Discovery of human disease genes

The canine narcolepsy gene encodes a receptor for a novel neuropeptide called hypocretin[3]. Emmanuel Mignot's group (Stanford University, Stanford, CA, USA) demonstrated that human narcolepsy involves the same neuropeptide system[1]. Mignot said, 'Human and dog genes have a very high homology, so finding the dog gene was instrumental in finding the cause of a disorder that affects one in every 2000 humans. New treatments for human narcolepsy based on this discovery will soon follow.'

Work by Matthew Binns (Head of the Genetics Section, Animal Health Trust) and colleagues, led by Matthew Breen in collaboration with researchers at the Sanger Centre (Hinxton, Cambridge, UK), could also lead to discoveries of human gene locations using reciprocal chromosome painting of dog and human chromosomes (reciprocal Zoo-FISH analysis; see Fig. 1)[4]. To make the paints, chromosomes from cells arrested during cell division are separated on the basis of their size and their guanosine–cytosine content, using the chromosome sorter at the Sanger Centre. This enables the separation of all the copies of the individual chromosomes, which are then labelled with a fluorescent stain. A complete set of these labelled chromosomes is available for both the dog and the human genome. If dog paints are added to the human karyotype, they will hybridize to any regions evolutionarily conserved between the two species. For example, if paints made from human chromosome 22 are added to the dog genome, they will 'light up' parts of dog chromosomes 10 and 26. Binns said, 'This means that we can say where in the dog genome are the genes that are the counterparts of those on human chromosome 22. So if you had a gene on 22 that you knew was associated with a human disease, and it was also a disease in dogs, this would short-circuit the process and allow you to head straight for the markers on the dog chromosomes.'

By using this method, Breen and his colleagues have identified 68 segments of the dog genome that have been evolutionarily conserved and are therefore common to the human genome. This technique should lead, they predict, to easier identification and cloning of genes that are present in both species.

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Lipid imbalance might contribute to cystic fibrosis

The discovery of a lipid imbalance in a mouse model of cystic fibrosis is opening the way for an entirely new treatment for patients with cystic fibrosis (CF). Researchers from the Harvard Medical School (Boston, MA, USA) have found that correction of this fatty acid abnormality reverses the pathology observed in a CF mouse model[1].

CF is the most prevalent lethal autosomal recessive disorder – among Caucasians, 1 in 29 people are carriers and the condition affects 1 in 2500 newborn babies. Patients with CF express a typical phenotype characterized by pancreatic insufficiency, ileal hypertrophy and recurrent pulmonary infections that ultimately lead to pulmonary failure and death. The median life expectancy of a patient with CF is now 31. Although great advances have been made in increasing this figure through improvements in nutrition and antibiotics, little progress is now being made. In 1989, the gene responsible for CF was characterized as an ATP-gated chloride channel that is regulated by cAMP-dependent protein kinase phosphorylation.

Lipid imbalance in cystic fibrosis

It is becoming apparent that chloride channels regulate many cellular functions, including the fluidity of cell secretions, inflammation and the production of certain fatty acids. All of these functions are aberrant in CF and this might explain the common symptoms.

The main investigators in the project, Steven Freedman (a gastroenterologist and cell biologist) and Juan Alvarez (a lipid expert), became interested in lipid imbalance after previous studies showed that CF patients show fatty acid defects, irrespective of their nutritional status[2]. Using University of North Carolina (UNC) mice that had the CF gene knocked out, the team determined whether there were any fatty acid-abnormalities in cells regulated by the CF gene, as previous studies had been con-

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fined to examination of plasma and not of tissues. 'The mice do not completely mimic what you see in humans. They have pancreatic and intestinal difficulties, but no lung problems. The explanation is that they appear to have back-up chloride channels that are not present in the intestine, but fully present in the lung', says Freedman.

Arachidonic acid and DHA

After the animals were sacrificed, their lungs, pancreas and ileum were removed and cells regulated by the CF gene isolated. Pancreatic duct and acini cells were isolated, together with a total lung cell suspension (after removal of the blood cells) and ileal mucosal cells. Lipids were then extracted from cell suspensions using chloroform and methanol. After methylation, the different fatty acid species present were measured using gas chromatography. Of the 50–100 fatty acids measured, only two were different between the CF and the wild-type mice. They found a twofold increase in phospholipid-bound arachidonic acid and a twofold decrease in phospholipid-bound docosa-hexaenoic acid (DHA) in all the cells taken from mice with CF. Furthermore, administration of 40 mg DHA to CF mice, given over a seven-day period, was enough to completely reverse the pathological changes observed in the pancreas and ileum.

As CF mice do not display pulmonary symptoms, it was necessary to induce pneumonia. Weight-matched wild-type and CF mice were given a dose of aerosolized *Pseudomonas endotoxin* (the most common bacteria found in human CF lungs) over 15 min for three days. The animals were then sacrificed and found to show an increased inflammatory response (indicated by the number of neutrophils present) compared with wild-type mice. However, when CF mice were treated with DHA, lung inflammation was identical to that seen in wild-type mice.

The team speculates that deficiencies in the CF gene could result in a primary defect in DHA biosynthesis, possibly leading to compensation by increasing arachidonic acid levels. The resultant fatty acid imbalance might explain some of the common symptoms of CF as too much arachidonic acid, through activation of prostaglandins, can increase inflammation. Moreover, prostaglandins would activate mucin secretion, increasing mucous production, while a relative absence of DHA would alter membrane function by decreasing the fluidity of the membrane.

Preliminary data, presented by the team at the *North American Cystic Fibrosis* conference (Seattle, WA, USA) in October 1999, shows similar results in humans with CF. In collaboration with Brian O'Sullivan (University of Massachusetts Medical Center, MA, USA), Freedman and Alvarez compared biopsy samples of nasal and rectal mucosa between ten CF patients and ten controls, and showed similar effects on DHA and arachidonic levels compared with CF mice.

Future studies

The team is currently in collaboration with Genzyme Corporation (Cambridge, MA, USA) and the Cystic Fibrosis Foundation (Bethesda, MD, USA) to bring a modified formulation of DHA to clinical trials. 'From our studies we know that the DHA has to be pure, as administration of some fatty acids actually worsened the disease in mice. As there are no pure preparations available, we are now developing our own', says Freedman. 'In addition, we will need to overcome problems with absorption, as most patients with CF have pancreatic insufficiency'. They are hoping to undertake a Phase I/II clinical trial, which will examine both safety and efficacy issues in mid-2000.

Freedman believes that DHA therapy might have implications for use beyond CF. Over 880 mutations in the CF gene have been described. While certain mutations lead to a classical form of CF, milder mutations result in other diseases. It is estimated that 60% of people with chronic or recurrent pancreatitis, a third with chronic sinusitis and 20% with asthma, as well as 30% of men attending fertility clinics all have some mutation in the CF gene. Hence, DHA therapy might have a use for all these gene defects.

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Collaboration…

Oxford Biomedica (Oxford, UK) and **AstraZeneca R&D** (Charnwood, UK) have signed a collaboration that will involve using BioMedica's LentiVector™ gene delivery technology that uses lentiviruses to deliver genes to primary cells involved in diseases such as asthma. Because this technology is being used in several other collaborations related to drug discovery outside the field of gene therapy, BioMedica are establishing a new drug discovery unit, which will focus on gene transfer, hypoxia control and macrophage delivery technologies.