Cystic fibrosis population carrier screening: Here at last—Are we ready?

Cystic fibrosis (CF) is typically described as one of the most common lethal autosomal recessive disorders in North America.[1] About 1 in 25–30 Caucasians are carriers of CF mutations, whereas the carrier frequencies in other ethnic and racial groups are lower. As in most recessive disorders, the initial sign of the carrier state in a family is often the unfortunate and unexpected birth of an affected child. In contrast to many other recessive disorders, the carriers of CF mutations have no biochemical or physiologic alterations by which they could readily be identified, as is done, for example, by observing decreased β-hexosaminidase A activity in carriers of Tay-Sachs disease or the abnormal β-globin in carriers of sickle cell disease. Thus, large-scale population screening to detect carriers and alert individuals and couples with no family history of their reproductive risk had to await the identification of the causative gene, *CFTR*, and the characterization of its mutations. This occurred, in a *tour de force* of gene mapping "the old-fashioned way," in 1989.[2–4] At the time, the achievement was heralded as the greatest triumph of pure positional cloning up to that point, and many observers assumed that translation of the discovery to practical screening programs was just around the corner. Yet today, more than 11 years later, general population screening for CF is still not in place, despite endless deliberation, several carefully conducted pilot studies, two NIH consensus conferences, and many new advances in the science and technology of gene-based testing. Why? Has there been an aberrant breakdown in the usual progression from scientific discovery to application? Or does the CF gene story tell us something more fundamental about the complexity of what used to be considered "simple" single-gene Mendelian disorders?

Certainly, the single biggest obstacle to implementing CF carrier screening has been the extreme mutational heterogeneity of the *CFTR* gene, boasting perhaps the greatest number of catalogued individual inherited nucleotide alterations of any gene yet described. With over 900 reported mutations (and still counting) (*http://www.genet.sickkids.on.ca/cftr*), the technical demands of screening become much more arduous than was initially anticipated when the 1989 studies reported one common mutation, ΔF508, in 70% of Caucasian carriers (but significantly less in other ethnic groups, as is now well documented[5,6]). But there are other thorny issues as well. One is the clinical variability of the disorder. Even though most CF patients have severe chronic problems and shortened life expectancies, and some even die early in infancy of meconium ileus, there are others with relatively mild or even subclinical symptoms. Moreover, the prognosis of CF is continually changing with the advent of new therapies (including the prospect of gene therapy). Thus, it is a stretch to try to fit CF into the successful carrier screening model of Tay-Sachs disease.[7,8] For some observers, it is hard to justify an expensive screening program, leading in most scenarios to termination of affected fetuses, for any disease not as uniformly devastating and hopeless as Tay-Sachs. Of course, if there were reliable predictors of severity and prognosis based on genotype, the options would be more straightforward, but unfortunately, the genotype-phenotype correlation in CF is quite soft, except for the consistent association of certain mutations with pancreatic insufficiency.[9] Indeed, some mutation combinations, including those that would be detected in the proposed screening program, do not cause CF at all, but rather male infertility due to congenital bilateral absence of the vas deferens (CBAVD).[10] Additional problems include the ethnic diversity and admixture of the U.S. population (complicating the selection of targeted mutation screening panels), the technical difficulty and varied nature of *CFTR* mutation tests as currently performed in laboratories around the country,[11] the perceived lack of adequate genetic counseling infrastructure in the country to handle the anticipated caseload, and a question of limited interest in screening among some target populations[12] and third-party payers.

Nevertheless, a general consensus has arisen over the years that the potential public health benefits of CF screening, even if not universally appreciated, were too significant to ignore. Screening in some form was inevitable, and some individual prenatal clinics and laboratories had already begun offering the option to selected couples and groups. Indeed, a recent conference of CF clinicians and researchers reached the conclusion that CF screening should proceed.[13] The only remaining question was how best to implement and deliver it. This question, in some but not all of its facets, was first addressed systematically in a series of pilot CF screening studies funded by NIH. Five studies were designated for random population screening of the type discussed here, two others focused on screening relatives of CF patients, and other notable studies were conducted outside the immediate NIH-sponsored consortium.

The population-based studies indicated markedly greater interest and uptake when screening was offered to individuals and couples who were already pregnant,[8,14–18] a finding not particularly surprising in light of earlier experience with Tay-Sachs disease and other screening programs.[7] This finding, along with the absence of reported adverse effects in any of the studies, clearly influenced the outcome of a consensus conference convened by NIH in 1997 to consider whether and how to proceed with nationwide screening. The recommendation of the consensus panel was that CF screening be offered to all

pregnant couples and those contemplating pregnancy.[19] It was recognized that such a large program would need to be phased in over time, and that important mechanistic details had to be addressed, including physician education, ethnic target populations, test reporting and counseling protocols, and the size and composition of the minimal core mutation panel. These and other related issues were considered at a follow-up NIH consensus conference in 1998,[20] and then a Steering Committee composed of representatives from the American College of Medical Genetics (ACMG), the American College of Obstetricians and Gynecologists (ACOG), and the National Human Genome Research Institute was brought together to develop guidelines for implementing a national CF carrier screening program. Subcommittees were appointed to work on three major aspects of the proposed program: (1) patient education and informed consent; (2) laboratory testing, interpretation, and reporting; and (3) provider education.

The report of one of these bodies, the ACMG Subcommittee on Cystic Fibrosis Screening, appears in this issue of *Genetics in Medicine*.[21] It represents the end result of many hours of deliberation on difficult and highly complex CF mutation-testing questions. The effort pushed the limits of our present knowledge of the molecular pathology of the *CFTR* gene, and as such may be expected to change as this knowledge continually expands. But it was recognized that to wait until most everything was known about this gene and its mutations would mean deferring a workable screening program essentially forever. It is hoped that the approaches outlined in the ACMG Subcommittee report will at last break the intellectual logjam and allow laboratories and primary care physicians to begin to implement CF screening and counseling.

Of greatest interest to the laboratories will certainly be the recommendations for selection of the minimal or core *CFTR* mutation test panel.[21] This is a crucial decision that will ultimately determine the test sensitivity in different ethnic groups and the requirements for reporting and counseling on the residual risks inherent in a negative screening result. It is a subject that, in the absence of any preexisting practice guidelines, has led to a proliferation of test panels that vary widely in the number and selection of mutations.[11] For this reason, it also carries major regulatory and economic implications, for once it becomes the recognized "standard of care," laboratories that wish to continue testing will adapt to it regardless of the cost, the technical challenge, or the availability (or lack thereof) of commercial test kits. As described in the ACMG Subcommittee report, a threshold of 0.1% frequency of mutations among American CF patients (out of >15,000 alleles examined[22]) was chosen as the criterion for inclusion of a mutation in the panel. This yielded a total of 25 mutations. Common Ashkenazi Jewish and African American mutations that reached the threshold frequency in the U.S. CF patients studied were included, while other prevalent ethnic-specific mutations that fell below that frequency were not. Mutations in other ethnic groups (e.g., Hispanic Americans), if found to exceed the same threshold, will be incorporated in the future as they are discovered. Use of a single pan-ethnic panel was felt to be technically more efficient than customizing multiple ethnic-specific panels, and would temper to some extent potential false-negatives due to inaccurate ascertainment of ethnicity during the test intake process. By the same rationale, universal screening was preferred over narrow targeting of specific ethnic groups, though Asian Americans, for example, should be informed of the low carrier detectability in their population with the current mutation panel. However, clinics and laboratories dealing with a population of defined ancestry may wish to modify or expand the test panel as appropriate for their specific patient mix.

The question of whether to test for mutations and polymorphisms associated with CBAVD was considered at length. Certainly one does not wish to screen for male infertility when the goal is to prevent CF; yet the problem arises because some of these mutations can also cause CF, depending on which alleles and polymorphisms they are coupled with (and whether in *cis* or *trans*).[23–25] As a compromise, we have recommended certain of these assays as reflex or second-tier tests. For example, the intronic 5T polymorphism, which by itself can cause CBAVD, is to be tested only if the initial core panel screen is positive for R117H, since that combination, as opposed to R117H alone, can produce classical CF.[24,25] The same is true for our recommendation of reflex testing for certain polymorphisms that can mimic pathologic mutations depending on the assay used. On the other hand, we do not recommend the routine offering of extended screening panels incorporating many more mutations than those listed in the report, even for those couples who test positive/negative on the initial screen and face some uncertainty as to their residual risk. The rationale, based on the extreme rarity of such additional mutations, is presented in the report. Appended to the Subcommittee report are model laboratory reports. These reports provide enhanced understanding (by both provider and patient) of the meaning and residual risk for several important combinations of mutations and polymorphisms that might arise from use of the test panel and reflex tests, for all the major ethnic/ancestral groups.

Another point of discussion by the ACMG Subcommittee was whether CF screening in the prenatal setting should focus on the mother and father individually or on the couple as a unit. Of the several approaches available, the Subcommittee favored two, depending on the clinical situation and target population. The first involves concurrent couple testing with reporting of both results; this would be of particular value in Ashkenazi Jewish individuals who may be tested simultaneously for several other diseases, increasing the likelihood of one member being a carrier.[8] The second approach, the two-step or sequential model, tests one partner first (typically the woman in a prenatal setting) and proceeds to the other partner, if available, only when the first test is positive. This model avoids the cost of specimen collection from the second partner in most cases, and always provides individual genetic testing results, which may be useful in future matings with a different partner and for more distant relatives who may be alerted that they are at risk. This approach may also be more practical in certain ethnic and/or socioeconomic settings. In the end, the Subcommittee elected not to mandate one or the other of these

approaches, leaving this decision up to the judgment of the practitioner.

With the publication of these recommendations (and those of the other subcommittees working under the joint Steering Committee), we feel many of the uncertainties that have impeded widespread adoption of CF carrier screening have been initially addressed. It is now expected that broad-based CF carrier screening programs will be in place and active by mid-2001, and ACOG is educating its members, with the help of material produced by the subcommittees, to prepare them as the primary providers of this service in the prenatal setting. This is not to say that all concerns have been dispelled. Even the most successful pilot programs were just that—pilots—federally funded or otherwise subsidized and conducted in the setting of research projects. There are still significant uncertainties about how successfully these programs will function in the real world. Nevertheless, the recommendations presented in this issue of the journal represent the Subcommittee's best initial effort at ensuring the appropriate use and "greatest benefit/least harm" for CF carrier screening programs.

CF is only the first of a number of single-gene disorders with high carrier frequency that will soon be upon us as potential candidates for large-scale population screening. Already hereditary hemochromatosis is being touted as potentially the first "adult PKU screen",[26] given the high carrier frequency of *HFE* mutations (10% in the Caucasian population) and the ready availability of an easy preventive intervention (phlebotomy) in those identified as affected prior to the development of irreversible organ damage. But if there is one lesson we have learned from CF, it is that single-gene defects are never as simple as they might initially seem, and no two are exactly alike in their complexities.[27,28] The *HFE* gene, like *CFTR*, has a predominant mutation (C282Y), but it is apparently only about 70% penetrant at most,[29,30] while the second mutation (H63D) is so common and of such low apparent penetrance (1–2%) that there is still debate over whether it may actually be a linked polymorphism.[31,32] Meanwhile, some heterozygotes for C282Y may exhibit iron overload.[29] Combine these observations with the other heterogeneous causes of hereditary and acquired hemochromatosis, along with the theoretical potential for insurance and employment discrimination in vast numbers of young adults testing positive, and you have a screening paradigm just about as complex as the one for CF.

Are geneticists ready for mass CF carrier screening? Efforts are under way to educate primary caregivers and public health officials about the complexities of population-based carrier screening for CF. Given what we now know about the *CFTR* gene and its phenotypic effects, it is certain that many complicated laboratory and counseling situations will arise, which will challenge the expertise even of competent clinical geneticists and genetic counselors. The stark fact is that, for all the attention CF has received in genetics circles since the cloning of the gene, most rank-and-file medical geneticists and genetic counselors are not particularly facile with the intricacies of CF mutations and unusual genotypes as outlined in the Subcommittee report. CF patients and their families are typically seen in pulmonary and gastroenterology clinics rather than genetics clinics, so aside from the setting of prenatal testing in the presence of a positive family history, which entails quite different issues than general population screening, most genetics professionals have not had to deal with this disorder on a day-to-day basis. The report in this issue should, therefore, serve as a "wake-up call" to educate our own ranks during the brief calm before the CF screening caseload hits like a tidal wave. Molecular genetics laboratorians will face a challenge of their own, as many of them will need to quickly gear up their CF test panels to include all of the mutations listed in the Subcommittee report. This process will face additional impediments as we await the appearance of commercial test kits, since the manufacturers too will have to retool their CF testing platforms, now under development, to conform to the recommended core mutation panel. Lastly, both laboratory and clinical geneticists will have to be cognizant of how to deal with unusual testing and counseling situations, novel genotypes, the uncertain clinical predictive value of many mutations, and complex issues of ethnicity and ancestry. Moreover, they will have to stay abreast of new information related to mutation frequencies, genotype/phenotype correlations, and other unexpected but relevant findings. We as the genetics professionals must be prepared to respond adeptly to the various challenges presented by the undertaking of this first nationwide DNA-based genetic screening program. Clearly, our ability to implement CF carrier screening successfully will in part determine the acceptance of future genetic screening programs by patients, physicians, and the society at large. The challenge is ours to meet, and it will fall to our professional genetics organizations to foster the continuing educational updates needed to maintain the knowledge-base required to deliver effective screening and counseling.

The ACMG Subcommittee members, who are the authors of the report in this issue, have been well aware during our lengthy deliberations of a certain impatience and even frustration by some within the genetics and obstetrical communities who have been anxious to move ahead with CF screening. It is hoped that the report will give the reader a feel for the many complex issues that had to be considered in developing these recommendations—issues for which there are no obvious answers, yet which have the potential to adversely affect millions of people if not worked through as thoughtfully and thoroughly as possible. Perhaps our frustration over the lengthy deliberative process should now give way to some amount of pride that the genetics community and other key professional groups came together to consider so carefully and in such great detail these many issues before launching headlong into a screening program of such unprecedented scope and complexity. While success is not guaranteed, we can now feel confident that the program has been fashioned with the highest possible level of scientific and professional scrutiny, and with input from a wide range of experts in the field. That is the process the ACMG and our colleagues in the other organizations chose to take, and it has now brought us, at long last, to this momentous point—the eve of nationwide CF carrier screening, the first

truly universal molecular genetic testing program of the genomic era.

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