G90D and a molecular mechanism for congenital night blindness. Nature 367:639–641

Schaeffer E, Smith D, Mardon G, Quinn, W, Zuker C (1989) Isolation and characterization of two new drosophila protein kinase C genes, including one specifically expressed in photoreceptor cells. Cell 57:403–412

Smith DP, Ranganathan R, Hardy RW, Marx J, Tsuchida T, Zuker CS (1991) Photoreceptor deactivation and retinal degeneration mediated by a photoreceptor-specific protein kinase C. Science 254:1478–1484

Xu S, Nakazawa M, Tamai M, Gal A (1995) Autosomal dominant retinitis pigmentosa locus on chromosome 19q in a Japanese family. J Med Genet 32:915–916

Xu SY, Schwartz M, Rosenburg T, Gal A (1996) A ninth locus (RP18) for autosomal dominant retinitis pigmentosa maps in the pericentromeric region of chromosome 1. Hum Mol Genet 5:1193–1197

Yamamoto S, Sippel KC, Berson EL, Dryja TP (1997) Defects in the rhodopsin kinase gene in the Oguchi form of stationary night blindness. Nat Genet 15:175–178

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0002-9297/98/6205-0030$02.00

Am. J. Hum. Genet. 62:1252–1254, 1998

Diversity of Cystic Fibrosis Mutation-Screening Practices

To the Editor:

As the most common lethal recessive disorder in North America, cystic fibrosis (CF) has been discussed as a potential target of nationwide carrier screening ever since the cloning of the causative *CFTR* gene in 1989 (Kerem et al. 1989). Practical implementation of such proposals has been impeded, however, by the extreme mutational heterogeneity of *CFTR* alleles within the carrier population, with upwards of 600 different mutations identified thus far (Zielenski and Tsui 1995). Aside from the most prevalent mutation, ΔF508, most of these alleles are extremely rare or even "private," although approximately half a dozen account for 1%–3% each of carriers in the general Caucasian population, and other subsets are relatively more common in other ethnic groups. Therefore, analysis of numerous mutations is required to reach satisfactory carrier detection levels. Studies in the general Caucasian population have revealed that at least 15–20 mutations must be tested to detect >80% of obligate carriers (Cystic Fibrosis Genetic Analysis Consortium 1994). An exception is the Ashkenazi Jewish community, in which the analysis of only seven mutations can detect ~97% of carriers, making high-sensitivity carrier screening more readily attainable (Eng et al. 1997). The range of mutations tested has steadily increased over the years, but there are still no authoritative national guidelines specifying a minimum number of CF mutations acceptable in a population screening panel (i.e., screening for unknown mutations in individuals with no family history and thus no index case with a known mutation). In the early months shortly after the cloning of the *CFTR* gene, screening for four to seven of the most common mutations was the norm, but as the total number of cataloged *CFTR* mutations has expanded, and additional ethnic-specific mutations have been identified, the size of available test panels also has increased.

The only way (theoretically) to detect all possible mutations would be to sequence the entire gene, but the cost of that approach would be prohibitive for population screening. Therefore, laboratories developing *CFTR* mutation tests have had to be creative in their choice of technique and their selection of appropriate mutation panels. Techniques used have included PCR amplification with electrophoresis and/or restriction endonuclease digestion of the products, dot blot hybridization with allele-specific oligonucleotide (ASO) probes, reverse dot blots, pooled ASO and probe-elution strategies, conformational analysis, and (still under development) various types of oligonucleotide microarrays (DeMarchi et al. 1994; Ravnik-Glavac et al. 1994; Wall et al. 1995; Shuber et al. 1997;). In the absence of guidelines, the choice of number and type of mutations in CF test panels has been left to the discretion of the individual laboratories.

Like the mutations themselves, the number of laboratories offering such testing has been increasing over the years, yet there has been no systematic survey of the range of CF mutations being tested across the country. For several years, the American College of Medical Genetics/College of American Pathologists (ACMG/CAP) Biochemical and Molecular Genetics Resource Committee has been administering a molecular genetics proficiency testing program for CF and other disorders, providing a means to collect such data. The 45 laboratories currently participating in the CF challenges represent most of those offering such testing in the United States.

As part of our March 1997 proficiency challenge, we included a survey questionnaire to ascertain the range of mutations tested by the laboratories offering cystic fibrosis mutation screening. Forty-three (96%) of the 45 participating laboratories subscribing to the CF challenge responded to this survey. Of those, there was a wide range in the number of mutations tested, from just 1 to 70. One laboratory offers testing for ΔF508 only, and one or two laboratories each offer testing for 5, 7,

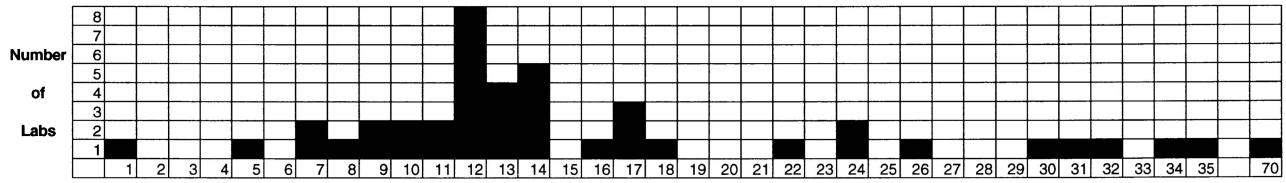

Figure 1 Number of CFTR mutations screened by participating laboratories.

8, 9, 11, 16, 17, 18, 22, 24, 26, 30, 31, 32, 34, or 70 mutations, with the median number centering around 12–14 mutations (fig. 1).

Aside from the absolute number of mutations, other notable findings in some of the laboratories' screening panels emerged from this survey. For example, 13 of the laboratories do not test for R117H, which many would feel is one of the relatively more common and important mutations, associated with both classical CF and congenital bilateral absence of the vas deferens (Jezequel et al. 1995). And only two of the other laboratories specifically indicated that they include testing for the intronic 5/7/9T polymorphism that markedly affects phenotypic expression of R117H and some other *CFTR* mutations (Kiesewetter et al. 1993; Chillon et al. 1995). Three laboratories do not include the prevalent W1282X Ashkenazi Jewish mutation, which would seem essential for any test panel directed at a North American urban population. Some of the laboratories included written comments that their panels cannot distinguish between mutations ΔF508 and ΔI507 (both 3-nucleotide deletions of adjacent codons) or G551D and R553X (two of the more common point mutations), which our ACMG/CAP committee already suspected, based on the results of our earlier CF challenges.

It is important to note that our survey addressed neither which laboratories are using their panels for testing of known mutations in at-risk relatives, as opposed to random proband or population screening, nor which particular ethnic groups, if any, are being targeted. These more limited and predefined applications would allow for more narrow test panels. In the same vein, it is possible that some of the laboratories in our survey perform CF mutation analysis primarily for research purposes. (The confidential structure of the CAP survey program precludes identification or contact of the individual laboratories by the resource committee.) Finally, although we believe this survey to be fairly comprehensive, there are undoubtedly some additional academic and/or commercial laboratories involved in CF testing that did not participate or respond.

In April 1997, the NIH convened a Consensus Conference on Cystic Fibrosis Testing. The consensus panel recommended that population-based CF screening be offered to all pregnant couples and those contemplating pregnancy in a program to be phased in over time (NIH Consensus Statement 1997). Without specifying a precise number of mutations, they stated that any test panel used should be capable of detecting $\geqslant 90\%$ of Caucasian carriers while achieving the best available sensitivity in other ethnic groups. For certain homogeneous ethnic groups, as few as five mutations would be sufficient to meet this criterion. But for general population screening in a country as heterogeneous as the United States, it is clear that many more mutations will need to be included.

In the absence of commercial test kits, setting up in-house multiplex testing for large numbers of *CFTR* mutations has proven to be a challenging and expensive exercise for most routine diagnostic molecular genetics laboratories, especially for those whose test volume does not lend itself to large-scale automation of the PCR and hybridization steps. A number of these laboratories have already abandoned CF mutation screening and refer their cases out to large reference laboratories that test as many as 70 or more mutations. The NIH panel's recommendation should exert even more pressure in this direction, although it might also inspire manufacturers to develop marketable *CFTR* mutation test kits. While most of the mutations beyond the first 10 or 20 are extremely rare, it could be argued that screening for fewer than 10–20 does not represent the current standard of care, unless the laboratory is restricting its testing to particular well-characterized ethnic groups such as Ashkenazi Jews. In any case, our experience suggests that more explicit guidelines specifying particular mutations and minimal test panel sensitivities for each ethnic population being screened would be prudent and helpful. Such guidelines could be recommended by professional practice organizations like the ACMG and CAP or through the NIH consensus conference process. In the meantime, this survey by the ACMG/CAP Biochemical and Molecular Genetics Resource Committee provides a valuable snapshot of the state and extent of CF mutation testing in the country at present and one that the broader medical genetics community should consider as

it ponders how best to extend these services to larger populations.

Acknowledgments

The authors thank the other members of the ACMG/CAP Biochemical and Molecular Genetics Resource Committee, Jill Kachin and other members of the CAP support staff, and all the participating laboratories in the CF proficiency testing program for assistance in the accrual and tabulation of these data.

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References

Chillon M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Romey M-C, et al (1995) Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med 332:1475–1480

Cystic Fibrosis Genetic Analysis Consortium (1994) Population variation of common cystic fibrosis mutations. Hum Mutat 4:167–177

DeMarchi JM, Beaudet AL, Caskey CT, Richards CS (1994) Experience of an academic reference laboratory using automation for analysis of cystic fibrosis mutations. Arch Pathol Lab Med 118:26–32

Eng CM, Schechter C, Robinowitz J, Fulop G, Burgert T, Levy B, Zinberg R, et al (1997) Prenatal genetic carrier testing using triple disease screening. JAMA 278:1268–1272

Jezequel P, Dorval I, Fergelot P, Chauvel B, Le Treut A, Le Gall JY, Le Lannou D, et al (1995) Structural analysis of CFTR gene in congenital bilateral absence of vas deferens. Clin Chem 41:833–835

Kerem B-S, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, et al (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245: 1073–1080

Kiesewetter S, Macek M, Davis C, Curristin SM, Chu CS, Graham C, Shrimpton AE, et al (1993) A mutation in CFTR produces different phenotypes depending on chromosomal background. Nat Genet 5:274–278

NIH Consensus Statement Online (1997) Genetic testing for cystic fibrosis. http://odp.od.nih.gov/consensus/statements/cdc/106/106_stmt.html (April)

Ravnik-Glavac M, Glavac D, Dean M (1994) Sensitivity of single-strand conformation polymorphism and heteroduplex method for mutation detection in the cystic fibrosis gene. Hum Mol Genet 3:801–807

Shuber AP, Mchalowsky LA, Nass GS, Skotetsky J, Hire LM, Kotsopoulos SK, Phipps MF, et al (1997) High throughput parallel analysis of hundreds of patient samples for more than 100 mutations in multiple disease genes. Hum Mol Genet 6:337–347

Wall J, Cai S, Chehab FF (1995) A 31-mutation assay for cystic fibrosis testing in the clinical molecular diagnostics laboratory. Hum Mutat 5:333–338

Zielenski J, Tsui L-C (1995) Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet 29:777–807

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0002-9297/98/6205-0031$02.00

Am. J. Hum. Genet. 62:1254–1258, 1998

Linkage Disequilibrium Analysis in a Recently Founded Population: Evaluation of the Variegate Porphyria Founder in South African Afrikaners

To the Editor:

Variegate porphyria (VP; MIM 176200) is relatively rare in most populations, but it is one of the most common autosomal dominant genetic disorders in South Africa (Dean 1971). The disease is characterized by a diversity of symptoms, including a variable picture of skin symptoms and acute attacks. By means of genealogical studies, the history of VP in South Africa can be traced back to the marriage of a Dutch couple in the Cape of Good Hope in 1688 (Dean 1971). This, along with the high prevalence of VP in South Africa, has promoted the founder-gene hypothesis for VP in this country.

Mutations in the protoporphyrinogen oxidase gene (PPOX), the seventh enzyme in the heme biosynthetic pathway, have been shown to be causative of VP (Deybach et al. 1996; Meissner et al. 1996; Warnich et al. 1996*b;* Lam et al. 1997). This gene has been mapped to chromosome 1q22 by FISH (Taketani et al. 1995), and the position has been confirmed by linkage analysis (Roberts et al. 1995). Three mutations have been described in South African VP patients, but one of these, a C→T transition at nucleotide position 452 (R59W), was found in ~90% of patients (Meissner et al. 1996; Warnich et al. 1996*b*). This mutation spanned a CpG dinucleotide, and, to exclude the possibility of a recurrent mutation, intragenic haplotype studies were undertaken. Mutation R59W was shown to be associated with one of four potential haplotypes defined by two diallelic polymorphisms in exon 1 (Warnich et al. 1996*b*), thus supporting the founder hypothesis. However, this was not totally conclusive evidence, since the alleles associated with the R59W mutation are also the common alleles in the normal population for each of the polymorphisms (L. Warnich, unpublished data).

If the high incidence of a genetic disease in a particular population is due to a founder effect, most cases studied