ABSTRACTS

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Application of genomic and molecular methods to fundamental questions in canine and feline reproductive health

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Molecular tools are becoming increasingly available to investigate the genetic basis of reproductive disorders in dogs and cats. Most of us can identify a disorder that we would like to approach with these methods. Clinicians play an essential diagnostic role in such studies, but also need to understand how these molecular tools can best be applied to advance our knowledge. The role of the clinician in establishing stringent diagnostic criteria that unquestionably identifies both affected and control animals should not be underestimated, as it is essential to the success of these studies. The collaborative efforts of molecular biologists and computational scientists are equally important to identify and utilize the appropriate molecular tools and analyses. We will illustrate how these tools can be used. Examples will include ongoing or published studies that investigate simple Mendelian or complex traits, and those that investigate gene expression differences between tissue samples.

The candidate gene approach has identified several mutations for disorders that are inherited as simple Mendelian traits. In most cases, considerable knowledge was available before the study that implicated deficiency of a specific protein, and thus a gene candidate. An example is Persistent Mullerian Duct Syndrome (PMDS) in the miniature schnauzer. Mutations in the human Mullerian Inhibiting Substance Receptor II (MISRII) were identified in affected humans, which led to identification of canine MISRII as a candidate gene. By sequencing canine MISRII exons, using DNA from affected and control dogs, the causative mutation was identified (1). That discovery led directly to development of a DNA test to identify carrier and affected dogs in this breed (2). However, the candidate gene approach is limited, as candidates have not been identified for most reproductive disorders.

An association study is a different approach that is used to narrow the mutation search from the entire genome to a chromosomal region, and eventually to a small interval of DNA, in which a causative mutation should be located. Genome wide association studies (GWAS) in dogs have been successful in identifying causative mutations for simple Mendelian traits. In contrast to linkage analysis, which requires DNA samples from a pedigree segregating the disorder, GWAS does not require development of pedigrees. The initial goal of a GWAS is to identify a chromosomal region associated with the disorder. Affected and control dogs are chosen from a single breed in which the disorder is highly prevalent (3). The number of animals that must be genotyped varies with the mode of inheritance. Genomic DNA from affected and control dogs is hybridized to a canine whole genome single nucleotide polymorphism (SNP) array. Statistical analysis of the genotypes identifies which SNP genotypes are highly associated with the affected and not the control phenotype. For a simple Mendelian trait, the SNPs with highest probability of association should cluster on one chromosome. After such a chromosomal region is identified, fine mapping of that region by additional SNP genotyping is used to identify a smaller region of DNA in which to search for a mutation. In fine mapping, DNA from additional affected dogs of the initial breed, as well as affected dogs from related breeds that have the identical disorder is used. Finally, sequencing the region of associated SNPs identifies a mutation that is shared by all affected dogs, but not by controls (3). Investigation of Canine XX DSD will be presented as an example of the GWAS method.

It is important to note that GWAS also has the potential to identify causative mutations for complexly inherited traits. At least one group is using this approach to investigate canine cryptorchidism. Another group has used association analysis to determine whether SNPs in candidate genes are associated with canine cryptorchidism (4). Potentially, both methods could be used to study the genetic basis for other reproductive disorders.

In order to establish a candidate mutation as the causative mutation, it is usually necessary to demonstrate how the mutation induces the pathology. One approach is to demonstrate an adverse effect upon the amount or timing of gene expression. Several methods are available for this purpose, including quantitative reverse transcription polymerase chain reaction (qRT-PCR), expression microarrays, and direct sequencing of RNA (RNA-Seq). The latter has several advantages. The sequence output from RNA-seq is called a transcriptome, which is a database of all messenger RNA transcripts that are present in the sample. The relative abundance of each transcript and differentially spliced transcripts can also be determined. For example, this method is being used to identify differences in gene expression in embryonic ovaries and testes during the sex determination period. These methods are also used to compare expression between tissue samples for other purposes, such as comparing neoplastic tissue to normal tissue from the same patient. By comparing the target tissue
and control tissue transcript sequences to genetic pathway databases on the internet, it is possible to identify which genetic
pathways in the target tissue have been altered.

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