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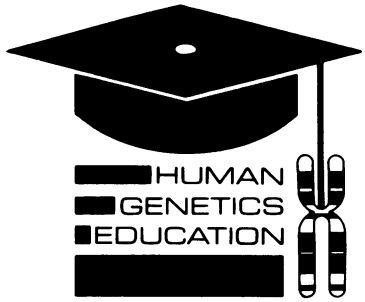
PCR-based Cystic Fibrosis (CF) Carrier Screening in a First-Year Medical Student Biochemistry Laboratory

Wayne W. Grody,*,† Kathryn E. Kronquist,*,‡,[1] Eryn U. Lee,§ John Edmond,§ and Leonard H. Rome§

*Division of Medical Genetics, and Departments of †Pathology and Laboratory Medicine, ‡Psychiatry, and §Biological Chemistry, University of California School of Medicine, Los Angeles



Introduction

The increasing pervasiveness of molecular biology and genetics in clinical medicine has been widely recognized and marveled upon. As sophisticated gene cloning and mapping techniques, now spurred by the Human Genome Initiative, lead to the identification of ever more disease-related genes, we are beginning to appreciate that virtually all disorders have an important genetic component. Even some of the major "somatic" disorders such as cardiovascular disease and cancer are coming to be thought of as genetic diseases that are diagnosable, and perhaps someday treatable, at the DNA level.

Many feel that medical school curricula have been slow to adapt to these changes and that at most institutions genetics is still given short shrift. Yet there is no question that today's medical students, who will be practicing in the 21st century, will have to be conversant with molecular genetic methods of diagnosis and, eventually, treatment. The few courses that are now offered do not seem to result in adequate knowledge retention even into the immediate postgraduate years. At our own institution, for example, we are often frustrated by clinical staff who order a complex Southern blot test (for example, a clonal gene rearrangement study on a suspected lymphoma) in our Diagnostic Molecular Pathology Laboratory and then call the lab the very next morning for the result, betraying little understanding of the nature of the test, let alone its interpretation.

At UCLA we developed several years ago a laboratory component to the basic first-year medical school biochemistry course (Biological Chemistry 204), which was designed to give the students a hands-on appreciation for some of the more commonly ordered clinical laboratory tests and the biochemical principles behind them. Laboratory exercises performed and interpreted by the students have included hemoglobin electrophoresis; iron, transferrin, and ferritin assays; fasting plasma glucose and insulin assays; creatinine clearance; urinary steroid analysis; and serum lipid profiles. Last year we introduced for the first time an exercise designed to acquaint the students with the newest area of laboratory medicine, DNA diagnostics. Of the many molecular biologic applications to medicine currently available, we felt that the most interest would be generated by focusing on the concept of molecular genetic screening and the revolutionary technique, PCR, that makes such screening feasible. Certainly this is the application with the most far-reaching societal and ethical implications and one in which the laboratory exercise would produce some personally meaningful results for the students.

Cystic Fibrosis (CF) Carrier Screening

CF is the most common lethal autosomal recessive disorder in the Caucasian population of North America, with an estimated carrier frequency of 1 in 25–30.

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Address for correspondence: Wayne W. Grody, Divisions of Medical Genetics and Molecular Pathology, UCLA School of Medicine, Los Angeles, CA 90024-1732.

[1] Present address: Department of Molecular Biology, Nichols Institute, San Juan Capistrano, CA.

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It is less common in African-Americans and Hispanics and quite rare in Asians. As is true for all autosomal recessive diseases, most CF patients are born to couples who do not know they are carriers and have no family history of the disorder. Since carriers of CF mutations are phenotypically normal, widespread carrier screening in high-risk populations has until recently not been possible.

The cloning of the CF gene on chromosome 7 in 1989 (Riordan et al. 1989; Rommens et al. 1989) and the discovery of a particular trinucleotide deletion (ΔF508) as the preponderant mutation in patients and carriers (Kerem et al. 1989) have now made heterozygote screening technically feasible, though not without controversy. The ΔF508 mutation, while prevalent, accounts for only about 70% of CF chromosomes; even adding to the screening protocol some of the more common of the more than 300 additional known mutations will raise the sensitivity level by only 10%–20%, depending on the ethnic group being screened. The suboptimal sensitivity of the test and the complexities involved in counseling those who test negative as to their degree of residual risk has led the medical genetics community to propose a moratorium on widespread CF carrier screening until its practicality can be demonstrated by pilot programs (Caskey et al. 1990). The results of those studies, currently ongoing and sponsored by the National Center for Human Genome Research, will likely have important implications not only for CF testing but also for screening of other, even more common, disorders for which DNA markers will become available in the future.

Aside from the cloning of disease-related genes, it is only through another great advance of molecular biology that we can begin to contemplate genetic screening on such a large scale. PCR, a process in which a small segment of DNA is replicated in exponential fashion by DNA polymerase initiating at oligonucleotide primers hybridizing to either end of the target sequence (Saiki et al. 1988), allows one to hone in on that particular region of the patient's DNA thought to harbor the suspected mutation, while ignoring the other 3 billion bp of the genome. The technique is far more rapid, sensitive, and cost-effective than the traditional Southern blot and thus is amenable to large-population screening. The extent to which PCR has already come to dominate research and clinical molecular biology cannot be overestimated. Since PCR is the emerging workhorse technique of the new era of molecular medicine, we believed that today's medical students would benefit from firsthand familiarity with this procedure.

Summary of Laboratory Exercise

The 1991–92 first-year class of the UCLA School of Medicine consisted of 149 students who were divided approximately equally among three separate laboratory sections of the biochemistry course. The entire class participated in a modified CF carrier screening test involving detection of only the most common CF mutation, ΔF508. The laboratory sessions were preceded by a lecture describing the clinical symptoms and genetics of CF, the rationale behind carrier screening, and the principles of PCR. A detailed experimental protocol was included in the laboratory manual used for the course (Rome and Edmond 1992, pp. IX-1–IX-14). The PCR utilized primers flanking codon 508 of the CF gene, producing an amplification product of 160 bp from a normal human genomic DNA template, and one of 3 bp fewer, or 157 bp, from target DNA containing the ΔF508 mutation (Chong and Thibodeau 1990). These products can be separated and visualized by PAGE and ethidium bromide staining. Target DNA from a heterozygous individual yields both bands, as well as a slower-migrating species produced by heteroduplex formation between the two PCR products.

Students prepared the target genomic DNA from 10 μl of heparinized blood at the time blood samples were drawn for another lab exercise. After crude extraction with Chelex-100 ion exchange resin (BioRad, Richmond, CA) (Walsh et al. 1991), PCR was performed in a DNA Thermal Cycler 480 (Perkin Elmer, Norwalk, CT) for 30 cycles at 94°C for 1 min (denaturation), 56°C for 2 min (annealing), and 72°C for 2 min (elongation). Polyacrylamide gels were loaded with 4 μl of each reaction mixed with 4 μl of 2× loading dye and electrophoresed at 400 V for 55 min. Gels were then stained in ethidium bromide–Tris borate–EDTA buffer solution for 20 min and photographed under UV light by using Polaroid 52 film. Copies of these photos were given to the students to analyze their results.

Results

The laboratory sessions generally went smoothly, with 100% participation. There were 24 PCR failures that were ascribed to faulty micropipetting by the students during DNA extraction and/or PCR setup. Most of these samples could be amplified successfully on repeat extraction. It should be kept in mind that the vast majority of the class had no prior laboratory experience; samples were loaded on the PAGE apparatus by the graduate teaching assistants rather than by the students, both because of the delicacy of the operation

and in order to maintain confidentiality (see below). The student body of UCLA's medical school is one of the most ethnically diverse in the country, and we thus did not anticipate many positive test results in this exercise. Two students were identified as carriers of the ΔF508 mutation; their results were confirmed by repeating the test on a fresh blood sample in UCLA's Diagnostic Molecular Pathology Laboratory, and they were offered genetic counseling if desired (both declined). A subsequent course evaluation survey indicated generally favorable reception by the students to this new facet of modern medical genetics and laboratory medicine. Negative comments seemed to center around the relatively sparse involvement in manual laboratory activities compared with some of the other class sessions. Examinations administered before and after the exercise demonstrated appreciable gain in conceptual knowledge of PCR and genetic screening.

Ethical Considerations

Genetic screening raises many ethical issues that are no less relevant for knowledgeable medical students than for naive patients. These issues were covered in depth in the lecture preceding the laboratory exercise and in the small-group discussion sessions held afterward. Indeed, many of the discussion questions presented to the students were designed to provoke a lively and even passionate debate over these issues. Just as in model genetic screening programs in real practice, we emphasized repeatedly that participation in this classroom version was strictly voluntary and did our best to avoid even a subliminal impression of coercion that might have been induced by the classroom setting. Regardless of one's personal feelings about being screened, however, we did feel that the other goal of the exercise—to gain firsthand experience with the PCR technique—was of value, and so any student who did not wish to test his or her own DNA was given the option to perform the exercise on an anonymous sample provided by the instructors. It is interesting that none took this option. Any student with a known family history of CF was asked to inform the instructors, as that would alter the risk of testing positive in the experiment.

While no separate informed consent form was composed for this laboratory section, an umbrella implied consent covered the course as a whole, and students were advised of the risk of anxiety and/or depression that could be evoked by the screening experience and even of the remote possibility of insurability problems for identified carriers. In order to maintain confidentiality and avoid potential stigmatization of identified carriers, PCR samples were number-coded prior to being loaded onto the polyacrylamide gel lanes, and only the teaching assistants who performed the actual loading knew the identity of the DNA in each lane. Students were also assured that their results would be held strictly confidential and not reported elsewhere and that they themselves were under no obligation to disclose such results to anyone else.

We are well aware that, despite all these precautions, one could still raise a number of objections on ethical grounds to this exercise as conducted. It could also be argued that a medical student's entire graduate and postgraduate education places him or her in the path of countless similar ethical risks, from which attempts to shelter would be coddling and unrealistic. Even within the defined biochemistry course under discussion, the students faced potential psychic traumas equal to or greater than learning that one is a CF carrier, such as when they measured their own cholesterol level and performed hemoglobin electrophoresis. On balance, we felt that, in light of its voluntary structure, the opportunity to become familiar with the power of PCR analysis and with the experience of genetic screening—both of which they will be ordering on their own patients in one setting or another—would be a valuable one.

Educational Considerations

To our knowledge, the experience at UCLA reported here is the first in which an entire large medical school class has performed PCR and human molecular genetic analysis in an individual, hands-on manner as part of their core training. It grew primarily from our desire to ensure that today's medical students be exposed, in a meaningful and memorable way, to an area of biology that is rapidly transforming all of medicine. We were fortunate that it fit well into the existing framework of UCLA's established and quite unique biochemistry laboratory course, in which students were already performing their own clinical laboratory tests for the more classical analytes. While we had earlier considered an exercise in DNA fingerprinting using the former workhorse of molecular biology, the Southern blot, we decided that the labor-intensive nature of that technique and its use of radioactivity would have been impractical in this setting. Our choice of PCR allowed for exposure to a true state-of-the-art technique and a genetic screening application that is much in the news and likely to assume even greater importance in the future.

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