse Mutational Mechanisms

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Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*; OMIM 602421) gene. Despite the extensive and enduring efforts of many CF researchers over the past 14 years, up to 30% of disease alleles still remain to be identified in some populations. It has long been suggested that gross genomic rearrangements could account for these unidentified alleles. To date, however, only a few large deletions have been found in the *CFTR* gene and only three have been fully characterized. Here we report the first systematic screening of the 27 exons of the *CFTR* gene for large genomic rearrangements, by means of the quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF). A well-characterized cohort of 39 classical CF patients carrying at least one unidentified allele, after extensive and complete screening of the *CFTR* gene by both denaturing gradient gel electrophoresis and denaturing high-performance liquid chromatography, participated in this study. Using QMPSF, some 16% of the previously unidentified CF mutant alleles were identified and characterized, including 5 novel mutations (1 large deletion and 4 indels). The breakpoints of these 5 mutations were precisely determined enabling us to explore the underlying mechanisms of mutagenesis. Although non-homologous recombination may be invoked to explain all 5 complex lesions, each mutation appears to have arisen through a different mechanism. One of the indels was highly unusual in that it involved the insertion of a short 41 bp sequence with partial homology to a retrotranspositionally-competent LINE-1 element. The insertion of this ultra-short LINE ('hyphen') element may constitute a novel type of mutation causing human genetic disease.