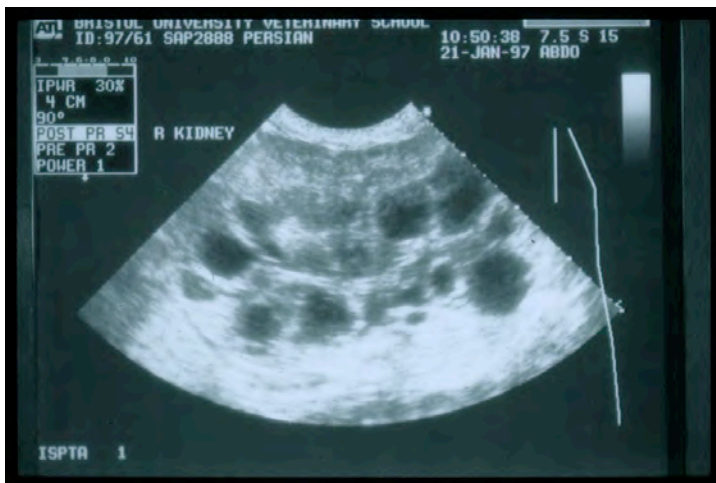


Feline polycystic kidney disease: from ultrasound to genetic testing

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Feline autosomal-dominant polycystic kidney disease (AD-PKD) is the most prevalent inherited genetic disease of cats. It affects Persians and related breeds, particularly Exotic Shorthairs. The worldwide prevalence of AD-PKD in Persians has been reported to be between 38% (USA)¹ and 49% (UK)². The disease is characterised by the presence of renal cysts that are usually detectable by ultrasonography. AD-PKD can lead to renal failure and premature mortality.

Until recently the only practical, non-invasive method available for the diagnosis of feline AD-PKD was renal ultrasound. While this is very reliable it does have its drawbacks: owners must transport their cats to the facility offering ultrasonography, registered scanning can only be carried out by an approved ultrasonographer and cysts can only be reliably identified in cats over 10 months of age.

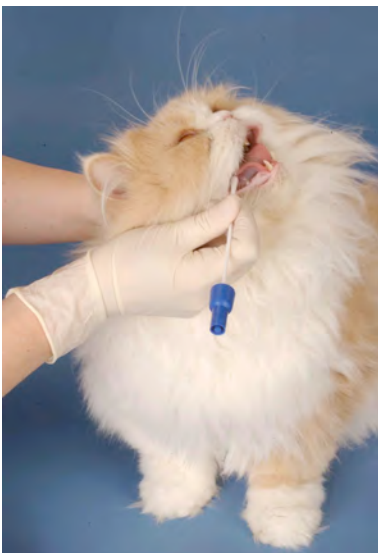


Ultrasound scan of a kidney from a Persian with AD-PKD. (Kindly supplied by Dr K. Bradley).

In 2004 the genetic mutation responsible for AD-PKD in Persians and related breeds was identified in a group of Persian cats from America³. A single nucleotide in the coding region of the PKD 1 gene was found to be different between affected and unaffected Persians. This single change (termed a single nucleotide polymorphism or SNP) causes premature termination of protein synthesis and results in the protein (called polycystin) being truncated by about 33%. It is this abnormal protein that is thought to cause the cysts present in the kidneys of affected cats. We have recently demonstrated that the same genetic mutation is present in the UK population of Persian cats⁴. Also, in agreement with the American results, we have shown that all AD-PKD Persians have one copy of the wild-type gene and one copy of the mutant gene, i.e. they are all heterozygous.

Now that the genetic basis of feline AD-PKD has been elucidated it is possible to diagnose the disease using tests based on molecular assays. All that is required from the cat is a sample of its genomic DNA. This can be from an anticoagulated (preferably EDTA) blood sample or from cheek epithelial cells collected using cytobrushes or cotton swabs (buccal samples). Once collected the samples are simply posted at ambient temperature to the laboratory. Here, the genomic DNA is released from the cells and purified to enable one of the molecular tests to be performed. The first step is to amplify the relatively small amount of genomic DNA to a level that can be worked with. This is done using the polymerase chain reaction (PCR). A pair of short single-strand DNA sequences (termed primers) are designed to bind close to, and on either side of, the AD-PKD mutation. After the addition of a thermostable DNA polymerase (an enzyme that synthesises DNA), the reaction is cycled through 40 repeats of heating and cooling; resulting in a million to billion fold amplification of the genomic DNA between the two primers. The resulting amplified DNA is called an amplicon. All that is now required is to detect the SNP in the amplicon and this can be done using one of several methods.

- The amplicon could be submitted for automated fluorescent DNA *sequencing* to determine its genetic sequence. In practice it is often difficult to discern the SNP and so sequencing is not used routinely.
- The amplicon could be subjected to *restriction fragment length polymorphism (RFLP) analysis*. This technique uses restriction enzymes that cut DNA strands at specific nucleotide sequences and gel electrophoresis to separate the subsequent DNA fragments. This technique is widely used to detect SNPs but it is time consuming and can give false results if the laboratory becomes contaminated with the amplicon.
- A *real-time PCR genotyping* method could be used. This method differs from the above two in that it combines the amplification and SNP detection steps into one. Two DNA probes are included in the PCR, one of which detects the wild-type sequence whilst the other detects the AD-PKD sequence. This results in a rapid, sensitive and specific assay that is much less likely to result in laboratory contamination.



Taking a buccal swab for AD-PKD genetic testing.
(Kindly supplied by Dr S. Tasker).

Recently we compared the results of ultrasound scanning, RFLP and real-time PCR genotyping for the detection of AD-PKD in 72 UK Persians and related breeds. Apart from 2 cats in which renal ultrasound diagnosis was equivocal, there was a 100% agreement between the three methodologies⁴. We have gone on to use the real-time PCR genotyping assay to determine the AD-PKD status of 506 Persians or related breeds from samples submitted to Langford Veterinary Diagnostics. The assay worked equally well with both buccal swab and blood samples and was able to reliably genotype every cat. Of the 149 cats identified to be AD-PKD positive all were heterozygous, supporting the hypothesis that homozygous AD-PKD kittens die *in utero*.

With the advent of molecular tests to determine the AD-PKD status of Persians and related breeds it should be possible to eradicate the mutation from the feline population by selective breeding. If an affected cat must be used for breeding, due to the presence of desirable traits, mating it with a wild-type cat should ensure some non-affected kittens are produced that can be identified by AD-PKD screening. The molecular tests are much more owner and cat friendly since they only require a small amount of easily obtainable feline genomic DNA. Additionally, testing can be done before the cysts become detectable by ultrasonography. However, this does not mean the end of ultrasound scanning for AD-PKD. Whilst the genetic test has advantages over ultrasound for initial routine AD-PKD diagnosis, ultrasound may still be used to monitor the severity of the disease in those cats that have the AD-PKD mutation. It may also be used to identify whether any cats exist with polycystic kidney disease caused by a different genetic mutation.

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Chris graduated in 1989 with a degree in Biochemistry from the University of Bristol. This was followed by a Wellcome Trust funded Ph.D to study the bovine renal sodium-dependent phosphate transporter. He was awarded a further two year Wellcome Trust funded fellowship in 1992 to clone and study the bovine renal sodium-dependent phosphate transporter at the molecular level. In 1995 he moved to the School of Clinical Veterinary Science, University of Bristol as a post-doctoral researcher to clone and sequence the genome of feline spumavirus. In 2001 he was promoted to Research Fellow. Over the past four years he has been extensively involved in the set up of real-time PCR assays for the detection and quantification of a wide range of feline pathogens. Some of these assays were recently used in a large epidemiological study to investigate the prevalence of feline herpes virus, feline calicivirus, *Chlamydomphila felis* and *Bordetella bronchiseptica* in 218 European catteries. These and other assays are currently being used by Langford Veterinary Diagnostics for the detection of feline pathogens. Recent work has focused on the development of a real-time PCR assay to detect feline AD-PKD.