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Cystic Fibrosis Mutation Analysis: How Many Is Enough?

To the Editor:

Cystic fibrosis (CF) is an extremely heterogeneous disease and one of the most common autosomal recessive diseases known to occur in the European Caucasian population.[1] It also occurs in individuals of other racial and ethnic backgrounds, though with lower incidence.[2] The gene responsible for CF, *CFTR*, was identified in 1989 along with the first discovered mutation.[3] Since the identification of the gene, more than 1,300 mutations have been identified in *CFTR*, most of which are very rare.[4] As more clinical laboratories began testing for CF mutations, it became evident that the menu of mutations tested for and the number of mutations tested would vary widely among laboratories.[5] In some cases, laboratories tailored their mutation panels to the local patient population served,[6] while in other instances, the choice of mutation panel was driven by technical capabilities, marketing concerns, or other factors.

The combination of this variability in practice, coupled with growing pressure to consider population carrier screening, led to the unprecedented call for some sort of nationwide policy on molecular genetic testing for CF mutations in the United States. In 1997, a National Institutes of Health consensus con-

ference convened to address this need.[7] Subsequently, the American College of Medical Genetics (ACMG) in conjunction with the American College of Obstetricians and Gynecologists (ACOG) released its recommendation that all pregnant couples and those planning pregnancy be offered screening with a minimum panel of 25 *CFTR* mutations.[8,9] This decree was embraced by some and challenged by others while the diagnostics industry scrambled to release analyte specific reagents (ASRs) modeled on these guidelines for *CFTR* mutation screening.[10]

As a result of the newly developed guidelines, laboratories were obligated to develop both diagnostic and carrier screening mutation panels that either were identical to or overlapped with respect to some mutations but needed to be extended or more comprehensive for certain applications. The inherent problems associated with the identification of one or two mutations for either carrier or diagnostic testing in heterogeneous subpopulations led to a number of publications calling for expanded mutation panels based on the subpopulation being tested.[11,12] Despite the many efforts to identify a standardized mutation panel for carrier screening, a number of reference laboratories consider the 25-mutation panel "minimalistic" and continue to call for expanded screening panels.

There is no doubt that the patient population served by a larger reference laboratory could and/or should be much more heterogeneous than a hospital-based laboratory. Because of this and the relatively homogeneous patient population at the institution of two of the authors (Dartmouth-Hitchcock Medical Center), we examined the feasibility of implementing a CF testing program using the 25-mutation panel for both diagnostic and carrier screening.

We retrospectively evaluated CF test results from 2002 through the first quarter of 2004. During this time, 532 patient specimens were sent out to a reference laboratory for CF mutation testing using an extended panel. Of these patients, 510 were ordered for CF carrier screening and 22 for diagnostic testing. CF screening was performed on 84 males and 426 females, while diagnostic testing was performed on 13 males and 9 females. In total, mutations were identified in 29 patients and included: (F508, (I507, G542X, G551D, R117H, A455E, N1303K, I148T.

Of interest to us was that all of the identified mutations were among those in the recommended 25-mutation panel. A review of the ethnicity of these patients indicated that the majority were Caucasians of European descent, 14 identified themselves as Asian, and one as African American. All of the mutations were identified in Caucasians. This rather homogeneous population prompted us to examine the financial ramifications of CF testing for an expanded mutation panel that did not serve our patient population. Each send-out test was examined for an extended mutation panel performed by a reference laboratory. The billable cost associated with this testing was in excess of $125,000 for 94.5% negative test results. The cost of performing the recommended mutation panel in-house, which would have detected all of the identified mutations, would have been less than $40,000.

While everyone is concerned about the economics of population based genetic screening, our data suggests that knowing patient population could have a significant impact on healthcare costs and calls into question both the medical and economic value of extended CF mutation panel screening in this setting. It is an inescapable fact of the CFTR gene that in the general Caucasian population, once one gets beyond the predominant (F508 mutation and a handful of secondary mutations of limited allele frequency (such as G542X, R117H, etc.), the remaining mutations are extremely rare. Moreover, with the exception of certain ethnic and racial minority groups (Ashkenazi Jews, African Americans), there are few if any 'ethnic' mutations one can contemplate adding to screening panels that will make much of a difference in practical carrier pick-up rates. The original 25-mutation panel derived by ACMG had as its criterion for inclusion any mutation that was present at a frequency of $>0.1\%$ in a cohort of genotyped affected CF patients maintained in the Cystic Fibrosis Foundation registry.[8] A level of 0.1% is already extremely low, and it is questionable how statistically significant a difference is between 0.1% and, for example, 0.05% in this limited and otherwise unselected and relatively uncharacterized population. In other words, at that low level of allele frequency, the impression of apparent mutation recurrence begins to merge with the general background 'noise' of even rarer mutations. Furthermore, these values were observed in an affected population and may not necessarily translate to reliably predictable carrier frequencies in the healthy screening population.[12] Indeed, once population carrier screening began nationwide and a much larger number of individuals were tested using the panel, the frequency of certain individual mutations turned out to be significantly lower (1078delT) or higher (I148T) than expected.[13] In fact, further study revealed that this latter allele is not a pathologic mutation at all but simply a benign polymorphism.[14,15] Such is the danger of including rare mutations that have not been extensively characterized from a genotype-phenotype perspective. One cannot help but wonder how many other mutations in extended screening panels may not be pathologically important, let alone economically dubious because of their extreme rarity.

Because the additional mutations in extended panels offered by some reference laboratories lie at this borderline horizon of allele frequency or below, their actual incidence in the general population is uncertain, and the choice for inclusion or exclusion of particular mutations is in some sense rather arbitrary. It would be difficult to argue against, or to defend, the substitution of 10 or 20 or 30 of these mutations with others of similar apparent frequency, or their removal altogether. At best a negative screen in an individual or couple already negative by the ACMG-25 panel provides an uncertain or even false sense of security, while a positive result for a very rare and unfamiliar mutation presents difficult genetic counseling issues in trying to predict phenotypic outcome for the family. And in practical, real-world experience as that related here, the additional yield of the extended panel beyond the ACMG panel is often negligible or nonexistent.

In the seemingly unending quest by testing laboratories and ASR vendors to add more and more mutations to screening panels, it is sometimes forgotten that the ACMG/ACOG guidelines and mutation panel were developed as a *screening* test for carriers, not as a comprehensive diagnostic test. It was recognized and acknowledged that some proportion of carriers, which varies by ethnic/racial group, would be missed, but that the minimal panel of 25 mutations represented an acceptable compromise between cost, sensitivity, and phenotypic predictive value. Our experience reported here, illustrating the significant cost yet minimal sensitivity differences between basic and extended panels, raises the question of whether the latter should continue to be pursued and marketed so aggressively. Perhaps our efforts and limited resources would be better spent expanding our screening panels to other diseases with mutations markedly more frequent than those in the extended CFTR panels.

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