

PET PROJECT

Stymied in the search for genes underlying human neuropsychiatric diseases, some researchers are looking to dogs instead. **David Cyranoski** meets the geneticist's new best friend.



Border-line: can collies help unpick the genetics of anxiety?

Solo takes a double dose of Xanax (alprazolam) for his nerves during the 4 July festivities in the United States. That is in addition to the antidepressant, fluoxetine or amitriptyline, that the 11-year-old border collie takes year-round. Fireworks just set him off, as do thunderclaps, gunshots — practically any explosive sounds — sending him into nervous fits. Panting and drooling with eyes dilated, he desperately searches for a place to hide. If another dog is nearby, he might attack. “It’s called anxiety redirection,” says Melanie Chang, Solo’s owner and an evolutionary biologist at the University of Oregon in Eugene.

As a postdoctoral researcher at the University of California, San Francisco, Chang helped to collect hundreds of border-collie DNA samples, including Solo’s, as part of a project studying the genes for noise phobia. She estimates that at least 50% of collies suffer from it, with 10% severely affected, sometimes injuring themselves or others in response to loud noises. Steven Hamilton, a psychiatrist at the University of California, San Francisco, who runs the project, says that he sees parallels between the dogs’ panic and human anxiety. And the same drugs work in about the same proportion of cases for man and beast. “It is easy to see similarities,” he says. A growing number of projects like Hamilton’s are underway to both help suffering dogs and untangle the roots of human neuropsychiatric disease.

The hunt for genes causing psychiatric problems in humans has been “hard work with slim pickings”, says Jonathan Flint, a geneticist at the Wellcome Trust Centre for Human Genetics in Oxford, UK. This is partly because human genomes are complex and these disorders are hard to diagnose consistently. But owing to 200 years of selective inbreeding, dogs have a bevy of breed-specific behaviours, and their genomes make it relatively easy to track down the genes responsible. “They are the only naturally occurring models of psychiatric disorders, and perfect for genetic mapping and cloning. It’s just beautiful,” says Guoping Feng, a mouse geneticist at the Massachusetts Institute of Technology in Cambridge, who is setting up collaborations with dog researchers.

Border collies were bred to herd grazing animals and to hear the calls of their masters from great distances. This, some have reasoned, might have produced hearing so sensitive that loud noises overwhelm some of the animals — inducing something akin to an anxiety disorder in humans. “In general, a lot of anxiety probably resulted from a long period of selection for dogs that can respond to human social cues,” says Chang. The provenance of other traits is less clear. Dobermann pinschers, for example, were bred to be faithful watchdogs but often have fixations and quirks akin to obsessive-compulsive

behaviour. And Dalmatians, bred for speed and endurance — probably so they could run with horses — tend to be aggressive.

Whether certain canine conditions arose by chance or are an unintentional outcome of selection for a specific quality is a matter of speculation. But behaviour problems are definitely frequent. Nicholas Dodman, an animal-behaviour specialist at Tufts University in North Grafton, Massachusetts, estimates that, at minimum, 40% of the 77.5 million dogs owned in the United States have some kind of behavioural disorder. Pet pharmaceuticals, including psychotropic drugs, are a thriving market. And sadly, many dogs with such problems are euthanized as a result of their temperament.

Researchers have good reason to believe that dogs will give up their genetic secrets more easily than humans. A study this year, for example, showed that variants at six locations in the dog genome could explain 80% of the variation in dog body size¹. In contrast, 294,831 common human variants, considered simultaneously, explained only 45% of height differences between humans².

But if the genetics of height is so different in dogs and humans, one might wonder why the genetics of anxiety, compulsion or aggression would be similar. Patrick Sullivan, a geneticist at the University of North Carolina in Chapel Hill, says that “behaviour that appears intriguingly similar in human and another species could have a completely different genetic architecture”, meaning that the same trait could map to different genes or to different parts of the brain. Proponents of canine studies suggest, however, that dog genes might hint at the pathways involved in human disease, and that might be enough.

Sleeping dogs don't lie

At least one success story shows that studies in dogs can lead to answers in humans. For decades, researchers vainly sifted through the DNA of human narcoleptics to find the genes behind

the sleep disorder. But many genes were involved, environmental factors were inconsistent and no clear mechanism emerged. “People were arguing whether it was an autoimmune disease, but no one knew what to do next. It was too difficult,” says Emmanuel Mignot, a sleep researcher at the Stanford University School of Medicine in Redwood City, California, with a background in molecular pharmacology.

But Doberman pinschers are often susceptible to narcolepsy, and they held the key. In 1989, Mignot started to use classical genetic techniques to breed narcoleptic Dobermanns and trace the inheritance pattern of the disorder. Without the benefit of modern genetic and genomic tools it took him ten years to zero in on the mutation that caused the disease, in a gene called hypocretin receptor 2 (ref. 3), which regulates the brain's uptake of the neurotransmitter hypocretin, also known as orexin.

Mignot did not find the same mutation in the corresponding human gene, but he did find changes in the hypocretin pathway⁴. “We started to measure hypocretin in cerebrospinal fluid. In narcoleptics, it was gone. It was striking,” says Mignot. Researchers are homing in on human gene mutations that lead to hypocretin depletion and to narcolepsy⁵, and drug companies are targeting hypocretin as a possible lead in the search for insomnia treatments.

Same dogs, new tricks

Since Mignot published his studies, the canine genome has been sequenced⁶. That has ultimately allowed researchers to quickly and easily compare the genomes of hundreds of dogs by looking at single nucleotide polymorphisms (SNPs) — single-letter changes in the genome that act as markers for inherited blocks of DNA.

The genome-wide association studies (GWAS) that researchers can carry out using these markers are much simpler in dogs than in humans. Most dog breeds are extremely homogeneous; individual animals in the same breed share significantly larger DNA blocks than are shared by any two humans. That means that researchers can look at fewer SNPs and fewer individuals to find a block of DNA that

associates reliably with a disease⁷. According to Kerstin Lindblad-Toh of the Broad Institute in Cambridge, Massachusetts, human GWAS might require 5,000 individuals with a trait of interest and 5,000 controls without it to show that the trait is convincingly associated with a particular genome region. Dog studies can sneak by on as few as a hundred cases and a hundred controls. And a study requiring hundreds of thousands of SNPs in humans might need only 15,000 in canines.

GWAS have proved successful in finding the genes for several dog traits that are relevant to human diseases, including the bone disorder osteogenesis imperfecta — pinned to the gene causing stubby legs in dachshunds⁸ — and the autoimmune disease systemic lupus erythematosus, which was shown in a study published this year to be controlled by five separate genes in Nova Scotia duck-tolling retrievers⁹. And more are coming. Anne-Sophie Lequarré, a veterinarian at the University of Liège in Belgium, coordinates the European dog-genetics initiative LUPA. The project, started in 2008 with a €12-million (US\$15.4-million) budget, brings together some 100 researchers to study single-gene and complex disorders — including cancer, cardiovascular disease and neurological disorders — by genotyping 10,000 dogs. Researchers involved will soon publish findings on two mutations in dog genes that cause disorders corresponding with human disease, says Lequarré: “The first results really show that once you find a mutation [related to a disease] in dogs, 90% of the cases involve the same gene in humans.”

Compulsive disorders may be among the first successes in unravelling human behavioural conditions through dogs. More than 60 studies on genes in mice thought to have a role in human obsessive-compulsive disorder (OCD) have so far failed to find significant, reproducible associations¹⁰. But there are lots of dogs with obsessive behaviour. A high proportion of bull terriers, for example, chase their tails relentlessly. Many large-breed dogs, such as




DOBERMANN PINSCHER

Breeding:
First bred as a guard dog around 1890 by tax collector Karl Friedrich Louis Dobermann.

Disorders:
Can suffer from narcolepsy, canine compulsive disorder, cervical vertebral instability, and the clotting disorder von Willebrand's disease.

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“Dogs are the only naturally occurring models of psychiatric disorders.”



DALMATIAN

Breeding:
Founded in the 1790s and used extensively as a carriage dog, running alongside horse-drawn coaches.

Disorders:
Predisposed to deafness, they can also suffer from hyperuricemia leading to urinary-tract stones and can be prone to aggression.

J. DANIELS/ARDEA.COM

Dobermanns, German shepherds, Great Danes and golden retrievers, chew their flanks or lick their legs until they lose hair, develop lesions and in some cases cripple themselves — a habit some compare with obsessive hand washing and other rituals of people with OCD.

In January, Lindblad-Toh and Dodman reported a link between canine compulsive disorder and a region on the dog's chromosome 7 (ref. 11). Their study was based on an analysis of 14,700 SNPs in the genomes of more than 90 compulsively chewing Dobermanns and about 70 controls. It linked the behaviour to variations in a 400-kilobase-long stretch of DNA. The connection between the variant that confers risk and the compulsive behaviour is not airtight, but it is good: 60% of the dogs that chewed their flanks, blankets and anything else they could get their teeth on had the variant, compared with 43% of those with milder chewing compulsion and just 22% of those with no signs of compulsive behaviour.

One gene in the targeted region has already captured the imaginations of other researchers. *CDH2* encodes the protein cadherin 2, which is involved in forming connections between neural cells. Deanna Benson, a neuroscientist at Mount Sinai School of Medicine in New York, says the possibility that cadherins are involved in OCD has inspired others.

Feng, who makes mouse models for OCD, is exploring the link. Last autumn, he and Lindblad-Toh struck up a collaboration to find brain circuitry related to compulsion that is shared by mice, dogs and humans. Feng is now knocking out *Cdh2* function in specific brain regions of mice to test whether that produces OCD-like behaviours.

Dogged progress

Lindblad-Toh is now seeking a tighter genetic fit to human OCD. Researchers approach dog genetic studies in two steps: first narrowing in on a large DNA chunk within one breed and then looking for overlap with that region in the DNA of dogs of other breeds with the same disease. Mignot used narcoleptic dachshunds

to home in on the mutation expressed by his sleepy Dobermanns. And by comparing DNA loci in flank-sucking German shepherds and tail-chasing bull terriers, Lindblad-Toh hopes to narrow the implicated region on chromosome 7 to a more manageable 10 kilobases. Similarly, Hamilton intends to broaden his noise-phobia studies from border collies to bearded collies and Australian shepherds that show similar anxieties.

But canine genetics is challenged by some of the same issues that have foiled researchers studying human illnesses. Diagnoses for neuropsychiatric disease are slippery. Schizophrenia, for example, could represent a collection of many different disorders, each with separate genetic and environmental triggers. And if the subjects grouped by symptoms have different underlying diseases, GWAS can become confused. "A few dogs can spoil a cohort," says Lequarré. She cites an epilepsy study that was not delivering any significant correlations. The researchers later found that some of the dogs in the disease group actually had a form of late-onset epilepsy that was different from that being studied. "Phenotyping is crucial. You need to have dogs that have exactly the same disease," she says.

LUPA is making an effort to clarify diagnosis. To identify neurological disorders consistently, the group selected veterinarians who follow standard procedures in parsing dog temperament. Standardization is the right approach, says Hamilton. For his work on collies, he leads owners through a 24-page questionnaire that elicits objective observations. "We don't ask, 'Is your dog aggressive?' We ask, 'When there is a thunderstorm, what does your dog do?'"

LUPA's neurological-disorder division is focusing on aggression in the English cocker spaniel and English springer spaniel, both given to sudden fits of rage. The researchers hope that the studies will identify mutations in genes related to human bipolar disorder, schizophrenia and other mental disorders involving aggression.

Excitement over dog models has been spreading. At the University of Tokyo's Laboratory of Veterinary Ethology, Yukari Takeuchi has collected DNA



ENGLISH COCKER SPANIEL

Breeding:
Developed in the nineteenth century to flush game out of the bushes and retrieve felled prey.

Disorders:
More prone than other breeds to epilepsy, and controversially, sudden-onset aggression or 'rage syndrome'.

J.-M. LABAT/ARDEA.COM

"For 10,000 years, dog has been man's best friend. Now dog is serving man again by helping us identify genes."

samples from 200 Japanese shiba inu and 200 labrador retrievers to look for the genes underlying the former's aggression and latter's lapses in concentration. It could help solve a practical problem, she says. Distracted retrievers do not make good guide dogs, and knowing the gene variant responsible could help breeders to limit the trait in their stocks¹².

Whether or not the dog studies live up to their promise for understanding and relieving human suffering, they are sure to benefit pets. Breeders are already taking notice of some of the gene variants that ravage certain breeds. For better and, in terms of scientific research, for worse, through screening and more selective breeding, the next

generations of border collies will probably have fewer anxiety-ridden dogs such as Solo who can be studied.

But Elaine Ostrander, dog geneticist at the National Human Genome Research Institute in Bethesda, Maryland, is confident that dogs have much to offer human health beyond the pleasure of warm fur and a cold, wet nose. "For 10,000 years, dog has been man's best friend. When we transitioned to hunter-gatherer, when we switched to agrarian, they were there. Now, in the genomic era, dog is serving man again by helping us identify genes," she says.

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GOLDEN RETRIEVER

Breeding:
Bred in the mid-1800s for hunting and retrieving shot waterfowl from long distances.

Disorders:
Although renowned for their friendly temperament, some are prone to aggression, dominance, seizures and a form of compulsive disorder.