Determination of White Spotting in Dogs: An Investigation of Candidate Genes

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Summary and Implications

In dogs, white spotting is of interest because in some cases of extreme white spotting, in which a dog is nearly entirely white, dogs may also have health disorders including blindness and deafness. Nine candidate genes were tested for association with spotting in dogs, including *SOX-10, MCOLN3, EDN3, KITLG, PAX3, MITF, ASIP, ADAMTS20, and SNAI1*. These genes were either ruled out (*SOX-10, MCOLN3, EDN3, KITLG, PAX3*), were not useful in the families in this study (*ASIP, ADAMTS20, SNAI1*), or studied further (*MITF*). A genetic mutation called a single nucleotide polymorphism (SNP) in the microphthalmia-associated transcription factor gene (*MITF*) was discovered and genetic tests were designed to classify the dogs (Beagle crosses and Newfoundlands) into their different genotypes (the combination of alleles located on paired chromosomes that determines a specific characteristic or trait). Studies showed an association between the genotypes and the observed white spotting in the dogs' coat color. Results suggest that *MITF* may predict spotting in these breeds.

Introduction

White spotting has been investigated in several species including the mouse, for which 10 genes with mutations causing spotting have been identified. In dogs, white spotting is of interest because extreme white spotting has been linked to health disorders including blindness and deafness in several breeds. The objectives of this study were: 1) to use a number of candidate genes known to be associated with spotting in other mammalian species, 2) to employ a limited genome scan to find SNPs (differences or variations in the DNA sequences) in each of the nine candidate genes, and 3) to perform association analyses on specific canine pedigrees for each gene.

Materials and Methods

To test associations between white spotting and the SNPs in the candidate genes, we used three families (Fig. 1), two families (A & B) which were part of a larger multigeneration family founded by a purebred Schipperke and two Beagle/mixed breed dogs, and one Newfoundland family (C). Within these pedigrees, homozygous normal (SS) and heterozygous animals (Ss) animals appeared completely or nearly completely black or otherwise solidly colored. Homozygous spotted (ss) animals had greater than or equal to 50% white color, with white extending over the trunk, and colored areas confined to large blankets or spots (Fig. 2). Heterozygous animals were classed as such because: 1) they produced at least one spotted offspring, or 2) they were obligate heterozygotes because they were produced from a spotted dog.

For this candidate gene study we used polymerase chain reaction (PCR), a technique in genetics that permits simple and efficient analysis of any short sequence of DNA. For all candidate genes, two "primers" that flank the beginning and end of the DNA stretch to be studied were designed from the canine genome sequence, which is now available on a public website. Amplified gene fragments from all three founder animals of the Schipperke and Beagle/mixed breed pedigree were sequenced to identify the genetic differences that existed in the amplified fragments. By comparing the DNA sequences of these animals, we were able to identify genetic differences, or SNPs, that existed in these animals. Using the SNPs identified in the founders, genetic tests were developed to determine the genotype of animals from the extended family. The resulting genotypes and spotting pattern of dogs were then analyzed to determine if there was an association between the gene and white spotting.

Results and Discussion

One SNP was discovered in *MITF* consisting of an altered nucleotide (a C to T substitution) which was found in intron 3 of the *MITF* gene. A genetic test was designed that recognized two forms of the gene, (C) or (T). Solidly colored animals (SS) were CC, obligate heterozygotes (Ss) were CT, and spotted animals (ss) were TT. Analysis of this SNP in our families demonstrated total agreement of genotypes with observed solid or spotting phenotypes, and this SNP was also in accordance with random samples from Beagles (5) and Boxers (2) that were also tested. Results suggest that this *MITF* marker may also predict spotting in these breeds. Other breeds (Great Dane and American Staffordshire Bull Terrier) did not show association between *MITF* and spotting. This research may be of interest to dog breeders for two primary reasons. First, dog breeders who work with breeds that have both solid colored dogs and spotted dogs and who would like to know the genotype of their breeding stock to select for (or against, as their breeding objectives dictate) the possibility of spotted phenotype in offspring may find this test useful. Second, there is evidence that extreme white spotting in some breeds is causally associated with deafness and other abnormalities.

This information might further assist breeders in selecting dogs to reduce the incidence of deafness.

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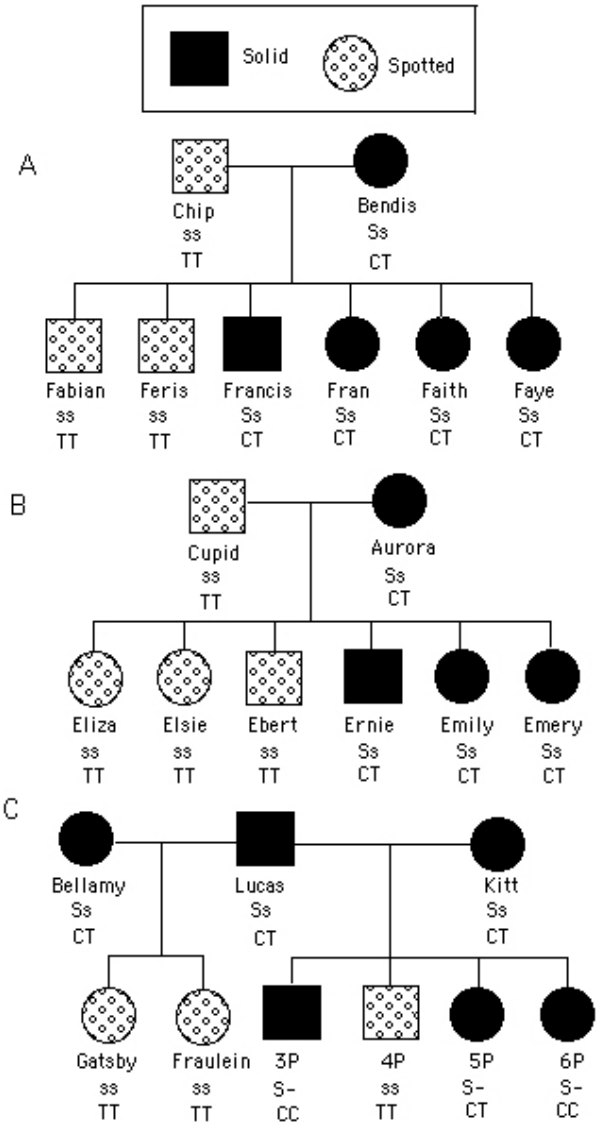

Figure 1. Pedigrees of two families (A & B) from a multigeneration pedigree founded by a Schipperke and two Beagle/mixed breed dogs and a Newfoundland family (C) with *MITF* SNP genotypes below.

Figure 2. Example of a spotted dog from the Schipperke x Beagle/mixed breed pedigree.