


Development and Evaluation of a PCR-based, Line Probe Assay for the Detection of 58 Alleles in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene, Xinjing Wang,1 Angela Myers,2 Randall K. Saij,2* and Garry R. Cutting1 (1 Johns Hopkins University School of Medicine, Baltimore, MD 21287; 2 Roche Molecular Systems, Alameda, CA 94501; * current address: Celera Diagnostics, Alameda, CA 94502; † address correspondence to this author: Johns Hopkins University School of Medicine, CMSC 9-120, Baltimore, MD 21287; fax 410-955-0484, e-mail gcutting@jhmi.edu)

Cystic fibrosis (CF) is the most common life-limiting recessive genetic disorder in Caucasians, with an incidence of ≈1 in 3200 newborns (1). The disease is less common in Hispanic Americans (1 in 9200), African Americans (1 in 15,000), and Asian Americans (1 in 31,000) (2). The diagnosis of CF requires the presence of one or more characteristic phenotypic features, a history of CF in a sibling, or a positive newborn screening test result plus laboratory evidence of cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction (3). Although sweat chloride testing continues to be the laboratory test of choice, DNA testing is growing in popularity, especially in situations where sweat testing is unavailable or ambiguous. In 1999, 9.1% of patients attending CF centers in the United States had DNA analysis as a confirmatory diagnostic test (4). Furthermore, DNA testing is the only method available that can readily identify the carriers of CF mutations and, therefore, is the only tool for CF carrier screening.

The gene responsible for CF, the CFTR gene, was identified by positional cloning (5, 6). Nearly 1000 putative disease-associated mutations have been reported in CFTR, but only a few dozen have a frequency >0.1% in the general population (1, 7). Thus, efficient and accurate population screening for CF requires an assay that can simultaneously detect the mutated and wild-type sequences of ~30 CF alleles. We have developed the Research Prototype Cystic Fibrosis Assay-31, which uses multiplex PCR (8, 9) and colorimetric detection with sequence-specific oligonucleotide probes immobilized in a linear array on nylon membranes (10), to type 28 CF mutations and 3 CFTR polymorphisms [Table 1; details of this procedure are described in a supplemental file, named “Cystic Fibrosis Assay 31 Procedure”, accompanying the online version of this Technical Brief (available at http://www.clinchem.org/content/vol48/issue7/)]. Probe sequences were optimized carefully to permit genotyping of all alleles under the same assay conditions. The procedure takes advantage of the fact that, under appropriate reaction conditions, a short oligonucleotide probe will hybridize to its target only when it is perfectly matched; a single base-pair mismatch is often sufficiently destabilizing to prevent a stable probe–target duplex from forming.

We used the Research Prototype Cystic Fibrosis Assay-31 to genotype 53 samples with CF mutations that were previously identified by other methods and 18 samples without CF mutations that were sequenced for entire coding regions of CFTR. The 53 positive samples

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>Size, bp</th>
<th>Mutations (polymorphisms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 13</td>
<td>598</td>
<td>2307 insA</td>
</tr>
<tr>
<td>Intron 8, exon 09</td>
<td>548</td>
<td>A455E, 5T (7/9 T polymorphism)</td>
</tr>
<tr>
<td>Exon 10</td>
<td>482</td>
<td>G480C, Δ1507, ΔF508 (F508C, I507V, I506V polymorphisms)</td>
</tr>
<tr>
<td>Intron 10, exon 11</td>
<td>433</td>
<td>1717–1G→A, G542X, G551D, R553X, A559T, R560T</td>
</tr>
<tr>
<td>Exon 19</td>
<td>420</td>
<td>R1162X, 3659delC</td>
</tr>
<tr>
<td>Exon 21</td>
<td>397</td>
<td>N1303K</td>
</tr>
<tr>
<td>Exon 20</td>
<td>359</td>
<td>S1255X, W1282X</td>
</tr>
<tr>
<td>Exon 07</td>
<td>328</td>
<td>1078delT, R334W, R347P</td>
</tr>
<tr>
<td>Exon 04, intron 4</td>
<td>288</td>
<td>R117H, 621+1G→T</td>
</tr>
<tr>
<td>Intron 14b</td>
<td>428</td>
<td>2789+5G→A</td>
</tr>
<tr>
<td>Intron 19</td>
<td>237</td>
<td>3849+10kbC→T</td>
</tr>
<tr>
<td>Exon 03</td>
<td>210</td>
<td>G85E, 405+3A→C</td>
</tr>
<tr>
<td>Intron 5</td>
<td>166</td>
<td>711+1G→T</td>
</tr>
<tr>
<td>Intron 16</td>
<td>139</td>
<td>3120+1G→A</td>
</tr>
</tbody>
</table>
contained 77 CF mutations and 7 polymorphisms. Regardless of the respective comparison methods used, a concordance of 100% was obtained for the detection and identification of CF mutations. In addition, no false-positive results were found during the evaluation or during the in-house validation of the assay. None of the CF mutations detected by the genotyping strips were incorrectly identified as other mutations. The assay accurately identified all cases in which 1 or more of the 28 mutations were present.

Fig. 1 shows the results for 33 samples containing each mutation or polymorphism detected by the assay. Sample 2 demonstrated simultaneous detection of three alleles; 5T, R117H, and 3659delC, consistent with the previously determined genotype. Sample 6 was correctly genotyped as a compound heterozygote for the common CF mutation F508C. Sample 28 demonstrated the detection of a homozygote for the R1162X mutation.

We also evaluated the effect of varying the concentration of the genomic DNA on the signals generated by the wild-type alleles. Tests with two samples using 6.25 ng, 12.5 ng, 25 ng, 50 ng, 100 ng, 200 ng, 400 ng, and 1 μg of genomic DNA demonstrated no difference in signal strength above 12.5 ng of DNA. The signal intensities of the wild-type alleles for A455E, G480C, I507/F508, and 2307insA probes were decreased when 12.5 ng of DNA was used, but could still be distinguished from background even at 6.25 ng of DNA. We concluded that the assay operates well over a wide range of genomic DNA concentrations, although at least 25 ng should be used to ensure robust signals for all alleles.

During the evaluation phase, a sample that was previously genotyped as a compound heterozygote, S549N/R553X, hybridized as expected with the R553X wild-type and mutant probes. However, the presence of the R553X and S549N mutations precluded hybridization to the G551D wild-type probe (data not shown). Thus, although the assay correctly identified the R553X mutation in a heterozygous state, the lack of hybridization with the G551D wild-type probe (absence of signal in the wild-type allele) indicated the need for additional analysis to determine the genotype of the sample. A sample with the...
I506V/ΔF508 genotype showed a similar result. In this case, one allele hybridized with the probe designed to detect the ΔF508 mutation. However, the other allele did not hybridize with the ΔF508 probe because it did not contain that mutant sequence, nor did it hybridize with the wild-type probe because the A→G transition (data not shown), which gives rise to the I506V polymorphism, prevents hybridization to that sequence. This limitation is not unique to the Research Prototype CF assay and is a characteristic of all mutation-specific assays in that sequence variations near the interrogated mutation affect test accuracy.

In what situations might this be a problem? One scenario is the incorrect diagnosis of a mutation carrier as a homozygote because he or she also bears a polymorphism in the wild-type CFTR gene that interferes with hybridization of the wild-type oligonucleotide. If this scenario occurs in the context of carrier screening, then homozygosity for a CF mutation would be unexpected, and clinical correlation would be indicated. If this situation occurred in a patient being evaluated for a diagnosis of CF (3), then the patient may be misdiagnosed. However, homozygosity for a rare mutation is unusual and can be confirmed by family studies. On the other hand, the ΔF508 mutation is the most common CF allele, and three polymorphisms (I506V, I507V, and F508C) can interfere with hybridization of the wild-type oligonucleotide sequence. Thus, the presence of oligonucleotides corresponding to the three polymorphisms in the Research Prototype Cystic Fibrosis Assay-31 test avoids misdiagnosis of ΔF508/I506V, I507V, or F508C compound heterozygotes.

The assay described here is an expanded version of a test designed for detection of the 16 most common CF mutations in Caucasians (11). The CF31 panel includes 22 CF mutations that are common in Caucasians and 6 that are common in African Americans (Fig. 1). Among the 28 mutations, 6 are common in Hispanic CF patients (12). The predicted coverage is 85% of the disease-associated mutations in Caucasians, 75% in African Americans, and ~50% in Hispanics. To accommodate screening for the panel of CF mutations recommended by the American College of Medical Genetics, a new linear array assay (Linear Array CF Gold 1.0) has been developed that uses the same methods and hybridization conditions (13). The Research Prototype Cystic Fibrosis Assay-31 is an accurate, rapid, and reliable standardized test and a highly specific tool for the simultaneous identification of mutations and polymorphisms in the CFTR gene.

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References

Effect of Corticosteroid Therapy on Serum Cystatin C and β2-Microglobulin Concentrations, Arend Bökemamp,1,2 Joanna A.E. van Wijk,3 Michael J. Lentze,1 and Birgit Stoffel-Wagner2 (1 Children’s Hospital, Medical Center of Bonn University, D-53113 Bonn, Germany; 2 Children’s Hospital, Vrije Universiteit Medical Center, NL-1007 MB Amsterdam, The Netherlands; 3 Department of Clinical Biochemistry, Medical Center of Bonn University, D-53105 Bonn, Germany; * address correspondence to this author at: Kindemreflogie, Vrije Universiteit Medisch Centrum, De Boelelaan 1117, NL-1007 MB Amsterdam, The Netherlands; fax 31-20-444-0849, e-mail bokenkamp@VUMC.nl)

Cystatin C, a cationic low-molecular-weight protein (M, 13 300) (1), has been described as a promising endogenous marker of glomerular filtration rate (GFR) in both adults (2, 3) and children (4). The correlation of serum cystatin C concentrations with the results of inulin and 51Cr-EDTA clearance examinations was superior to the correlation obtained with serum creatinine (2–4). The gene for cystatin C is expressed in all nucleated cells (5) and bears the characteristics of a housekeeping gene (6). Therefore, the cystatin C production rate is assumed to remain constant (3). This is supported by clinical evidence from several studies (7, 8). In renal transplant recipients, however, increases in serum cystatin C concentrations out