Painting a brighter future for dogs and humans

Dogs might be best friends with the human race for more than the usual reasons. The project to map their genome, which has been running for five years, has already made it possible to identify a human gene for narcolepsy[1], and is likely to continue to hasten the discovery of many genes involved in human diseases. The aim of the canine project is to map the linkage markers throughout the dog's 40 chromosomes to aid in the identification of genes that cause some of the 300 or so inherited diseases that afflict dogs.

Researchers have been creating a low-resolution map, under the direction of Elaine Ostrander (Director of the Genetics Program, Fred Hutchinson Cancer Research Center, Seattle, WA, USA)[2], in collaboration with investigators at Cornell University (Ithaca, NY, USA), the University of Pennsylvania (Philadelphia, PA, USA), the Animal Health Trust (Newmarket, UK) and the University of Rennes (Rennes, France).

Scientists present at the *Molecular Genetics and Canine Genetic Health* conference (31 October–1 November 1997) held in St Louis (MO, USA) agreed that it would be useful to have a family of reference DNA, so that markers could be placed on a common map. To provide this, researchers at Cornell University, led by Gus Aguirre and Greg Ackland, agreed to release their data on a set of dog pedigrees they had previously developed, and which already had several markers in place. Ralston Purina (St Louis, MO, USA) offered to fund the maintenance and distribution of these reference material samples, and this began in spring 1999. An oversight committee was also set up to ensure that researchers could only use this limited DNA resource for identifying markers, and that all information obtained from its use is made public, and within a suitable time period. This information is freely available at http://www.fhcrc.org/science/dog_genome/dog. html, which contains current and previous versions of the canine map, as well as the sequences of primers defining each of the markers.

Markers being used include genes (if their locations are known), but are more often microsatellite markers, which hybridize with areas of hypervariable polymorphisms. Some researchers, however, such as those at the Animal Health Trust, are using a set of bacterial artificial chromosomes made by Pieter De Jong's group at the Roswell Park Cancer Institute (Buffalo, NY, USA). These contain large fragments of dog DNA, isolated from a single male Doberman, which have been cloned into a bacterial vector.

Health and welfare of companion animals

One of the long-term aims of the project is to obtain genetic screening tests that will improve the health and welfare of companion animals by selective breeding. The collaborators hope that they will soon identify the genes involved in diseases such as hip dysplasia, epilepsy, cancer, allergies, hypothyroidism, progressive retinal atrophy, autoimmune and heart diseases, and eye disorders. Genetic tests have already been produced for cystinuria in Newfoundlands, von Willebrand's disease in Manchester terriers, poodles, Pembroke Welsh corgis and Scottish terriers, and congenital stationary night blindness in briards. The American Kennel Club's Website (http://www.akcchf.org) lists all the tests that are available clinically, and gives details of the companies providing these tests, such as Vetgen (Ann Arbor, MI, USA), PennGen (Philadelphia, PA, USA), Optigen (Ithaca, NY, USA) and Genesearch (Rockville, MD, USA).

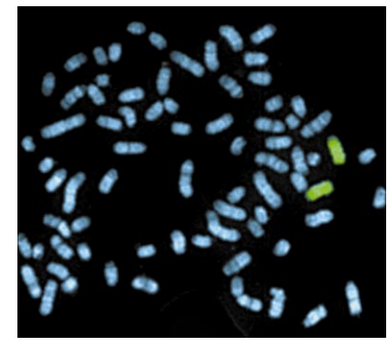

Figure 1. *Chromosomes of the domestic dog counterstained with DAPI (4′,6-diamidino-2-phenylindole; in blue). The dog has 38 pairs of non-sex chromosomes (autosomes) and the two sex chromosomes. All the autosomes are very similar in appearance and are, thus, quite difficult to identify with complete confidence. The Animal Health Trust has developed a set of chromosome-specific 'paint' probes that, when used in Fluorescence in situ hybridization (FISH) analysis, enables complete confidence when identifying individual chromosomes. In this image, the two chromosomes appearing green are the two copies of dog chromosome number 12. They appear green because the cell has been exposed to a dog chromosome 12-specific paint probe labelled with a green fluorochrome.*

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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.

REFERENCES

1 Nishino, S. *et al.* (2000) Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*, 355, 39–40

2 Werner, P. *et al.* (1999) Anchoring of canine linkage groups with chromosome-specific markers. *Mammalian Genome* 1, 814–823

3 Lin, L. *et al.* (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98, 365–376

4 Breen, M. *et al.* (1999) Reciprocal chromosome painting reveals detailed regions of conserved synteny between the karyotypes of the domestic dog (*Canis familiaris*) and human. *Genomics* 61, 145–155

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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