Cystic fibrosis: after the gene

The isolation of the cystic fibrosis (CF) gene^{1–3} has ended one chapter in the history of CF research. This tour de force was accomplished by walking and jumping over 280 kb of the genome, and represents the first time a major disease gene has actually been cloned without either biochemical or cytogenetic assistance. Although a great achievement, it merely marks the beginning of an era of experiments to find out how this disease is caused and how treatment may be improved.

The comprehensive nature of the analyses that have just been reported on the CF gene^{1–3} will probably convince even the most sceptical that the groups from Toronto and Michigan have indeed found the right gene. Three key items of evidence are as follows.

(1) The identification of the same 3 bp deletion in the gene, resulting in the loss of a single phenylalanine residue at amino acid 508 (ΔF_{508}) in nearly 70% of CF chromosomes analysed (the deletion was not found on nearly 200 normal chromosomes).

(2) The mRNA is expressed at highest levels in the pancreas and nasal polyps, at lower levels in sweat gland duct epithelial cells, lung, colon, placenta, liver, and parotid glands, but apparently not at all in brain, skin fibroblasts, or lymphoblastoid cell lines.

(3) The 170 kd protein predicted from the CF gene sequence is likely to be an integral membrane protein. It has two potential membrane spanning domains; sequences resembling ATP binding folds; consensus sequences for phosphorylation by protein kinase A and potential substrate sites for protein kinase C. In other words, the protein has all the attributes that might have been expected from the electrophysiological abnormalities observed in CF epithelia.^{4–7} It is likely to be a transmembrane regulator of ion movements, hence the name CFTR, and shows substantial homology with members of the family of multidrug resistance proteins.

The CF gene is large, 250 kb genomic length, with 24 exons producing a message of 6.5 kb, nearly half the length of the giant dystrophin molecule. About 70% of CF chromosomes carry the same mutation, apparently on the same haplotype. Without this individual mutation, the gene frequency would be 1/120 and the trait frequency an unremarkable 1/150 000. This is still compatible with the unusual frequency of the disorder being the result of either genetic drift or heterozygote advantage; further work will be needed to resolve this important issue in population genetics. Haplotype disequilibrium analysis hints at a limited number of common mutants and there is a suggestion that different mutants may account for the forms of the disease with, and without, pancreatic insufficiency.

The clinical genetic implications of the cloning of the CF gene are obvious. It will immediately give even more reliable prenatal diagnosis of CF and carrier detection within the extended families of CF patients. At present, only the one major mutation that occurs on 70% of chromosomes has been reported. The combined power of the polymerase chain reaction and methods of direct detection of mutations will undoubtedly show the nature of other CF gene mutations in the next couple of years. Population screening for heterozygotes, therefore, becomes more and more practicable. Whether screening should be instituted soon, with a significant false negative rate owing to an inability to detect minor mutations, is the subject of much discussion. In practice, the inertia in instituting large scale screening is likely to ensure that other mutations in the CF gene will be defined well before much screening is done. In the meantime, genetic centres may use the time profitably to come up with some effective strategies as to how they are actually going to cope with the enormity of the task: there are more than two million CF carriers in the UK.

Where next? In terms of understanding how the CF gene functions *in vivo* the fundamental experiments will probably involve cell biological systems that are likely to express the basic defect, such as human epithelial cell cultures.^{8–10} One of these systems, the sweat gland duct epithelium, has already played a key role in cloning the gene.² The immediate priorities are probably to raise monoclonal antibodies against the CFTR protein and to purify it. The substantial homology with members of the family of multidrug resistance proteins may provide useful information on protein structure and function.¹¹ Isolation or creation of full length cDNA clones for the CF gene will enable key complementation experiments to be done. These will show whether the electrophysiological defect, measured as abnormal regulation of chloride ion transport in

the apical membrane of CF sweat gland duct epithelial cells, can be corrected by insertion of a vector expressing the normal counterpart of the CF gene into these cells. Another potentially exciting avenue of research will be to create transgenic mice, carrying a mutated human CF gene (from cross species homologies at a DNA level it already seems likely that the mouse has its own counterpart of the CF gene). The frustration of CF researchers in not having an animal model may well be nearing an end.

As the experiments start, using the whole armamentarium of current molecular genetic techniques to try and understand how the mutation in the CF gene results in the electrophysiologically measurable basic defect, let us not forget that what actually kills CF patients is the mucus that deposits in their lungs and in their pancreatic ducts.

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References.

- ¹ Rommens JM, Iannuzzi MC, Kerem B, *et al.* Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989; **245**:1059–65.
- ² Riordan JR, Rommens JM, Kerem B, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; **245**:1066–73.
- ³ Kerem B, Rommens JA, Buchanan D, *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; **245**:1073–80.
- ⁴ Quinton PM. Chloride impermeability in cystic fibrosis. *Nature* 1983; **301**:421–7.
- ⁵ Sato K, Sato F. Defective beta-adrenergic response of cystic fibrosis sweat glands in vivo and in vitro. *J Clin Invest* 1984; **73**:1763–71.
- ⁶ Widdicombe JH, Welsh MJ, Finkbeiner WE. Cystic fibrosis decreases the apical membrane chloride permeability of monolayers cultured from cells of tracheal epithelium. *Proc Natl Acad Sci USA* 1985; **82**:6167–71.
- ⁷ Knowles MR, Strutts MJ, Yankaskas J, *et al.* Abnormal respiratory epithelial ion transport in cystic fibrosis. *Clin Chest Med* 1986; **7**: 285–97.
- ⁸ Lee CM, Carpenter F, Coaker T, Kealey T. The primary culture of epithelia from the secretory coil and collecting duct of normal human and cystic fibrotic eccrine sweat glands. *J Cell Sci* 1986; **87**:695–703.
- ⁹ Harris A, Coleman L. Establishment of a tissue culture system for epithelial cells derived from human pancreas. *J Cell Sci* 1987; **87**: 695–703.
- ¹⁰ Harris A, Coleman L. Ductal epithelial cells cultured from human epididymis and vas deferens: relevance to sterility in cystic fibrosis. *J Cell Sci* 1989; **92**:687–90.
- ¹¹ Higgins CF, Gallagher MD, Mimmack ML, Pearce PL. A family of closely related ATP-binding subunits from prokaryotic and eukaryotic cells. *Bioessays* 1988; **8**:111–6.