Animal Health Trust

• The Animal Health Trust is a registered charity that works to reduce the impact disease and injury have on the welfare of companion animals.

• Founded in 1942 by Reginald Wooldridge, President of the National Veterinary Medical Association (now the British Veterinary Association).

• He wanted to apply the same advances being made in human medicine through research, to veterinary medicine in order to improve the health and welfare of animals.

• It has an active research unit that focuses on inherited and infectious diseases.

• It provides a clinical referral service for veterinary surgeons in practice
  – dermatology, internal medicine, neurology, oncology, ophthalmology, orthopaedics, soft tissue surgery, hydrotherapy

• Over 250 veterinary surgeons, scientists & support staff
Canine Genetics at AHT

• The broad aim of the Canine Genetics Research group at the Animal Health Trust is to investigate the genetic basis of important inherited diseases in dogs and identify genetic variants that underpin those diseases.

• Our definition of important is a disease that is painful, blinding, requires surgical or medical intervention or otherwise reduces the quality or length of life of affected dogs.

• An immediate-term objective of our research is to develop DNA tools that dog breeders can use to reduce the prevalence of disease in future generations of dogs and that veterinary surgeons can use to help diagnose disease.

• A longer-term aim is for our findings to improve our understanding of disease aetiology in dogs and other species.
## Costs and Funding

### Research Costs – 2018

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<tr>
<th>Description</th>
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<tr>
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<td>Consumables &amp; data storage</td>
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<td><strong>TOTAL</strong></td>
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### Funding

- American Kennel Club Canine Health Foundation
- Dogs Trust
- **Kennel Club Charitable Trust**
- PetPlan Charitable Trust
- Vets4Pets
- Breed Clubs and Individuals
Fundraising

Support us
Discover more →

Donate
Fundraise
Come to an event

Other ways you can help
Play our lottery

Fact
Our fundraisers have a phenomenal impact on our work. We
DNA Tests Developed 2005 - 2017

Over 60,000 dogs tested

- Total mutations identified
- Breeds with at least one DNA test
<table>
<thead>
<tr>
<th>Disease</th>
<th>Year DNA test launched</th>
<th>Number of breeds to benefit</th>
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<td>2005</td>
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<td>Miniature Longhaired Dachshund, English Springer Spaniel</td>
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<tr>
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<td>2006</td>
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<td>Staffordshire Bull Terrier, French Bulldog, Boston Terrier</td>
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<td>2008</td>
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<td>Australian Shepherd</td>
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<td>2009</td>
<td>17</td>
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<tr>
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<tr>
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<td>Cavalier King Charles Spaniel</td>
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<tr>
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<td>progressive retinal atrophy</td>
<td>2011</td>
<td>3</td>
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<td>Parson Russell Terrier, Jack Russell Terrier</td>
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<td>Petit Basset Griffon Vendeen</td>
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<tr>
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<td>Basset Fauve de Bretagne</td>
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<tr>
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<td>Giant Schnauzer</td>
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<tr>
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<td>2017</td>
<td>1</td>
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<tr>
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<td>2017</td>
<td>1</td>
<td>Norwegian Buhund</td>
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<tr>
<td>OSD</td>
<td>2017</td>
<td>1</td>
<td>Northern Inuit</td>
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</tbody>
</table>

Progressive Retinal Atrophy in the Shetland Sheepdog

Rebekkah Hitti
PhD Student
What is PRA?
What is PRA?

Basic anatomy of the dog retina

Normal retina, tapetum, dog. H&E stain, GCL - ganglion cell layer, I - inner segments, INL - inner nuclear layer, IPL - inner plexiform layer, NFL - nerve fiber layer, OPL - outer plexiform layer, OSM - outer nuclear layer, OS - outer segments, T - tapetum, arrow (short) - retinal epithelium, arrow (long) - middle limiting membrane, arrowhead (upper) - outer limiting membrane, arrowhead (lower) - inner limiting membrane

Cone Rod
What is PRA?

Normal

PRA affected
PRA in the Shetland Sheepdog

• Currently a DNA test for one form of PRA in the Shetland Sheepdog is available
  – CNGA1-PRA DNA test
  – Average age of clinical diagnosis for this form of PRA was reported to be 5 years

• However, this does not account for all types of PRA seen in the breed
  – Similarly seen across breeds, PRA can be caused by different mutations within the same breed
PRA in the Shetland Sheepdog

- Shelties clear of the CNGA1-PRA mutation have been diagnosed with PRA
  - Another form of PRA in the breed

- Tested for 25 mutations that cause retinal degeneration in other breeds
  - CLEAR of these mutations

- Give A Dog A Genome
  - Whole genome sequence one PRA-affected Sheltie clear of known mutations

Update to come!
DNA – what is it?

- DNA is responsible for every characteristic of you and your dog that is not determined by the environment

- Traits, including disease, can be due to a mixture of genetics and the environment

- A genome is an organism’s complete set of genetic instructions
Canine Genome

2.4 BILLION letters!

1 bp = 1 mm the DNA in the dog would stretch from Land’s End to John O’Groats and back again...

...or 1 bp = 1 letter
Harry Potter series x 440
2.4 BILLION letters!

1 bp = 1 mm the DNA in the dog would stretch from Land’s End to John O’Groats and back again...

...or 1 bp = 1 letter

Harry Potter series x 440
Whole Genome Sequencing

Reads the entire DNA sequence (genome) of the dog

Whole genome sequencing (WGS)
- Reads the entire DNA sequence (protein-coding regions and non-coding regions)
Mutations

A mutation is a permanent change to the nucleotide sequence of an organism’s DNA

Substitution

Insertion

Deletion

Inversion
PRA Research in the Shetland Sheepdog

- One PRA case diagnosed around the age of 8
  - Later onset to what has been noted previously in CNGA1-affected dogs

- Collect samples from this dog, related dogs and other control dogs to look at their genetic differences

- Whole genome sequencing
PRA Research in the Shetland Sheepdog

Raw sequence data received from laboratory

Sequence aligned to Boxer reference genome

List of variants created

Each variant is annotated with its details, chromosome and position, location within a gene, predicted effect on protein
PRA Research in the Shetland Sheepdog

DNA Collection

- Buccal cheek swabs
- PRA affected Shetland Sheepdog
- Control dogs

WGS

- Sequencing carried out by Edinburgh Genomics, University of Edinburgh

Variant Filtering

- Autosomal recessive (2 copies of the mutation required to present with disease)
- Effect on the protein sequence
- AHT Give a Dog a Genome bank
- Shared consortium (DBVDC)
Our PRA Research in the Shetland Sheepdog

- Genomes from 186 dogs were used for this study to compare to the Shetland Sheepdog PRA case.

- This catalogue of genetic variants in dogs is used to filter out benign neutral variants that are unlikely to be involved in disease.

- A bank of 648 genomes was also used to further exclude neutral variants.

- Candidate genes are those associated with human retinal degeneration.
Follow-up work

Mutation validation – where today comes in!

• Eye testing to support DNA samples submitted from your dogs is crucial to our research – Thank you!

• Screen your dogs DNA, and more control dogs, for our candidate variant to determine it is not present in clinically clear dogs (that are old enough to show clinical signs of this new form of PRA)

• This will help us offer a DNA test for this form of PRA
  – This will prevent any affected dogs being born
  – Stop the condition becoming widespread in the breed
The impact of DNA testing...

Frequency of RCD4-PRA mutation in Gordon Setters

DNA test made available
Acknowledgements

• Shetland Sheepdog Breed Club
  – Marion Withers
• All Sheltie owners (you!)
• Animal Health Trust
  – Canine Genetics
• Kennel Club Charitable Trust
• University of Cambridge
Give a Dog a Genome

Generating a stake-holder funded bank of whole-genome sequences with which to elucidate benign and disease-associated variation within the canine genome

Louise Burmeister, Ellen Schofield, Rebekkah Hitti, Christopher Jenkins, Carol Brunt, Bryan McLaughlin, James Oliver, Louise Pettitt, Sally Ricketts and Cathryn Mellersh

Kennel Club Genetics Centre, Animal Health Trust, United Kingdom
Canine Genetics at AHT

• The broad aim of the Canine Genetics Research group at the Animal Health Trust is to investigate the genetic basis of important inherited diseases in dogs and identify genetic variants that underpin those diseases.

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• A longer-term aim is for our findings to improve our understanding of disease aetiology in dogs and other species.
Give a Dog a Genome

• On 25\textsuperscript{th} January 2016 the Kennel Club contacted the Breed Health Co-ordinators of all 215 registered breeds, inviting them to participate in \textbf{Give a Dog a Genome}.

• In 2015 the Kennel Club Charitable Trust awarded the KCGC £50k to spend on \textbf{Whole Genome Sequencing} (WGS).

• This was sufficient funding to sequence \textit{\approx}25 whole genomes.

• We invited each breed community to donate £1000

• We promised to match each donation with £1000 from the KCCT funding to fund the sequencing of the whole genome of a dog of that breed.
Press release

Human genome: UK to become world number 1 in DNA testing

From: Prime Minister's Office, 10 Downing Street, Department of Health, NHS England, The Rt Hon David Cameron MP, George Freeman MP and others

History: Published 1 August 2014
Part of: Helping more people survive cancer, increasing research and innovation in health and social care, investing in research, development and innovation, others

£300 million investment that will transform how diseases are diagnosed and treated announced by the Prime Minister today.

The UK is set to become the world leader in ground-breaking genetic research into cancer and rare diseases, which will transform how diseases are diagnosed and treated, thanks to a package of investment worth more than £300 million, the Prime Minister will announce today.

The 4 year project will allow scientists to do pioneering new research to decode 100,000 human genomes - a patient’s personal DNA code. The landmark project is on a scale not seen anywhere else in the world.

It is part of the Prime Minister’s commitment to ensure the NHS as well as the UK’s research and life science sector is at the forefront of global innovation.
Coding DNA

DNA: CGATGTCAATAGCACC

mRNA: GCUACAGUUAACGUGG

Protein: ATVTTW
### Genetic Code

- **AUG** used to both initiate protein synthesis and direct the incorporation of methionine at internal positions of the polypeptide chain.

- three special ‘stop’ codons that do not code for an amino acid but signal the termination of translation.
A mutation is a permanent change to the nucleotide sequence of an organism’s DNA

Types of mutation:

**Insertion**  – the addition of one or more extra nucleotides into the DNA

**Deletion**  – the loss of one or more extra nucleotides from the DNA

**Substitution**  – the exchange of a single nucleotide

**Inversion**  – the ‘flipping over’ of a stretch of nucleotides

Mutations can be small-scale, involving single, or small numbers of nucleotides, or large, involving hundreds, or thousands of nucleotides.
Variation

- There is a **lot** of variation within the canine genome.
- Most of that variation is **neutral**.
- Some of the variation is **positive**, or **advantageous**.
- Some of the variation is **deleterious** (disease-causing).
- The challenge is to identify the deleterious variants within all the background, non-deleterious variation.
How much DNA is there?

- The canine genome contains $2.4 \times 10^9$ nucleotides.

- This is nearly two and a half billion ‘letters’ of DNA.

- If each one measured 1 mm the DNA in the dog would stretch from Land’s End to John O’Groats and back again.

- There are about 20,000 genes.
Finding a disease mutation using whole genome sequencing
• In 2004 the Canine Genome Sequencing Project produced a high-quality draft sequence of a female boxer named Tasha, known as CanFam1.0

• By comparing Tasha with many other breeds, the project also compiled an initial set of ~2.5 million single nucleotide polymorphisms (SNPs)

• The current version of the canine reference genome is CanFam3.1
<table>
<thead>
<tr>
<th></th>
<th>CASE</th>
<th>REF</th>
<th>DOG 1</th>
<th>DOG 2</th>
<th>DOG 3</th>
<th>DOG 4</th>
<th>DOG 5</th>
<th>DOG 6</th>
<th>DOG 7</th>
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<td><strong>CGAT</strong>CATGATCAACTAGACTAGGCATGACATACCGATCGATCGAATCGATCGT**</td>
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Affected Dog = **CASE**
It’s a bit more complicated than that…

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<th>Sample ID</th>
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The Genome Bank

• The genomes of two different dogs will differ in ~2-3 million places.

• The more breeds that are represented in the bank of genomes the more variation across the genome we will capture.

• The more variation we capture the more effectively we can filter out benign (or neutral) variants.

• The more effectively we can filter out neutral variants, the more quickly (& cheaply) we will be able to identify disease mutations.

• Sequencing the genomes of single representatives of >75 different breeds will capture far more variants than multiple dogs of the same breed.
WGS comparisons: effect of the number of controls on filtered variants
Give a Dog a Genome

Breed Recruitment & Funding

Health Questionnaire & Disease Selection

Sample Collection & DNA QC

Whole Genome Sequencing

Use & Sharing of WGS Data

Defining features of GDG:

★ Engagement of breeds involved
★ Collaboration & shared resource
Give a Dog a Genome

- Sequenced a total of 89 dogs from 77 breeds
  - 82 have been sequenced and the data analysis is underway
  - 7 are in the final stage of sequencing and the data will be available shortly.
- 69 Affected with something:
  - 16 Idiopathic Epilepsy
  - 9 PRA (including a Sheltie)
  - 7 Hereditary Cataracts
  - 2 Glaucoma
- 20 Healthy controls
- Data from 29 dogs shared with 17 scientists from Europe and USA
Any questions?