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PCR-Based Screening for Cystic Fibrosis Carrier Mutations in an Ethnically Diverse Pregnant Population

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Summary

As the most common lethal autosomal recessive disorder in North America, cystic fibrosis (CF) is an obvious candidate for general population carrier screening. Although the identification of the causative gene has made detection of asymptomatic carriers possible, the extreme heterogeneity of its mutations has limited the sensitivity of the available DNA screening tests and has called into question their utility when they are applied to patients with no family history of the disease. The purpose of this study was to determine the technical feasibility, patient acceptance and understanding, and psychosocial impact of large-scale CF carrier screening in an ethnically diverse pregnant population. A total of 4,739 pregnant women attending prenatal clinics located in both an academic medical center and a large HMO were invited in person to participate. Of this group, 3,543 received CF instruction and assessments of knowledge and mood, and 3,192 underwent DNA testing for the six most common CF mutations, by means of a noninvasive PCR-based reverse-dot-blot method. Overall participation rates (ranging from 53% at the HMO to 77% at the academic center) and consent rates for DNA testing after CF instruction (>98%) exceeded those of most other American studies. The PCR-based screening method worked efficiently on large numbers of samples, and 55 carriers and one at-risk couple were identified. Understanding of residual risk, anxiety levels, and overall satisfaction with the program were acceptable across all ethnic groups. Our strategy of approaching a motivated pregnant population in person with a rapid and noninvasive testing method may provide a practical model for developing a larger CF screening program targeting appropriate high-risk groups at the national level, and may also serve as a paradigm for population-based screening of other genetically heterogeneous disorders in the future.

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Introduction

Cystic fibrosis (CF) is a relatively common autosomal recessive disorder, with carrier frequencies as high as 1/25–1/30 in the Caucasian population of North America (Boat et al. 1989). Although more aggressive antibiotic and other treatment strategies have emerged over the years to increase the life expectancy (Cystic Fibrosis Foundation 1986; Hubbard et al. 1992), the disease in its classic form remains one of chronic infection and debilitation, years of frequent hospitalizations, and, ultimately, early death, with a tremendous cumulative psychological and financial toll on the family and on society. As is typical of recessive disorders, the majority of affected patients are born to couples who do not know that they are at risk. Since heterozygotes are phenotypically normal, population-based carrier screening has not been possible previously.

The identification and cloning of the CF gene in 1989, as well as the finding of a particular three-nucleotide deletion (designated "ΔF508") in ~70% of Caucasian CF carriers (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989), raised the possibility of identifying couples at risk, who then could be offered genetic counseling and prenatal testing if desired. The initial discovery of the ΔF508 mutation in a high percentage of CF chromosomes engendered hope that the remaining patients and carriers would all express perhaps one or two other common mutations—but such was not to be the case. It is now known that there are >500 uncommon mutations, many of which are probably family specific (Beaudet 1990; Davies 1992; DeMarchi et al. 1994). Thus, any practical screening program for detection of a few of the more common mutations in unsuspecting carriers falls, unavoidably, below the level of sensitivity (usually 95%–99%) generally acknowledged as the minimum for a clinical laboratory test to be acceptable. Screening for the ΔF508 mutation alone will identify only ~50% of Caucasian couples at risk of producing a CF child (Ten Kate 1990), and introducing tests for 5–10 of the more prevalent additional mutations will increase this sensitivity only to ~81%. (The

detection level of individual carriers, without regard to spouse or partner, is of course higher, 68% and 85%, respectively, although this will vary by ethnic group [Beaudet 1990; Cystic Fibrosis Genetic Analysis Consortium 1990; Cutting et al. 1992; Grebe et al. 1994].) With refinement of multiplex PCR, pooled allele-specific oligonucleotide (ASO), and automation strategies (Chehab and Wall 1992; Davies 1992; Wall et al. 1995), some reference laboratories now boast detection of ≥30 mutations, but most of the additional ones are so uncommon that they add only incrementally (at best ~5%) to the overall sensitivity of the test (Davies 1992).

This state of affairs has led to a serious ethical dilemma and much debate within the clinical genetics community (for reviews, see Wilfond and Fost 1992; Williamson 1993). Should mass carrier screening be withheld until the sensitivity can be increased to a more acceptable level, as initially recommended by the official consensus statements of The American Society of Human Genetics (ASHG) (Caskey et al. 1990; American Society of Human Genetics 1992), the National Institutes of Health (NIH) workshop report (Workshop on Population Screening for the Cystic Fibrosis Gene 1990), and others (Gilbert 1990; Biesecker et al. 1992)? Or is it equally unethical to withhold such a powerful tool from even the fraction of couples who might benefit (Brock 1990; Schulman et al. 1990)? The point has been raised that screening for neural tube defects by maternal serum alpha-fetoprotein began when the detection level was only 70% and that even a reduction, as opposed to outright abolition, of genetic risk is useful to society (Brock 1990).

One point that all factions in the debate agreed on was the need for pilot studies of CF carrier screening, and in fact this was a key proposal of both the ASHG and NIH consensus statements. Moreover, psychosocial, ethical, and counseling aspects of such screening were considered to be just as essential for pilot study as were the more practical and technical laboratory aspects (Caskey et al. 1990). Even if carrier-detection methods were 100% sensitive, a screening program on the scale proposed for CF would be unprecedented and would raise many complex issues not considered before, since previous carrier-screening programs have focused on more narrowly defined segments of the population. Experience derived from these earlier programs (Tay-Sachs, sickle-cell anemia, and thalassemia) indicated that the meaning of the carrier state is misunderstood by many patients and that informing a patient that he or she is a carrier can be psychologically devastating (Whitten 1973; Childs et al. 1976*b*; Zeesman et al. 1984). Such reactions may vary significantly among different target populations, depending on ethnic group, level of education, socioeconomic status, religion, and perception of the clinical burden of the disease in question, and it may be modified or ameliorated by appropriate pre- and posttest counseling (McCrae et al. 1973; Childs et al. 1976*b*; Loader et al. 1991; Weil 1991). Finally, there is the very real risk of socioeconomic stigmatization of identified carriers, with discrimination in matters of marital choice, insurability, and employment (Gostin 1990; Billings et al. 1992).

In response to these considerations, the National Center for Human Genome Research (NCHGR) inaugurated, in 1991, a series of pilot CF carrier-screening studies designed to address these concerns. A consortium of seven research teams was funded: five of them were to address, by random population-based screening in those with no known family history of CF, the issues raised above, and the other two were to target CF patients' relatives, who are at much higher a priori risk of carrying the mutant gene. (Two other studies, with somewhat different goals, have been funded more recently.) Our UCLA study, a member of the first group, stands somewhat apart from the others, in the size of the sample, choice of target population, and the inclusion of substantial numbers of racial and ethnic minorities. Our Southern California target population is among the most ethnically diverse in the United States and includes large numbers of minority groups, such as Hispanic Americans, which hitherto had not been studied extensively for either their allele frequencies of CF mutations or their response to screening and counseling. We have been exploring the technical feasibility and patient acceptance of a rapid and noninvasive yet unavoidably noncomprehensive PCR-based mutation-detection method. Pre– and post–DNA test questionnaires have been used to determine the level of understanding of and the emotional response to the implications of the DNA findings across the various ethnic and socioeconomic groups. Multiple approaches to pre- and posttest counseling have been compared to determine their effectiveness. Attitudes about genetic screening and CF screening in particular, as well as the impact of positive and negative results on those tested, have been compared among the different groups and between our private and HMO-based subject populations.

Subjects and Methods

Subject Recruitment

Mindful of the stated consensus within the genetics community—that carrier screening for recessive disorders should concentrate on persons and couples of childbearing age—we recruited our subjects from prenatal (obstetric) clinics. Women attending these clinics, >18 years of age and <19 wk pregnant, were invited to participate by one of our genetic counselors or project staff. At this point they were told only that they would be participating in a study to evaluate a new test for the

CF gene, that the test was free of charge and noninvasive, and that the testing and questionnaire procedures would take ~30 min to complete. Those deciding to participate completed an informed-consent form and then filled out a demographic-survey form prior to receiving CF instruction and the subsequent survey instruments (see below); women answering affirmatively to a question about family history of CF were excluded from the study. Those declining to participate in the study were asked to fill out a brief questionnaire assessing their reason(s). In order to comparatively assess optimal settings for delivery of this sort of genetic service, we recruited subjects from two different types of health-care milieus: a large academic medical center (UCLA), and a large HMO (Kaiser Permanente Medical Centers of Southern California). All procedures were approved by institutional review boards of the respective institutions.

CF Instructional Tools

We produced both a brochure and an 8-min videotape (both English and Spanish versions) describing the nature and incidence of CF, its anticipated prognosis in the coming years, and the advantages and imperfections of the currently available DNA screening method. The CF carrier frequencies and relative test sensitivities in various ethnic populations are described in the brochure and are shown graphically in the video. Some carrier frequencies (and test sensitivities) demonstrated include 1/25–1/30 (85%) for Caucasians of northern European descent, 1/40–1/50 (≈60%) for Hispanics, 1/65 (<50%) for African Americans, etc. Each of these aspects is presented in an emotionally neutral, nondirective, and noncoercive manner. CF patients shown briefly in the video are depicted neither as overtly disabled nor as athletic superstars. These materials were first pilot-tested repeatedly on students, nurses, and actual patients, assisting us in tailoring them to an acceptable length, tone, and educational level. At the conclusion of the CF instruction session, subjects were given the opportunity to ask questions of the genetic counselors and the option to proceed with the DNA testing, at which point a second informed consent was obtained. Reasons for declining were recorded also.

Assessment Instruments

A series of questionnaires assessing clinical knowledge of CF, mood state, and health-belief perceptions relevant to genetic screening were administered before and after the instruction sessions and after receipt of the results of the DNA test. The overall framework for this sequence is shown in the appendix. Knowledge of both the clinical symptoms and genetics of CF was assessed before and after the instruction, with important questionnaire items specifically targeted at measuring subjects' understanding that a negative DNA test result does not completely eliminate carrier risk. Also tested was understanding of the ethnic differences in incidence of CF, the meaning and implications of the carrier state, and attitudes regarding the severity and emotional burden of the disease and regarding genetic testing in general (Tatsugawa et al. 1994). Questionnaire items were written in either a true/false or agree/disagree format. A total of 17 knowledge questions and 29 attitude items were asked at the various time points.

For mood assessment before and after the instructional and DNA testing interventions, we adapted the Spielberger State-Trait Anxiety Inventory (Spielberger 1985) and a coping-style assessment to the setting of CF screening. In order to develop predictive models of the various subject groups' likelihood of consent to screening, we constructed questionnaire items to test two health-behavior models: the Health Belief Model (Rosenstock 1966) and the Theory of Planned Behavior (Ajzen 1985). These models relate motivation toward positive health behaviors to such attitudes as perceived vulnerability to the disease, its perceived severity, and belief that one can successfully accomplish the preventive behavior. More details and results of this aspect of the study are being presented elsewhere (Fang et al., in press).

Specimen Collection and DNA Analysis

For specimen collection, we employed gentle scraping of the buccal mucosa by a single standard Pap smear–type cytobrush (Medscand) (Richards et al. 1992; Thomson et al. 1992). After the sample was taken, the brush was placed in a 15-ml plastic conical centrifuge tube, was stored, if necessary, in the refrigerator, and then was transported, dry and at room temperature, to our laboratory, usually within 24–48 h. DNA was extracted into 1 ml H_2O by inversion for 25 min at room temperature. The solution was then microfuged for 3 min at 12,000 *g*, and 20 μl of supernatant was retained. This was heated with 2 μl of 10 mg proteinase K/ml, in 180 μl H_2O, for 1–2 h at 37°C. The solution was microfuged for 5 min at 12,000 *g*, and 20 μl of supernatant was retained. The mix was then incubated with 180 μl of 10% Chelex-100 ion-exchange resin (Bio-Rad) for 25 min at 56°C, vortexed for 10 s, boiled for 8 min, vortexed, and spun at 12,000 *g* for 3 min. Three microliters of the resulting supernatant was used for each PCR.

To evaluate an efficient protocol for DNA testing, we employed a rapid, nonelectrophoretic, nonisotopic reverse-dot-blot system being developed by Roche Molecular Systems. In this method, oligonucleotide probes complementary to the six most common CF mutations (ΔF508, G542X, G551D, R553X, W1282X, and N1303K) and their corresponding normal alleles are

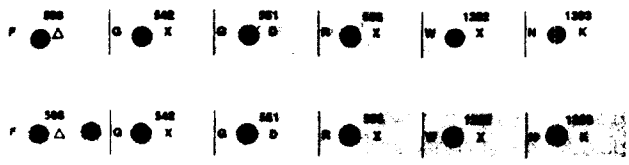

Figure 1 Detection of CF mutations by reverse-dot-blot hybridization. ASO probes complementary to the normal and mutant sequences are located on the left and right, respectively, for each of the six mutation loci. The subject tested on the upper strip is not a carrier for any of the six mutations screened; the subject on the lower strip is a ΔF508 heterozygote. (Test strips courtesy of Roche Molecular Systems, Inc.)

bound to a filter membrane (in the form of a strip), to which biotin-labeled PCR products amplified from the subject's DNA are hybridized (Saiki et al. 1989; Chehab and Wall 1992). Hybridization is detected by addition of a streptavidin–horseradish peroxidase complex and the appropriate enzyme substrates to produce a blue color (fig. 1). Because the colorimetric signal tends to fade after initial development, the strips were photographed with Polaroid black-and-white film to create a permanent record. All positive test results were confirmed by retesting of a second specimen obtained from the subject.

Results Reporting and Counseling

At this point our triage bifurcated, depending on whether the subject tested positive or negative for one of the screened CF mutations in the DNA test. The vast majority of our subjects testing negative were sent a detailed letter of test results and explanation by mail, along with a posttest questionnaire assessing both the level of understanding of the meaning of a negative result and the degree of anxiety provoked. Paradoxically, the nature of CF DNA testing is such that it is easier to explain the meaning of a positive result than to explain the subtleties of the residual risks that accompany a negative result. Because it was recognized that the emotional reaction to a positive result could be far greater and could lead to reproductive interventions, reporting and counseling of those who tested positive was conducted in person. The subjects were contacted by telephone by one of our genetic counselors and were invited to return to the clinic for repeat confirmatory testing, in-depth counseling, and, if desired, testing of the reproductive partner. The subjects testing positive also were given the posttest knowledge–assessment and mood-assessment surveys. They typically were told of the repeat-confirmation result (no discrepancies ever occurred) during the same counseling session at which the partner's results were presented. Those whose partner also tested positive (only one couple in our study) were counseled as to the options and procedures for prenatal diagnosis.

Management and Analysis of Data

All data were coded from questionnaires onto an IBM PC Dbase database. The Statistical Analysis System (SAS) package of programs (SAS/STAT, version 6.04; SAS Institute) was used to conduct all statistical analyses. CF allele frequencies in the various ethnic groups studied were calculated as proportions reporting the ethnic group in both parents. Ninety-five percent confidence-interval estimates were calculated for all carrier frequencies (Sachs 1982).

Results

Demographics

To date we have approached 4,739 potential subjects, of whom 3,688 (78%) consented to participate. One hundred forty-five subjects failed to complete all instructional materials, usually for logistical reasons within the clinics. The resulting study cohort (n = 3,543) was as follows: 50% non-Hispanic Caucasian, 28% Hispanic American, 11% Asian American, 7% African American, 1% Native American, and 3% other, not specified, or mixed ethnicity.

Understanding of CF and the DNA Test

Across all subject groups, baseline knowledge of the genetics of CF (recessive inheritance, ethnic differences, etc.) was poorer than knowledge of the disorder's clinical features. Delivery of instruction in the manner described above appeared to be both efficient and effective, with correct-answer scores in both categories increasing by 30%–100% between the pre- and postinstruction knowledge assessments (fig. 2). In a subset of subjects carefully matched for age, ethnicity, socioeconomic status, and educational level, there were no significant test-score differences between those receiving instruction by video and those using the brochure, whether the language was English (48 subjects) or Spanish (8 subjects) (data not shown), a finding matched by another group in our consortium (Hannig et al. 1994). By the end of testing, only 7% of subjects completing all questionnaires evinced inadequate understanding of the residual risk inherent in a negative DNA test result.

Consent to Screening

Overall our study population showed a surprisingly high interest in CF screening, with as many as 98% of those who completed the educational intervention consenting to the DNA test. The small proportion who chose not to be tested after the CF instruction session most often stated as their reason low perceived risk based on ethnic origin. In contrast, we had a somewhat lower proportion of potential subjects consenting to enter the study when they initially were approached. Individuals' overall willingness to commence the question-

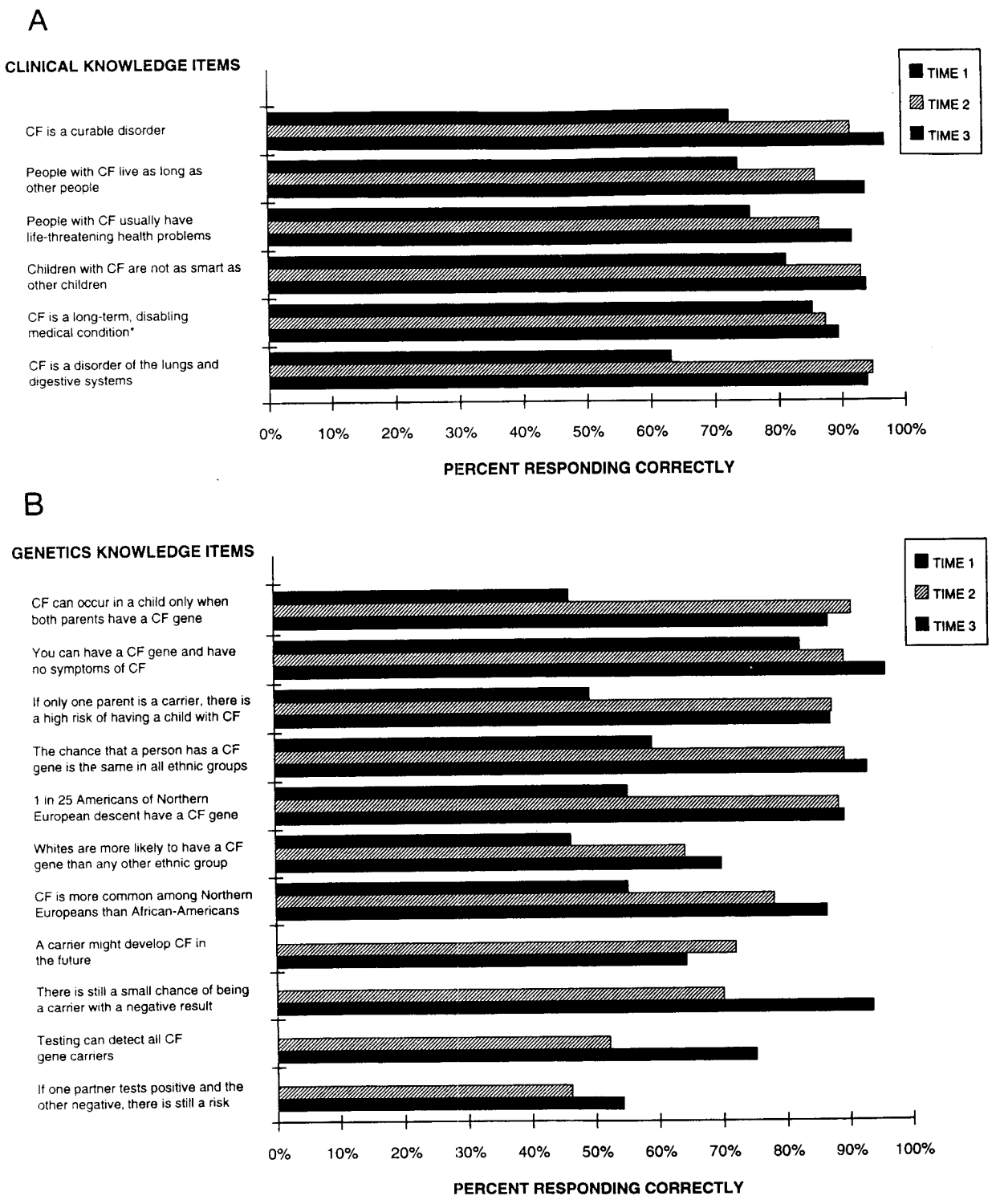

Figure 2 Subjects' knowledge of clinical (*A*) and genetic (*B*) aspects of CF, assessed before (TIME 1) and after (TIME 2) the CF educational intervention and after receipt of the results of DNA testing (TIME 3). Only subjects completing the assessment tools at all three time points are included in this figure. Differences for all items but one (which is marked by an asterisk [*]) were significant to $P < .05$.

Table 1

Reason for Declining to Participate in Original Protocol

Reason	% of Total
Lack of time	40.4
Burden of questionnaires	27.1
Need more information	20.0
Lack of concern—no history of CF	12.3
Lack of concern/other reasons	14.0
Too anxious at time of visit	10.6
Must consult with partner first	9.4
Other	20.0

Table 3

Post–DNA Test Knowledge Scores: Original Protocol versus Streamlined Protocol

	Original Protocol	Streamlined Protocol
No. of subjects	455	251
No. of correctly answered questions:[a]		
Mean	8.76	7.27
Median	9.0	8.0

[a] Of 17 questions asked.

naires and CF instruction when first invited ranged from 77% at the academic center to 53% at the HMO. The UCLA clinic is slower paced than the busy Kaiser sites, and, indeed, at all sites the most common reason given by individuals declining to enter the study was lack of time (table 1). It is noteworthy that 27% of decliners stated that they would have participated if they could have taken the laboratory test only, without having to answer any questionnaires. Since any national program ultimately instituted is likely to be far more streamlined, perhaps consisting of only a short brochure and the DNA test itself, we subsequently initiated an abbreviated recruitment protocol along these lines. As expected, this produced higher willingness to commence CF instruction but lower subsequent consent for DNA testing, perhaps because these subjects had less time and attention invested in the program by that point or because the shorter protocol captured a larger number of subjects who were ambivalent at the start. Overall consent rates were still considerably augmented, however, especially in the busy HMO setting (table 2), and effectiveness of CF knowledge retention was not significantly compromised, with those in the streamlined protocol answering correctly, on average, only one less questionnaire item (of 17) than a complementary set of subjects in the original protocol who otherwise were matched for age, ethnicity, and educational level (table 3).

Under the original protocol, consent to participate in the study was relatively greater among Caucasians, Native Americans, and Asian Americans (~60%–70%) than among African and Hispanic Americans (51%–54%), and this correlated broadly with educational level as well (data not shown). Consent was also relatively greater among those women attending clinics specifically for other prenatal diagnosis procedures (76%) than among those undergoing routine prenatal care (51%). The former group may be already more attuned to, aware of, and motivated toward prenatal and genetic testing than is the latter. However, these differences tended to disappear when the streamlined protocol was used (table 4).

Mutation Detection

To date, 3,192 subjects have undergone DNA testing for the six mutations. We have identified a total of 55 carriers, giving an overall carrier frequency in this ethnically diverse cohort of 1/58 (1.7%; 95% confidence interval 1.3%–2.2%). The distribution of mutations in the identified carriers is presented in table 5. Not surprisingly, the ΔF508 mutation was most prevalent, followed

Table 2

CF Screening Consent Rates

	% (No.) Consenting		
Category	Academic Center	HMO	Overall
Willing to commence protocol:			
Original protocol	76.6 (685)	53.2 (620)	64.5 (1,305)
Streamlined protocol	88.2 (304)	93.0 (1,608)	92.1 (1,912)
Overall			77.8 (3,217)
Willing to have DNA test after education:			
Original protocol	99.1 (634)	96.0 (513)	97.9 (1,147)
Streamlined protocol	87.5 (276)	84.4 (1,446)	84.8 (1,722)
Overall			90.1 (2,869)

Table 4

Consent Rates for Entering Study, by Ethnicity, Protocol, and Reason for Visit

	% (No.) of Subjects						
Category	Asian American	African American	Hispanic American	Caucasian	Native American	Routine Prenatal Care	Prenatal Diagnosis
Original	60.4% (113)	54.2% (84)	51.4% (358)	69.7% (707)	70.0% (7)	51.1%	75.5%
Streamlined	93.7% (237)	92.9% (156)	96.9% (563)	93.3% (889)	100% (16)	95.4%	90.3%
Overall	79.6% (350)	74.3% (240)	72.1% (921)	81.1% (1,596)	88.5% (23)	77.3%	79.4%

by the W1282X Ashkenazi Jewish mutation (10% of our subjects were from this ethnic group). Questionnaire information on maternal and paternal ethnicity and countries of origin of those tested revealed no CF mutations among 269 African Americans (calculated carrier frequency <1.2%) or 26 Native Americans (calculated carrier frequency <3.2%) and revealed one ΔF508 mutation, in a woman of mixed German and Japanese ancestry, among 332 Asian Americans (calculated carrier frequency <0.9%). Of the 1,040 subjects reporting Hispanic ancestry, nearly two-thirds were Mexican American. The eight carriers identified (encompassing three different mutations) produced carrier frequencies of 0.3%–1.5% among Hispanic Americans and 0.2%–1.5% (95% confidence limits) among Mexican Americans. Of the 1,851 subjects with non-Hispanic Caucasian ancestry, 47 carriers encompassing four mutations were identified (2.1%–3.9%). The 456 subjects with parental descent from the British Isles yielded carrier rates of 0.7%–3.6%, and, among 9 women reporting ancestors from Australia or New Zealand, 2 New Zealanders carried the ΔF508 mutation. Our 365 Jewish individuals produced a carrier frequency of 1.1%–5.5%, primarily for the expected W1282X mutation. The low carrier yield observed for the Asian American and Native American populations in our screening test is consistent with results of earlier studies (Grebe et al. 1992; Curtis et al. 1993).

Table 5

CF Carriers Identified

Mutation	No. of Individuals Positive[a]
ΔF508	41
W1282X	10
G542X	2
G551D	1
N1303K	1
R553X	0

[a] Of 3,192 individuals tested.

For the 55 carriers, 47 male partners presented themselves for testing. Reasons given by the eight partners who did not pursue testing included geographic unavailability (three cases), inconvenience of the return visit (one case), spontaneous (one case) or elective (two cases) termination of the pregnancy for unrelated reasons, and lack of concern with regard to degree of risk (one case). Only one at-risk couple emerged, in which both the man and woman were carriers of mutation ΔF508. They opted to proceed with prenatal diagnosis and, when the fetus was found to be homozygous for ΔF508, to undergo termination. Despite the unwonted nature of the circumstances, both were extremely grateful to our program for identifying them as at risk for having a child with a disease for which they had no family history.

The multiplex PCR and reverse-dot-blot hybridization system, in conjunction with our specimen-collection technique, worked efficiently and accurately in our hands. All those who tested positive were confirmed by retesting them with a fresh DNA sample from the subject, and no discrepancies were observed. The entire testing procedure takes 6–8 h to complete, and a single technologist can comfortably handle as many as 50 tests/d. Likewise, the buccal brush sample-collection technique has proved advantageous for screening large numbers of subjects at geographically dispersed sites. As noted, the brushes can be transported and stored at room temperature, and we have demonstrated DNA stability and extractability for as long as 1 mo. With the use of Chelex-100 ion-exchange resin (Walsh et al. 1991) in the extraction protocol (to remove PCR inhibitors), overt amplification failures, easily detected by the absence of blue color on both the mutant and normal allele spots of the test strip, have been rare (<0.5%). And we have found that even many of these "failures" often can be salvaged by re-treating the DNA sample with Chelex-100 and/or proteinase K, without having to go back to the patient to collect a fresh specimen.

Impact and Follow-up

In follow-up questionnaires, the vast majority (>98%) of subjects have expressed a high degree of

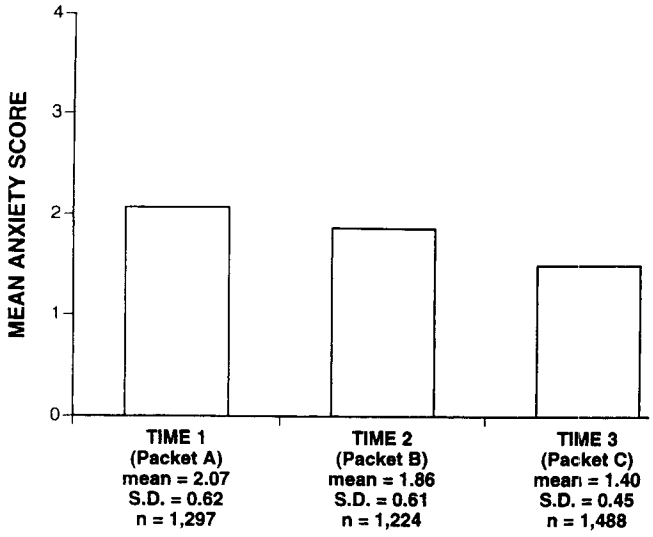

Figure 3 Mean state anxiety scores of subjects assessed before (TIME 1) and after (TIME 2) the CF educational intervention and after receipt of the results of DNA testing (TIME 3). Only subjects completing the assessment tools at all three time points are included in this figure. Results were significant to $P < .001$.

satisfaction with the screening program and results reporting. In general, no increase in anxiety levels appeared to have been evoked by either the CF educational intervention or the DNA testing. On the contrary, mean anxiety scores actually decreased somewhat after the instruction and again after subjects received their DNA test results (fig. 3). Similarly, we have detected no significant residual change in anxiety levels, nor evidence of stigmatization or discrimination among those testing positive, although the numbers involved in the latter group are small and the follow-up period still is limited. Although this group expectedly showed concern while awaiting their partners' test results, the vast majority (97%) were reassured by the partner's negative outcome and remained emotionally unaffected for the duration of the pregnancy (table 6). In fact, this feeling of relief even translated into a false sense of security (i.e., belief that the partner's negative test result reduced to zero the risk to the fetus) in fully 40% of this subset of subjects, as compared with only 7% among those women who themselves tested negative. Most (93%) of the women who tested positive stated that they would have pursued prenatal (fetal) testing had the partner's result been positive, although only half said that they would have considered abortion of an affected fetus; none of the women who tested positive and had partners who tested negative requested prenatal testing (and it was not offered). Although we do keep the testing confidential, most subjects stated little concern about others (family members, friends, or physicians) knowing their test results; indeed, all of our interviewees who tested positive had confided in their relatives and physicians (table 6). However, 30% of those who tested negative and 5% of those who tested positive said that they would refuse testing if results were to be given to their health-insurance carrier.

Discussion

Precedents and expectations for significant differences between ethnic and educational groups, in their understanding, utilization, and response to genetic screening, have been detailed above in the Introduction. The clinical nature and demographics of CF and its yet imperfect direct DNA test for mutation detection are likely to make these factors even more crucial in the contemplation of large-scale population screening for this disease trait. Prior to the initiation of the NCHGR-sponsored pilot screening studies, we had insufficient experience on which to predict the feasibility and impact of applying a complex and <100%-sensitive carrier-testing protocol to such a huge population as would be envisioned for CF. Although we may derive some hints from ongoing experience with maternal serum alpha-fetoprotein screening, Huntington disease testing, and HIV testing, a CF screening program would be unique in terms of the sheer numbers of people being analyzed directly at the DNA level. More harm than good will ensue if the implications of testing results are not assimilated in a comprehensible way or if they raise the specter of stigmatization or discrimination by mates, peers, employers, or insurers. All of these considerations take on yet another slant in light of the tremendous changes taking place in the U.S. health-care system, in which preventive medicine, of which genetic screening can be considered a part, is likely to assume greater importance, and in which insurance and reimbursement mechanisms are likely to change.

Considering that the lifetime cost for medical care of a

Table 6

Follow-up of 30 Identified CF Carriers

Attitude	% Agreeing
Worried while awaiting partner's results	70
Partner worried while awaiting his results	45
Reassured by partner's negative result	97
No longer worried about baby's CF risk	71
Would pursue prenatal diagnosis if partner tested positive	93
Would consider termination if fetus were affected with CF	52
Shared results with partner, doctor, relatives	100
Would have had test even if it would be reported to insurer	95
Believed that there was no risk of CF after partner tested negative	40

CF patient may be >$200,000 (Wilfond and Fost 1990), some mathematical models have produced a highly favorable cost-benefit analysis for the institution of widespread DNA screening for CF (Chapple et al. 1987); others have argued just the opposite, primarily citing the overwhelming burden on counseling resources (Wilfond and Fost 1990, 1992). Some authors have cited the advent of novel, specific therapies for CF, now that the molecular defect is known, as a reason to question whether elective abortion of an affected fetus can be justified currently (Chapple et al. 1987; Kerem and Lynch 1991). Indeed, engineered animal models of the disease now exist (Snouwaert et al. 1992), and gene-replacement strategies (Rosenfeld et al. 1992; Crystal et al. 1994) are well underway, although early results have been discouraging (Knowles et al. 1995).

Preliminary pilot studies clearly have been required and have been funded by NIH in order to gain some sense of the technical and psychosocial feasibility of such screening on a national level in the United States. This paper reports on one of these studies, aimed at assessing the practicality, understanding, psychosocial impact, and acceptance of CF carrier screening conducted on a large and ethnically diverse pregnant population. Given both the increasing ethnic heterogeneity of the U.S. population at large and the continuing admixture of its constituent groups (which tends to blur ethnic allele frequencies), we felt, even though we are well aware of the lower CF mutation-detection rate in some of these groups, that this strategy would be helpful toward developing a practical paradigm for expansion of such programs to the national level.

As a key part of this study, we have pilot-tested novel methods for sample collection, patient education, and mutation analysis, which have proved expedient for the screening of large populations. The nonisotopic reverse-dot-blot system, used in collaboration with Roche Molecular Systems, proved to be sufficiently rapid, reliable, and easily interpretable for such use. Although in theory it is no more efficient than the pooled direct-dot-blot systems favored by a number of large reference laboratories (Shuber et al. 1993), we have found this approach both convenient and prudent in its provision of individualized patient results and internal controls against amplification failures. Furthermore, the system does not appear particularly prone to PCR contamination artifacts, since we have observed none thus far in using standard laboratory techniques (Kwok 1990). The choice of six mutations was made by the NIH consortium members, with the recognition that, as testing technology and knowledge of the CF gene continue to evolve, testing for additional mutations likely will be indicated in a future large-scale program. In this regard, we have recently pilot-tested a 16-mutation reverse-dot-blot system, also developed by Roche, with excellent results. Similarly, our buccal brush specimen-collection technique provided adequate substrate for PCR amplification, was less threatening for the subjects (see below), and facilitated collection, storage, and transport from the many non-UCLA clinic sites involved in our study. On the basis of the small aliquot of extracted sample volume required for analysis of the six mutations, we estimate that as many as 200 additional PCR tests could be performed on the same buccal brush specimen, easily incorporating any number of other CF mutations to be included in future screening programs.

One striking aspect of our findings has been the relatively high level of interest in CF screening. The screening rates that we observed are higher than had been expected both a priori and on the basis of the consent rates observed by some of the other studies in the NIH consortium. The latter have ranged from as low as <1% to as high as 57% (NIH Cystic Fibrosis Studies Consortium, 3d meeting, September 8–9, 1993; Tambor et al. 1994; Clayton et al. 1996; Loader et al. 1996). Indeed, an unexpected hindrance to our testing of various health behavior theories as possible predictors of consent to screening has been the extremely high rate, across all ethnic groups, of consent to DNA testing once the individuals have undergone CF instruction (Fang et al., in press; table 5). As described above, 98% of our subjects have consented to the DNA test after having undergone our standard protocol of CF instructional intervention (table 2). Even if we consider only our consent rates with regard to entry into the study for education and assessment, our proportion (65% overall, 77% at our major center) still generally exceeds those observed by others in this series of studies. Furthermore, 38% of our decliners indicated that it was the questionnaires, not the DNA test, that dissuaded them—a statement borne out by the even higher consent rates (92%) observed when we reduced the questionnaire component. And these results have emerged despite the fact that our target population is the most ethnically and socioeconomically diverse of all the studies—an aspect that we would expect to *reduce* consent rates, given (*a*) the correlation that past programs have seen between socioeconomic level and interest in genetic screening (Childs et al. 1976*a*; Whitten et al. 1981; Yuen et al. 1988) and (*b*) the fact that our non-Caucasian subjects are fully informed that the DNA test will be less sensitive for their spectrum of CF mutations.

There are a number of attributes of our approach that differ from those of one or more of the other pilot studies and that could be invoked to try to explain these discrepancies. First, the specimen-collection technique that we used was noninvasive and painless; subject responses uniformly indicated great appreciation for this aspect, as well as some aversion to tests requiring phlebotomy (in fact, the first question that subjects typically asked

when approached was whether the procedure involved a blood test). Second, the DNA test has been offered free of charge; our subjects' responses to questioning revealed an aversion to testing when costs were >$25 (data not shown). Third, our subjects were approached in person by one of our genetic counselors, project coordinators, or trained assistants in the respective clinics, rather than by mail, by posted notices, or through contact with primary-care providers. Even though this first contact was noncoercive and verbalized nothing about CF other than the invitation to participate in a research project to evaluate a new method of screening, we believe that it served to humanize the encounter and to pique more interest than would be engendered by a mass mailing to which the subject or provider must take the initiative to respond. This is consistent with the findings of those who have tried the latter approaches (Watson et al. 1991; Tambor et al. 1994; Clayton et al. 1996; Loader et al. 1996). Finally, there was our strategy of predominantly targeting pregnant women in prenatal clinics. In agreement with what others have noted in past programs, we feel that this population is among those most predisposed to be receptive and motivated toward genetic screening. Our experience matches that of the most successful genetic screening program to date, Tay-Sachs disease screening, in which even well-informed at-risk couples typically wait until pregnancy to be tested (Blitzer and McDowell 1992). It also reflects the high response rates to prenatal CF screening programs in Europe (Mennie et al. 1992, 1993*b;* Harris et al. 1993; Jung et al. 1994), as well as in a recent large American study (Witt et al. 1996), although, to our knowledge, the concept has not been tested previously in a population as ethnically diverse as the one that we have studied. Indeed, our consent rate corroborates that (78%) observed by Witt et al., who used a similar direct, in-person prenatal approach. We also extend those findings to other ethnic groups, to an academic health-care setting, and to use of a noninvasive sampling method and a comprehensive information-effectiveness and psychosocial assessment program developed in concert with the goals of the NIH CF consortium and administered to all of our subjects.

Our initial choice of prenatal clinics as our primary recruitment setting was largely one of logistics and practicality, as a means to accrue large numbers of subjects of reproductive age whose mates then would be readily accessible. We are well aware of the objections that can be raised to this approach, including issues of timeliness of intervention, range of reproductive options that can be offered, and subliminal sexual prejudice inherent in placement of the initial burden wholly on women. However, the choice does seem to be a successful one in terms of numbers of appropriate individuals approached and level of participation.

Another possible objection to the prenatal screening approach is the theoretical risk of inducing unnecessary anxiety in the women who test positive. Given the population carrier frequencies for CF mutations (~1/30 in North American Caucasians, less in other ethnic groups), the vast majority of the male partners of those women will test negative. Even with the inability to detect all possible mutations, the resulting risk of an affected fetus in such positive/negative couples is well below the general a priori population risk and, in most cases, below the risk of fetal harm from amniocentesis or chorionic villus sampling. Consequently, the testing will have raised anxiety in a couple for whom no further prenatal intervention will be offered or recommended. To circumvent this problem, Wald (1991) has proposed a couple-based screening model for CF, in which DNA from both parents is tested simultaneously and results are reported as positive only if *both* individuals are found to carry one of the tested mutations. Some subsequent studies have favored this strategy (Doherty et al. 1994; Livingstone et al. 1994), although it remains controversial (Miedzybrodzka et al. 1991; Asch et al. 1993), and others have reported that it may even result in *heightened* anxiety among those testing negative (Miedzybrodzka et al. 1995).

We declined to employ the Wald model in our study, for several reasons: (1) we feel that it is fundamentally unethical not to divulge the results of any clinical laboratory test to the patient who consented to it; (2) we are concerned about the long-term social stability—and, hence, the clinical accessibility—of this two-person biological unit called a "couple"; and (3) the couple model precludes any opportunity for relatives of identified carriers to be tested, thus diminishing the potential impact and cost effectiveness of population-based screening. Moreover, as has been noted above, we have not detected any undue or irreversible anxiety among those of our subjects testing positive, a finding consistent with other large studies that have used the sequential testing model (Watson et al. 1992; Mennie et al. 1993*a;* Witt et al. 1993, 1996).

Our study was not designed to directly address the cost effectiveness of population-based CF screening, in terms of costs per affected fetus detected (and presumably terminated) versus those for lifetime care of a single CF patient. Extending the screening to relatives of identified carriers would be expected to enhance the cost-benefit ratio of such screening, although further exploration of this assumption likewise fell outside the scope of our study. The technical costs inevitably will decrease with advances in technology (e.g., DNA chips), so that the availability of genetic-counseling resources is likely to be the most important limiting factor. Our study demonstrated that less costly ancillary counseling and instructional approaches were generally effective in con-

veying the necessary information, without provoking undue anxiety, depression, or stigmatization.

In summary, our approach to CF screening as presented here has resulted in relatively high uptake in an ethnically diverse western U.S. population, with generally satisfactory understanding of the subtleties of the genetics and test results and with no adverse psychosocial consequences detected thus far. Although we remain concerned about the small minority of subjects who continue to misunderstand the residual risk inherent in a negative test result, this proportion may represent a practical minimum, given the diversity of educational levels in our country; it is nevertheless lower than that reported in some large European studies (Watson et al. 1992; Bekker et al. 1994). In general our experience with the use of modern DNA technologies and ancillary counseling modalities indicates that multiplex population screening for such molecularly heterogeneous genetic traits need not overwhelm either laboratory or genetic-counseling resources. Indeed, these results suggest that a larger CF carrier-screening program could be initiated by use of this model, and, especially, they would seem to justify its being routinely offered to such high-risk ethnic groups as non-Jewish Caucasians of northern and eastern European descent and Ashkenazi Jews. We further believe that the CF model may provide an especially powerful paradigm for genetic screening in general, by virtue of its relatively high carrier frequency among broad segments of the population, the preponderance but not ubiquity of one mutation, and the speed and sensitivity of the relevant DNA analysis methods. Such findings should help us to formulate a national policy for application of the powerful yet still imperfect molecular-genetic techniques available for detection of CF mutations, and they may serve as a guide for delivery of DNA-based population screening for other common genetic disorders and cancer predispositions in the future.

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Appendix A

Study Design and Measurement Framework

Time 1—Packet A: Preinstruction
- Background questionnaire: demographics
- Mood assessment: state anxiety and affect balance
- CF-knowledge questionnaire: true/false items
- Health-beliefs questionnaire: agree/disagree items

Intervention: CF information (video/brochure)

Time 2—Packet B: postinstruction
- Mood assessment: state anxiety and affect balance
- CF-knowledge questionnaire: true/false items
- Health-beliefs questionnaire: agree/disagree items
- Intention questions

Intervention: DNA-testing procedure

Time 3—Packet C: postscreening
- Negative results—by mail
  - Mood assessment: state anxiety and affect balance
  - CF-knowledge questionnaire: true/false items
  - Protocol assessment and screening attitudes
- Positive results—on site
  - Personal counseling
  - Mood assessment: state anxiety and affect balance
  - CF-knowledge questionnaire: true/false items
  - Protocol assessment and screening attitudes

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