Man's Best Friend Becomes Biology's Best in Show: Genome Analyses in the Domestic Dog[*]

Heidi G. Parker[1], **Abigail L. Shearin**[1,2], and **Elaine A. Ostrander**[1]

Elaine A. Ostrander: eostrand@mail.nih.gov

[1]Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892

[2]University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pennsylvania 19104

Abstract

In the last five years, canine genetics has gone from map construction to complex disease deconstruction. The availability of a draft canine genome sequence, dense marker chips, and an understanding of the genome architecture has changed the types of studies canine geneticists can undertake. There is now a clear recognition that the dog system offers the opportunity to understand the genetics of both simple and complex traits, including those associated with morphology, disease susceptibility, and behavior.

In this review, we summarize recent findings regarding canine domestication and review new information on the organization of the canine genome. We discuss studies aimed at finding genes controlling morphological phenotypes and provide examples of the way such paradigms may be applied to studies of behavior. We also discuss the many ways in which the dog has illuminated our understanding of human disease and conclude with a discussion on where the field is likely headed in the next five years.

Keywords

GWAS; linkage disequilibrium; genomics; canine; domestication; complex traits

> "All knowledge, the totality of all questions and all answers is contained in the dog."
>
> – Franz Kafka

INTRODUCTION

History of the Domestic Dog

Dogs and humans have been traveling the globe together since prehistoric times. It has been so long in fact that neither of us remembers exactly how or when we met. With the genomic tools in place to trace common haplotypes, build pedigrees, and use molecular methods to disentangle ancestries, we are driven to understand the beginnings of our relationship with the domestic dog, in hope that it will provide illumination as to our own ancestors' travels, evolution, and genetics.

---

[*]The U.S. Government has the right to retain a nonexclusive royalty-free license in and to any copyright covering this paper.

Fossil evidence places dogs in close proximity with humans as early as 31,000 years before present (YBP) in an Aurignacian period cave located in what is now Belgium (47). Additional evidence was found in France placing dogs with humans 26,000 YBP, and dog fossils have been found in Russia dated approximately 13,000–17,000 YBP (46, 131). In all of these sites, the dog fossils are as large or larger than modern wolves, making the classification as domestic dog difficult and controversial. Whether these fossils represent the earliest domestic dogs or extinct varieties of wolves, multiple pieces of evidence suggest that dog-like animals and humans have shared space for over 30,000 years and that the dog-wolf ancestor showed much greater variability than do modern wolves.

Molecular calculations regarding the time since domestication vary greatly and tend to agree only that the critical events occurred prior to 15,000 YBP. Mitochondrial DNA (mtDNA) analyses have suggested 100,000 YBP, 40,000 YBP, and 16,300 YBP (114, 134, 156). Comparisons of nuclear genomic data from wolves, dogs, and coyotes suggest a possible domestication time of 18,000–27,000 YBP (9000 generations) (88). All of these estimates require assumptions to be made regarding the number of independent events, the size of the critical population, mutation rate since domestication, and degree of backcrossing between domestic and wild progenitors, which is hypothesized to be extensive given the diversity that exists in modern dogs. The fact that the genome of modern domestic dogs contains both dog-specific alleles and haplotypes derived from wolves further supports the backcrossing hypothesis (5, 157).

Although phylogenetic studies based on several independent molecular datasets agree that the gray wolf is the direct ancestor of the dog, the details remain elusive (2, 39, 88, 156). mtDNA analyses of a large number of Asian dogs compared with dogs from around the world indicate a high level of diversity in the East Asian population, suggesting that that area was a major site for critical domestication events (114, 134). However, studies of African village dogs reveal a level of heterogeneity similar to that found in Asia, suggesting that a detailed analysis of other indigenous populations may yield novel theories (17). An early Asian domestication fits well with the introduction of old world dogs into the new world via migration across the Bering strait 12,000–14,000 YBP (86), but it does not explain the near simultaneous appearance of domesticated dogs throughout geographically distinct areas of Europe. Such an event would require dogs to travel without human companions, bringing into question their domestic status.

We showed in a genome-wide haplotype analysis of 912 domestic dogs and 225 wolves collected from all over the world that most modern domestic breeds share genetic signatures, to the greatest extent, with wolves from the Middle East (158). However, breeds like the Akita, which are believed to have originated in Asia, share a disproportionate number of haplotypes with Chinese wolves, whereas European breeds such as the Staffordshire bull terrier share more haplotypes with European wolves. Overall, these data suggest that wolves from many geographical regions contributed to the development of the modern dog, either through backcrossing and/or multiple domestication events (156, 158) (Figure 1).

Small dogs, morphologically distinct from wolves, were first found in human proximity in burial sites in the Levant area of Israel, south of the Mediterranean, dating approximately 12,000 YBP (147). At this point in time, and in this region, little confusion exists as to what is dog and what is wolf, as the size and shape of both had changed considerably from earlier fossil remains. It is therefore interesting that the haplotype at the insulin-like growth factor 1 (*IGF1*) locus that Sutter et al. associated with small size in the domestic dog (143) is found exclusively in wolves from this region of the world (51). This unexpected finding suggests that the genetic variants at *IGF1* that contribute to small size, a feature often associated with domestication, likely developed in the Middle East. In an independent line of study,

evidence suggests that the retrogene responsible for chondrodysplasia, which defines breeds such as the dachshund, basset hound, and corgi, also arose in this region (118), implying that the Middle East was critical for breed development through selection of specialized morphologic traits.

Sequence analysis of mtDNA from ancient dogs and wolves reveals haplotypes that are not present in modern populations of either, indicating a loss of diversity in both species since the division (47, 154). These changes affect our ability to develop precise timelines and trees describing the domestication events and subsequent evolution of the dog. As a result, there remain unanswered questions regarding dog domestication. For instance, we do not know what effect domestication has on the genome of an organism. It has been suggested that there is a relaxation of selection after domestication, leading to the build-up of mutations that would otherwise be cleared through purifying selection (13).

This idea fits well with our perception of dogs and wolves. Domestication would likely change the dog's living environment and food source, which could alter their energy consumption, change behavioral patterns, and allow less wilderness-ready individuals to thrive and reproduce under human protection. In addition, constant contact with roving human groups would have had a drastic effect on canine diversity through selective breeding, migration, hybridization, invasion, and decimation or assimilation of local populations (95). One can easily imagine the result would be the development of large numbers of nonlethal mutations for breeders to select from. Though these changes may greatly affect our ability to develop a precise timeline of domestication, they do create the opportunity to identify critical genetic changes that occurred to create our treasured modern companions. The domestic dog, then, holds a unique position as the darling of geneticists studying variation, who seek to discover genes and sequence-level changes associated with morphology, susceptibility to simple and complex diseases, and behavior.

CANINE POPULATION STRUCTURE

In 70 AD, Columella identified three specific types of dog: the guard dog, the shepherd, and the hunting dog that "does not help the farmer but actually lures him away from his work." This was the first written description of dog proto-breeds, with sizes, shapes, and behaviors related to human needs. But it wasn't until 200–300 YBP that the majority of dog breeds were established leading to the approximately 400 varieties that compose today's modern population.

In the early 1900s, registering bodies such as the American Kennel Club (AKC) in the United States and the Kennel Club in the United Kingdom were created (4). Rules were developed to further separate breeds by requiring the parents of each new puppy to be a registered member of the same breed in order for the puppy to be eligible for registration. In addition, well-documented standards were established, requiring specific size, shape, color, and sometimes behavior from each qualifying dog (4). As a result, each dog breed represents an isolated breeding population, with a constellation of traits maintained under strong selection that define each breed (110, 112) (Figure 2). Nowhere else but among domestic dogs can members of the same species coexist that differ in size by more than 40-fold as do the largest breeds, such as the Great Dane, and the Chihuahua, which is among the smallest of the breeds (112, 161).

Studies using microsatellite markers have shown that each breed has a unique genetic signature based on allele frequency and distribution that allows for singular identification of nearly every breed (116, 117). Cluster analysis of microsatellite data from as few as five dogs per breed allows breeds to be clustered into five major groups characterized by shared ancestry, usually following geographic, morphologic, and/or occupational boundaries (116,

117). These findings have recently been further refined using haplotype data constructed from approximately 48,000 single-nucleotide polymorphisms (SNPs). Phylogenetic analysis of haplotype sharing using 80 breeds with approximately 12 unrelated individuals per breed divides the population of modern dogs into approximately eight groups, each of which share common signatures of variation across the genome (158).

Not only is there a tremendous amount of population structure found between breeds, but stratification within breeds exists as well. Such variation often results from breed popularity and popular sire effects, as well as pressure on the part of the breeding community to propagate certain traits (degree of spotting, fur length, tail or ear position, etc.). By way of example, Mosher et al. identified strong signals of nonrandom mating and stratification based on racing performance levels in a study of whippets segregating a mutation in the myostatin gene (*MSTN*) that increases muscle mass (103). In a homozygous state, the mutation creates an excessively muscled dog that has an increased tendency to cramp when the muscles are stressed. In a heterozygous state, however, the musculature is only mildly exaggerated, and there is a measureable and reproducible positive effect on running speed (103). The investigators found that there was a preference for crossing dogs with the mutation within racing lines versus those that lacked the mutation in order to produce faster puppies with a more muscled appearance. This lack of random mating created substructure in the breed apparent in regions separate from the original mutation (103).

Within-breed stratification has also been described in the context of geography (126). Quignon et al. studied several breeds from Europe and the United States, assessing allele frequency and distribution of 722 SNPs from four unlinked loci (126). They found that stratification depends on population size and breeding strategies, with some breeds having experienced sufficient outbreeding to exist as a single breed across continents, whereas others are as divergent as distinct breeds. Also, Bjornerfeldt reported breed stratification based on color preferences in the poodle (12). The use of the poodle breed to map a disease gene would therefore require careful correction for the overrepresentation of one color within selected datasets.

CANINE GENOME ARCHITECTURE

Genes and the Sequence

When the sequence of the boxer was released in 2005 (88), it was estimated that the dog genome contained 19,300 genes, which is less than estimates from other sequenced species (currently 22,320 in human, 23,062 in mouse, and 24,147 in zebrafish; Ensembl database (http://www.ensembl.org). To determine if the lower numbers reflected a true loss of genes in the dog or, rather, an artifact of sequence assembly, Derrien et al. (35) examined 400 missing genes through multiple species alignment among dog, human, rat, and mouse. They compared orthologous genes and built multiple pairwise synteny maps that allowed them to infer short orthologous intervals that targeted a putatively missing canine gene (35). These data were also compared with the deep radiation hybrid (RH) map of the dog, upon which sequences from nearly 10,000 genes were localized (63).

Of the 400 missing genes that were the primary focus of the study, 70% were found, but had been miscalled. Another 12% could not be found due to poor sequence or assembly problems in the syntenic regions. Sixty-nine genes were recorded as lost because they were either totally undetected or had become pseudo-genes (35). Based on accumulation of mutations within the latter category, these genes are estimated to have lost function as many as 170 mya, well before the division of the order Carnivora from other mammalian groups (39). Although it is possible that the loss of these genes provides a selective advantage in

dogs and other carnivores, it is more likely that these findings reflect a loss of redundancy in the genome.

Linkage Disequilibrium

The creation of the breeds has altered the genome structure of the dog, affecting linkage disequilibrium (LD), haplotype structure, heterozygosity, and possibly even the rates of mutation (13, 33, 95). LD, the nonrandom association of alleles at two or more loci, can be greatly altered by population bottlenecks, small numbers of founders, and admixture. Two studies, the first by Sutter et al. in 2004 (144), followed by Lindblad-Toh et al. in 2005 (88), measured LD from five and ten loci in multiple breeds. Both studies demonstrated that, on average, LD in dogs is as much as 100-fold greater than that observed across the human population. As a result, genome-wide association studies (GWAS) in the dog require only tens of thousands of markers compared with the million or so needed for comparable human studies (88, 144). Lindblad-Toh and colleagues also calculated LD across breeds and found levels to be similar to human (~10 kb), suggesting that short-range LD in the dog arises from the ancient bottleneck created by domestication and is shared among the breeds, whereas long-range LD is the product of recent breed creation (88). An extension of the LD studies into a larger number of dog breeds shows a range of within-breed values from 20 kb in the Labrador retriever to 4.5 Mb in the Pekingese (50), although measures were variable across the genome (88, 144). Not surprisingly, LD in dog breeds displays significant correlation with demographic history, although there are outliers: breeds that display higher or lower levels of LD than expected based on population history (Figure 3). This demonstrates the complex factors contributing to breed formation, including hybridization, migration, and evolving selective pressures.

Interestingly, Gray et al. detected a similar range of values in a diverse collection of wolf populations. As with dogs, the degree of LD correlated closely with both population size and severity of bottleneck (50). Although dog populations are considered artificial because of man's interference, they can still provide accurate models of extreme events in natural populations and inform genome-based conservation efforts.

Haplotype Structure

The haplotype structure of the dog was elucidated in conjunction with the prior LD analyses (88, 144). In ten regions dispersed over the genome and spanning approximately 100 kb each, Sutter et al. reported that less than three haplotypes made up the majority of chromosomes in each breed. In addition, only 4.5 haplotypes were found in 80% of chromosomes in all five breeds tested, and on average, each breed pair shared more than 50% of their haplotypes (144). The degree of sharing reflected the common history of some breeds. For instance, the Labrador and golden retriever breeds shared the highest number of haplotypes, and the Japanese Akita and the Swedish Bernese mountain dog, which have no known common heritage, shared the fewest. As is evident in the following sections, these findings have strong implications for fine mapping loci of interest using combinations of breeds that share a trait from a common origin.

Homozygosity and Selection

In the analysis of the 7.8x boxer sequence, Lindblad-Toh and colleagues noted extensive regions of homozygosity interspersed with highly heterozygous regions along each chromosome (88) (Figure 4). The homozygous regions were on average sixfold longer than the heterozygous regions and covered 62% of the boxer genome. Closer examination suggests that extensive stretches of homozygosity exist in all dogs, with distinct patterns for each breed (88).

Based on the assumption that artificial selection has shaped the canine genome, Akey et al. examined 21,000 SNP genotypes from 10 breeds and assessed signatures of selection through examination of population differentiation statistics in one Mb windows across the genome (3). Out of 1933 windows, 155 showed significant homozygosity in at least one breed. Examination of some of those regions revealed genes that were plausible candidates for breed specific traits. For example, one such region in the Chinese shar-pei breed contained an intronic deletion associated with skin wrinkling in the HSA2 locus, which contains the gene for hyaluronic acid synthatase. Interestingly, excessive wrinkling of the skin, which typifies the shar-pei breed, correlates with increased serum hyluronic acid levels (166).

Canine SNP Resources

In 2005, the 7.8x genome sequence of a domestic dog, a boxer, was made available (88), following release of the 2x sequence of the standard poodle (82). The combination of these two resources, along with one Mb of sequence from nine additional breeds and a group of wild canids, generated more than 2.5 million SNPs that could potentially be used for trait mapping (88). To date, four whole genome SNP chips have been made available [Affymetrix, Santa Clara, CA (http://www.affymetrix.com); Illumina, San Diego, CA (http://www.illumina.com)], with increasing numbers and quality of SNPs on each new release.

CAUSES OF GENOMIC VARIATION

Retrotransposons

In 1992, Minnick and colleagues identified the first canid-specific repeat element, a widely dispersed short interspersed nuclear element (SINE) (101). Termed SINEC_Cf, these repeats represent about 7% of the dog genome sequence (82) and are highly active. Indeed, more than 6000 SINEs were found to be heterozygous in the canine reference sequence alone (88). Comparing the boxer and poodle reference sequences, Kirkness et al. reported 11,000 bimorphic SINEC_Cf elements and predicted that another 10,000 are likely to exist. These numbers are in stark contrast to the less than 1000 active SINEs estimated in the human genome (96). In addition to the SINE population, long interspersed nuclear elements (LINEs) are more active in the dog than in humans, and evidence suggests that SINEs are transposing additional flanking chromosomal sequences in conjunction with the retrotransposons (81).

Polymorphic SINEs have already been implicated in both morphology and disease in the dog (Table 1). For instance, Clark et al. showed that insertion of a SINE element into the SILV gene causes merle coat patterning in the Shetland sheepdog (31).

Copy Number Variation

In 2004, two large-scale human genome analyses revealed that copy number varations (CNVs), duplications or deletions of entire stretches of genomic DNA, are common throughout the normal human genome and are a major source of individual variation (reviewed in 167). To date, more than 38,000 CNVs have been identified in the human genome, and some of the most exciting studies in the past year are those that tested for an association between CNVs and phenotype.

Multiple approaches have been used to assess CNV in the dog genome. The first two efforts, that of Chen and colleagues (29) and Nicholas et al. (107), both assessed multiple different breeds compared with the boxer. Chen and colleagues used a commercially developed whole genome aCGH array, whereas Nicholas et al. developed a custom aCGH array based on

segmental duplications predicted from the canine sequence. Chen identified 155 CNVs in nine dogs with an average size of approximately 300 kb and found multiple indications for breed or breed-group specificity. Nicholas's targeted approach enabled the development of a denser array that identified 3,583 CNVs in 678 unique regions from 17 domestic dogs and a wolf. The average size of the CNVs was 33 kb. Approximately 38%, however, could not be assigned to a chromosome using the current CanFam2 assembly. Both studies agree that larger numbers of dogs and markers would be needed to develop an accurate picture of CNV patterning in the domestic dog.

A study by J.D. Degenhardt, E. Karlins, A. Auton, T.C. Spady, P. Quignon, et al. (unpublished data) utilized much greater numbers of dogs. This study assessed CNVs in 781 dogs from 75 breeds at approximately 125,000 markers based on intensity data from the Affymetrix v.2 canine SNP chip. By increasing the number of dogs, the authors were able to identify 9,789 CNVs in 1,220 regions. Approximately 8% of the CNVs had been identified previously in one of the two earlier studies, whereas more than 90% were unique and 76% overlapped genes (J.D. Degenhardt, E. Karlins, A. Auton, T.C. Spady, P. Quignon, et al., unpublished data). Analysis of the SNPs surrounding the CNVs showed a marked decrease in LD compared with LD observed between SNPs. In addition, many of the CNVs were not shared by closely related breeds, suggesting that they are frequently recurring events, more likely to share identity by state rather than by descent.

Slippage

Arguably, the most interesting questions in canine research today are centered on an effort to understand the source of the phenotypic variation that characterizes breeds. The active SINE family may play a role in increasing the frequency of nonlethal mutations available for breeders to select on, as may a relaxation of selective pressures (33, 81). Fondon and colleagues, however, propose that an abundance of repetitive elements in developmental genes may provide the answer (43). They identified polymorphic repeats in the coding sequence of 36 developmental genes likely to be important in morphologic evolution and compared them with orthologous repeats in human genes. Indicated by expansion or contraction of the repeat, they found a significant increase in selection of divergence in 29 of the 36 dog genes examined in at least a subset of breeds. Variation in the number of unit repeats in the coding regions of the *Alx-4* (aristaless-like 4) and *Runx-2* (runt-related transcription factor 2) genes were specifically associated with significant differences in limb and skull morphology (43).

In a subsequent study, Laidlaw et al. sequenced 55 coding repeat regions in 42 species representing 10 major carnivore clades and found that dogs possess a genome-wide increase in the basal germ-line slippage mutation rate compared with other Carnivoran families and primates (85). In addition, when comparing orthologous microsatellite sequences in dog and human, they found that the reported increase in purity in dog repeats is a genome-wide phenomenon and is not specific to a few genes (85). This implies that the increase in slippage rates may have contributed to the malleability of the canine genome and, as such, could account for the high level of phenotypic variation in breeds.

MORPHOLOGY AND BREEDS

Identifying genes that are responsible for the morphologic features that define breeds is a major goal for many in the canine genomics community. Thus far, interest has focused on genes that control body size, leg length, and variations in coat. In each case, not only have the genetic underpinnings been unraveled, but contributions have been made to understanding mammalian developmental biology as well.

Body Size

The first efforts to find loci associated with body size were undertaken by Chase et al. in the Portuguese water dog (PWD) breed (27). These researchers sampled DNA and a set of five radiographs from over 500 dogs. From the radiographs they derived a set of 92 skeletal metrics that they used to establish phenotypes for a linkage study. Key to their success was a principal component analysis (PCA) used to identify groups of traits that were coregulated. They mapped several sets of traits, with the strongest result, PC1, describing overall body size. PC1 is now known through analyses of several datasets to be controlled by four to six loci (16, 71), the strongest of which was initially defined as a 4 Mb region on canine chromosome 15 (CFA15).

Sutter et al. followed up this preliminary observation by analyzing CFA15 in large and small PWDs and a multi-breed dataset of large and small breeds (143). Although the four million bases included several genes, the presence of a selective sweep in 14 small breeds highlighted *IGF1* as the causative gene. Interestingly, analysis of multiple small breeds, like the toy poodle and Pomeranian, revealed that most individuals were homozygous for an identical haplotype, suggesting that the critical mutation(s) likely occurred early in the domestication process (143). When large breeds, such as the St. Bernard and Newfoundland, were examined, two other haplotypes dominated, offering other routes for the *IGF1* gene to increase skeletal size.

The results of the *IGF1* study were exciting for several reasons. First, it demonstrated that the canine system was indeed sufficiently powerful to decipher the genetics of traits that have proven intractable in humans. Second, it suggested that dogs can bridge human studies, which are notoriously underpowered, and mouse studies, which often lack refined phenotypes. Finally, it demonstrated the power of the multi-breed approach (88, 110, 112) (Figure 5), which takes advantage of the fact that many mutations are likely to have occurred early in domestication, and are then propagated through the independent meiotic events leading to breed formation. This offers an efficient way to fine-map regions identified in GWAS studies. Other studies of both morphology and disease had made the latter point with regard to single gene traits (49, 73, 117), but this powerful demonstration of quantitative trait locus (QTL) fine mapping of a morphologic trait opened the door for scientists to study the genetics of literally any breed-defining trait.

In order to create a dataset that would allow us to tackle this problem on a large scale, approximately 900 dogs representing 80 breeds were genotyped using the Affymetrix v.2 canine SNP chip, generating a dataset of nearly 50,000 informative SNPs per dog (16). The number of lineages was maximized by genotyping a dozen unrelated dogs from each breed. The resulting data, termed CanMap, was analyzed to identify dozens of loci for morphologic traits, including body size, leg length and width, tail and ear position, skull shape, etc.

The Canine Coat

The genetics of coat color has been studied extensively in the dog (11, 31, 73, 76, 124, 136) and, more recently, the wolf (5). Although melanin production through the melanocortin 1 receptor (*MC1R*) controlled by agouti signal peptide (*ASIP*) has been well described in many species (69, 72, 142, 150), a recent canine study has identified a new gene in the color pathway. Black coat color in most dogs is not controlled by mutations in *ASIP* or *MC1R* (77, 78). Rather, dominant black is caused by a variant in β defensin 103 (*CBD103*), a protein previously associated with immune function (21). This finding further supports the role of melanocyte signaling in immunity and adds a new dimension to the study of coat color.

More recently, studies of canine coat length and texture, beginning with simple Mendelian traits segregating in single breeds, have been undertaken. Hillbertz et al. identified a 133 Kb

duplication on chromosome 18 that associated perfectly with the presence of the ridge of hair growing in the opposite direction along the spine of the Rhodesian ridgeback (62). In addition, Drögmüller and colleagues found a frameshift variant in the *FOXI3* gene that segregated with the hairless phenotype, also called ectodermal dysplasia (CED), in the Chinese crested breed (37). Both of these mutations have important health implications as dogs with the ridge are predisposed to a congenital abnormality called dermoid sinus, which is a neural tube defect, whereas the hairless mutation is homozygous lethal. These provide interesting examples of deleterious traits that have piggybacked with genes under selection for desirable traits. As canine researchers map additional traits under selection, the identification of other deleterious loci is sure to follow.

The most comprehensive study of canine coat structure has been undertaken by Cadieu et al., who did complimentary GWAS analyses in single breeds where specific coat types segregated and in the larger CanMap dataset, and then looked for overlapping peaks of association (20). They identified genes and variants controlling curl, length, and furnishings, which is a pattern of hair growth on the face and legs of some dogs typified by a mustache. Two of the three genes identified, fibroblast growth factor 5 (*FGF5*) and keratin 71 (*KRT71*), had been previously associated with hair growth in dog and mouse, respectively (65, 130).

However, the identification of r-spondin 2 (*RSPO2*), which is strongly associated with the furnishings growth pattern, was a surprise. A 167 bp insert in the 3′ untranslated region (UTR) of *RSPO2* was found in all dogs with furnishings in a dataset of over 1000 dogs of varying phenotypes. Although not previously associated with hair growth, *RSPO2* synergizes with Wnt to activate β-catenin (75), and Wnt signaling is required for establishment of hair follicles (6, 32). In addition, the Wnt/β-catenin pathway is involved in the development of hair-follicle tumors, or pilomatricomas (26). In dogs, these tumors occur most frequently in breeds with furnishings (100).

Examination of dogs with a variety of coat types demonstrated that variant alleles at these three genes create seven different coats and can explain 95% of the fur phenotypes observed in the nearly 1000 dogs studied (20) (Figure 6). Thus far, only a few breeds, such as the exceptionally long coated Afghan hound and the curly-coated retriever, are not explained by the three-gene model, suggesting that a small number of additional genes contributing to subtleties of fur growth remain to be found.

One of the advantages of conducting morphologic studies in dogs is that there is strong phenotypic uniformity within breeds. As a result, mapping can sometimes be done using breed average data presented through the registering kennel club as a phenotype (111). Jones et al. demonstrated this in an analysis of 148 breeds (71), identifying the same loci for body size and other morphologic features as had been published previously, but they also reported putative loci for newly considered traits such as longevity and behavior. This suggests that the dog system holds promise for understanding some of the most basic questions that occupy today's scientists (112).

CANINE BEHAVIOR

Three different aspects of behavior in dogs have attracted modern geneticists: behaviors associated with personality, such as loyalty and protectiveness; behavioral disorders, including rage and obsessive-compulsive disease (OCD); and breed-defining behaviors, including herding, drafting, and pointing (45, 109, 119). Little is known about the genetics of personality traits in dogs. The work of Hare et al. (52) establishes that dogs are able to relate to humans in ways unique from what even primates can achieve, but the underlying

genetics has yet to be tackled in a major way. Thus far, the most is known about anomalous behaviors, as they often mimic human disorders.

Behavioral Disorders

Dogs and humans share a number of behavioral disorders, including anxiety, rage, compulsive behavior, and attention deficit disorder. Many show evidence of heritable components through pronounced breed disposition (14, 108, 113). To date, candidate gene analyses based on human studies have been the primary route to finding mutations associated with canine behavior (57, 58, 145, 149). Recently, however, the first GWAS for a behavioral disorder in dogs was completed, identifying a region on CFA7 associated with a flank-sucking compulsive disorder in Doberman pinschers (36). No mutation has been identified to date, but the most promising candidate in the region is a neuronal adhesion protein *CDH2*.

Canine compulsive disorders mimic human obsessive-compulsive disorders at a pharmacologic and phenotypic level. For instance, they respond to treatment with clomipramine hydrochloride, a serotonin-reuptake inhibitor. One of the most interesting phenotypes described in the literature is that of tail chasing, observed primarily in the bull terrier (102). It will be interesting to see if GWAS identify the same locus in the bull terrier as the Doberman, or if other genes in the relevant pathways are found.

In addition to compulsive diseases, some effort has gone into the study of hyperactivity and aggressive behavior in dogs. For instance, an association between variants in the dopamine D4 receptor gene and impulsivity was reported in the German shepherd dog (59). Aggression has been studied in several breeds based on biochemical observations. Reisner et al. (128) reported decreased concentrations of a major metabolite of seratonin in cerebrospinal fluid of dominant aggressive dogs, and Badino et al. (8) found modifications of serotonergic receptor concentrations in the brains of aggressive dogs. In response, Van den Berg et al. (152) evaluated four serotonergic genes with respect to aggressive behavior in golden retrievers. Using mutation screens, linkage analysis, association studies, and quantitative genetic analysis, no obvious associations were found. However, in a study of Shiba Inu, Takeuchi et al. reported that polymorphisms in the *SLC1A2* gene were significantly associated with aggression towards strangers (145).

One interesting phenotype that has been well described is owner-directed aggression (127), which occurs in nearly half of all springer spaniels and at a lower frequency in related breeds like the English cocker spaniel (125). Manifesting as unprovoked biting or other threatening behaviors, owner-directed aggression is the major behavioral problem many experts report they encounter. Although training and environmental factors clearly play a role, the overrepresentation of some breeds with the phenotype suggests a clear rationale for a GWAS.

Breed-Associated Behaviors

Setting aside the issue of anomalous behaviors, studies show that there are both behavior and personality traits associated with specific breeds (18, 53, 54, 132, 146). Simply put, border collies do not herd sheep because they are raised on sheep farms; rather, they are raised on sheep farms because they herd. In addition pointers point, retrievers retrieve, and mastiffs guard, all because those traits are part of their breed expectations, meaning strong and continuous selection in the underlying breeding program (Figure 2). Although one recent study has suggested genomic regions where loci may exist that are associated with some of these behaviors (71), no genes have been identified in large part because of the difficulties associated with breaking these complex traits into quantifiable phenotypes.

A promising new approach derives from a recent study of performance-related traits in racing Alaskan sled dogs (68). Racing dogs are divided into distance and sprint categories. The latter perform short (<30 mile) runs, whereas the former race for hundreds of miles over multiple consecutive days, as in the renowned Idi-tarod race. Huson et al. studied the genomes of sprint and distance racing dogs and identified purebred signatures within these highly mixed populations associated with specific traits, such as endurance, work ethic, and speed (68). Follow-up GWAS studies are now in progress to identify the underlying genes. What is likely to emerge for each trait is a complex picture of multiple interacting genes. An understanding of how the network of genes functions will establish a new paradigm for studying behavioral genetics.

CANINE DISEASE MAPPING INFORMS HUMAN DISEASE

The study of complex human disorders has been stymied by the small size of human families for linkage studies, the limited size of case groups for GWAS, and the inability to accurately phenotype many complex diseases. Attempts to stratify human conditions based on their clinical presentation, response to treatment, or long-term outcomes, thus simplifying the underlying locus heterogeneity, have been only partially successful.

Genetic studies of canine disease have been of proven value in three major ways (138). First, because human and canine disorders are similar from a medical perspective, the identification of canine disease genes often offers unique opportunities to test new therapies. Second, canine genetic studies often identify genes or gene families that were not previously associated with disease. Finally, identification of disease genes in the dog has foretold new ways in which mammalian genomes can be perturbed to produce phenotypes of interest.

Many canine diseases are caused by mutations in the same genes as the corresponding diseases in humans. Canine X-linked hemophilia A is caused by a gene inversion in factor VIII, the same gene that causes the human disease (94). X-linked severe combined immune deficiency, observed in both basset hounds and humans, is caused by a 4 bp deletion in the *IL2-R gamma* gene in dogs, which produces a truncated protein, as does the human mutation (61). Several breeds of dog share a predisposition for adult-onset insulin-dependent diabetes, including the Samoyed, Tibetan terrier, and Cairn terrier, and alleles at the canine DRB and DQA loci are associated with the disease (24). Indeed, the canine DLA locus appears to be associated with several diseases, including early-onset systemic lupus erythematosus (SLE), as seen in the Nova Scotia duck tolling retriever, which is associated with variant alleles in DLA class II genes (163). By way of a final example, mutations in the *SLC3A1* gene cause type 1 cystinuria in humans and a similarly severe form of the disease in the Newfoundland (60).

Canine Disease Studies Offer Relevant Animal Models for Human Disease

Cancer and autoimmune disease in the dog often recapitulate human disorders so closely that they become models for the development of new therapies. Cancer generally occurs spontaneously in the dog, with a clinical presentation, histology, disease progression, and response to treatment similar to human, e.g., transitional cell carcinoma of the bladder, non-Hodgkin's lymphoma, chronic myelogenous leukemia, osteosarcoma, and melanoma (19, 48, 104, 115). In addition, several breeds exhibit a strong genetic predisposition for a particular cancer, suggesting a genetic component. For instance, Scottish and West Highland white terriers have a high incidence of transitional cell carcinoma of the bladder (48); the Scottish deerhound, Irish wolfhound, and Rottweiler frequently get osteosarcoma (138); and mammary cancer is often diagnosed in English springer spaniels (40).

In some cases, an understanding of the underlying genetics has begun, and it is likely that the dog condition will be useful for informing us about comparable human diseases. For instance, canine mammary cancer in English springer spaniels from Sweden shows an association with the *BRCA1* and *BRCA2* loci (129). Two loci for Addison's disease have been mapped in the Portuguese water dogs; one is in the DLA region, the other is near the immunosuppression locus *CTLA-4* (28).

In the case of Duchenne X-linked muscular dystrophy, the dog model, golden retriever muscular dystrophy (137), is so similar to the human disease that multiple labs are actively studying it. The disease is caused by loss of the dystrophin protein, a critical component of the dystrophin-glycoprotein complex (64). In dogs, the underlying mutation is in a consensus splice acceptor site, leading to exon 7 skipping, disruption of the open reading frame, and premature termination of translation (66, 137). In contrast to an existing mouse model, the affected dogs are analogous to humans displaying progressive muscle wasting, with degeneration and fibrosis. As a result, they are frequently used to test novel therapeutic interventions, often via bone marrow transplant, that may prove useful for human patients (reviewed in 160).

Canine Disease Genetics Highlights New Genes and Pathways

Retinitis pigmentosa, the leading cause of blindness in both purebred dogs and humans, is a constellation of diseases that in the dog is referred to as progressive retinal atrophy (PRA). Although multiple causative genes have been described in man and dog (reviewed in 122), many forms of the disease in both species remain unmapped. Of interest to researchers in both fields is the identification of causative genes in the canine that were not previously recognized as contributors to the human disease. Progressive rod-cone degeneration (prcd), a late onset form of the disease, affects multiple breeds, including poodles, cocker spaniels, and Labrador retrievers. A linked marker was found in 1997 (1), and the region was narrowed to 106 Kb through LD mapping in multiple breeds after several painstaking years (49) (Figure 5). The underlying canine mutation, which occurs in a novel 54–amino acid protein, was described shortly thereafter (165) and appears to be important for both canine and human disease.

Although useful for identifying specific disease genes, canine mapping studies are even more useful for revealing whole pathways relevant to a human condition. The best example is that of narcolepsy in the Doberman pinscher, which is caused by a fully penetrant autosomal recessive SINE insertion in the hypocretin/orexin receptor 2 gene (*HCRTR2*) (25, 70, 87). Human narcolepsy is rarely attributed to a mutation in the *HCRTR2* gene but is commonly caused by a decreased number of hypocretin cells. The hypocretin/orexin signaling pathway is implicated as the decreased cell number likely results from a disrupted hypocretin/orexin pathway (25). This was the first gene identified that conferred a risk for a sleep disorder, highlighting a pathway that had not previously been implicated in the studies of human sleep.

The same argument can be made regarding copper toxicosis, which was mapped in the Bedlington terrier breed (164), and is caused by a mutation in the *MURR1* gene, now called *COMMD1* (151). Although virtually nothing was known about the gene when it was linked to copper toxicosis in 2002, it is now recognized as a regulator of copper metabolism (34, 44).

Canine Mapping Studies Reveal New Ways In Which the Genome Can Be Altered

Finally, canine studies suggest new ways in which mutations can change phenotype. For instance, studies of autosomal recessive progressive myoclonic epilepsy (PME) in the

miniature wire-haired dachshund (MWHD) reveal a disease very similar to human Lafora disease, which is caused by mutations in the EPM2 genes: *EPM2a* and *EPM2b* on HSA 6q22–24 (140). Lohi et al. (92) mapped a locus for PME in the MWHD to CFA35 in a region syntenic to HSA 6p21–25. Sequencing of canine *EPM2b* revealed a dodecamer repeat at the 5′ end that normally exists in 2–3 copies. MWHD exhibiting PME, however, possess 19–26 copies of this repeat. Subsequent studies revealed a 14-copy repeat in an affected basset hound. The mutation apparently affects expression, as *EPM2b* mRNA levels in the skeletal muscle of affected dogs are expressed at levels 900 times lower than what is observed in normal canine skeletal muscle (92). Although expansions of trinucleotide repeats have been associated with human disease, this is the first example of a dodecamer repeat causing a disorder.

Even studies of morphology reveal new disease mechanisms. For example, Parker et al. recently showed that expression of a retrogene encoding a complete copy of the coding sequence of fibroblast growth factor 4 (*FGF4*) is strongly associated with chondrodysplasia, the short-legged phenotype that is a requirement for approximately 20 breeds including the dachshund, corgi, and basset hound (118). Interestingly, the retrogene has none of the original regulatory machinery, but genes surrounding it are expressed in fetal chondrocytes, as is the retrogene. Expressed retrogenes have been found in insects, but this is the first time, to our knowledge, that one has been identified that controls a major phenotype in a mammal. Not only is this gene now a candidate for cases of human asymmetrical dwarfism in which the phenotype is not explained by known genes, but the novel disease mechanism must now be considered in cases where sequencing of candidate genes does not reveal underlying mutations (118).

CONCLUSION

The American writer, Corey Ford, tells us that, "Properly trained, a man can be dog's best friend." If that is the case, then in the last several years, canine researchers have distinguished themselves. We have sequenced the genome of the dog (88) and found the mutations causing dozens of diseases, and, as a result, genetic tests are widely available for many diseases.

Consequently, we have come to recognize the enormous value of the canine system for understanding the human species. We have demonstrated that many of the genes causing dog disorders cause similar diseases in humans, begun to unravel the genetics of a multitude of complex traits, and discovered new ways in which the genome can be perturbed to cause changes in phenotype. At the most fundamental level, we have undertaken comparative genomics, discovering how subtle changes in the genome cause a wealth of changes in the species, offering a smorgasbord of variations for breeders to select from as they propagate existing breeds and develop still newer varieties. We now understand why big breeds are big and little ones are small, and we will, shortly, understand much more about the genetics of the canine form.

Our primary goals for the next five years are threefold. First, we will continue to disentangle the genetics of complex traits and use the canine system to understand the genetics of common diseases like heart disorders and diabetes. Second, there remains in the 400 or more dog breeds an extraordinary amount of untapped variation, simply because the phenotypic measures have not been developed to quantify it. As they become available, we can expect to see the canine genetic system applied to the study of everything from tail length to metabolic rate. Finally, there remain to be studied truly complex quantitative traits such as behavior and response to drug therapies. As we develop an ever more sophisticated

understanding of genetic variation in the canine genome, the genes controlling those traits will be found as well.

In the end, however, what will be the most difficult to understand is our own relationship with the dog. Unwavering loyalty, compassion, and blind adoration are not traits we can map with our genomic tools, and for now we must be content with that. In the words of American author Margery Facklam, "We give dogs time we can spare, space we can spare, and love we can spare. In return, dogs give us their all. It's the best deal man has ever made."

Acknowledgments

Glossary

Haplotype	combination of alleles at multiple loci on the same chromosome that segregate together
YBP	years before present
Mitochondrial DNA (mtDNA)	circular molecule of DNA contained within the mitochondria of the cell rather than the nucleus and inherited through the maternal lines
Allele	one specific variation at a site where there are two or more possibilities (polymorphic site)
Locus	a specified position in the genome
Single-nucleotide polymorphism (SNP)	one nucleotide position in a DNA sequence that has more than one possible base
Linkage disequilibrium (LD)	Nonrandom association of alleles from adjacent loci on a chromosome; caused by a variety of reasons and indicates reduction in recombination between the loci
Genome-wide association study (GWAS)	a scan of the entire genome using a large number of markers in multiple unrelated individuals designed to identify regions segregating with measurable traits
Genotype	the combination of two alleles carried by an individual at a particular locus
Short interspersed nuclear element (SINE)	a very common type of small (100–500 bp) retrotransposon
Long interspersed nuclear element (LINE)	a large retrotransposon that carries a promoter and codes for the proteins required to replicate and reinsert itself into the genome
Copy number variation (CNV)	section of genomic DNA that has undergone duplication or deletion
Phenotype	an observable and/or measurable trait

| **Principal component analysis (PCA)** | mathematical method for grouping correlated variables to create uncorrelated sets, reducing the complexity of a dataset while minimizing information loss |
| **Polymorphism** | variation in DNA sequence from a reference sequence; location in the DNA sequence where more than one allele exists in a population |

LITERATURE CITED

1. Acland GM, Ray K, Mellersh CS, Gu W, Langston AA, et al. Linkage analysis and comparative mapping of canine progressive rod-cone degeneration (prcd) establishes potential locus homology with retinitis pigmentosa (RP17) in humans. Proc Natl Acad Sci USA. 1998; 96:3048–53. [PubMed: 9501213]

2. Agnarsson I, Kuntner M, May-Collado LJ. Dogs, cats, and kin: a molecular species-level phylogeny of Carnivora. Mol Phylogenet Evol. 2009; 54:726–45. [PubMed: 19900567]

3. Akey JM, Ruhe AL, Akey DT, Wong AK, Connelly CF, et al. Tracking footprints of artificial selection in the dog genome. Proc Natl Acad Sci USA. 2010; 107:1160–65. [PubMed: 20080661]

4. American Kennel Club. Breed Registry Data Base. New York: AKC; 1999.

5. Anderson TM, vonHoldt BM, Candille SI, Musiani M, Greco C, et al. Molecular and evolutionary history of melanism in North American gray wolves. Science. 2009; 323:1339–43. [PubMed: 19197024]

6. Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. Dev Cell. 2002; 2:643–53. [PubMed: 12015971]

7. Awano T, Johnson GS, Wade CM, Katz ML, Johnson GC, et al. Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. Proc Natl Acad Sci USA. 2009; 106:2794–99. [PubMed: 19188595]

8. Badino P, Odore R, Osella MC, Bergamasco L, Francone P, et al. Modifications of serotonergic and adrenergic receptor concentrations in the brain of aggressive *Canis familiaris*. Comp Biochem Physiol A Mol Integr Physiol. 2004; 139:343–50. [PubMed: 15556390]

9. Baldeschi C, Gache Y, Rattenholl A, Bouille P, Danos O, et al. Genetic correction of canine dystrophic epidermolysis bullosa mediated by retroviral vectors. Hum Mol Genet. 2003; 12:1897–905. [PubMed: 12874109]

10. Benson KF, Li FQ, Person RE, Albani D, Duan Z, et al. Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. Nat Genet. 2003; 35:90–96. [PubMed: 12897784]

11. Berryere TG, Kerns JA, Barsh GS, Schmutz SM. Association of an Agouti allele with fawn or sable coat color in domestic dogs. Mamm Genome. 2005; 16:262–72. [PubMed: 15965787]

12. Bjornerfeldt S, Hailer F, Nord M, Vila C. Assortative mating and fragmentation within dog breeds. BMC Evol Biol. 2008; 8:28. [PubMed: 18226210]

13. Bjornerfeldt S, Webster MT, Vila C. Relaxation of selective constraint on dog mitochondrial DNA following domestication. Genome Res. 2006; 16:990–94. [PubMed: 16809672]

14. Blackshaw JK, Sutton RH, Boyhan MA. Tail chasing or circling behavior in dogs. Canine Practice. 1994; 19:7–11.

15. Boudreaux MK, Catalfamo JL. Molecular and genetic basis for thrombasthenic thrombopathia in otterhounds. Am J Vet Res. 2001; 62:1797–804. [PubMed: 11703027]

16. Boyko A, Quignon P, Lin L, Schoenebeck J, Degenhardt JD, et al. A simple genetic architecture underlies quantitative traits in dogs. PLoS Biol. 2010 In press.

17. Boyko AR, Boyko RH, Boyko CM, Parker HG, Castelhano M, et al. Complex population structure in African village dogs and its implications for inferring dog domestication history. Proc Natl Acad Sci USA. 2009; 106:13903–8. [PubMed: 19666600]

18. Bradshaw JW, Goodwin D. Determination of behavioural traits of pure-bred dogs using factor analysis and cluster analysis; a comparison of studies in the USA and UK. Res Vet Sci. 1999; 66:73–76. [PubMed: 10088716]

19. Breen M, Modiano JF. Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans: man and his best friend share more than companionship. Chromosome Res. 2008; 16:145–54. [PubMed: 18293109]

20. Cadieu E, Neff M, Quignon P, Walsh K, Chase K, et al. Coat variation in the domestic dog is governed by variants in three genes. Science. 2009; 326:150–53. [PubMed: 19713490]

21. Candille SI, Kaelin CB, Cattanach BM, Yu B, Thompson DA, et al. A β-defensin mutation causes black coat color in domestic dogs. Science. 2007; 318:1418–23. [PubMed: 17947548]

22. Capt A, Spirito F, Guaguere E, Spadafora A, Ortonne JP, Meneguzzi G. Inherited junctional epidermolysis bullosa in the German Pointer: establishment of a large animal model. J Invest Dermatol. 2005; 124:530–35. [PubMed: 15737193]

23. Casal ML, Scheidt JL, Rhodes JL, Henthorn PS, Werner P. Mutation identification in a canine model of X-linked ectodermal dysplasia. Mamm Genome. 2005; 16:524–31. [PubMed: 16151697]

24. Catchpole B, Kennedy LJ, Davison LJ, Ollier WE. Canine diabetes mellitus: from phenotype to genotype. J Small Anim Pract. 2008; 49:4–10. [PubMed: 17617163]

25. Chabas D, Taheri S, Renier C, Mignot E. The genetics of narcolepsy. Annu Rev Genomics Hum Genet. 2003; 4:459–83. [PubMed: 14527309]

26. Chan EF, Gat U, McNiff JM, Fuchs E. A common human skin tumour is caused by activating mutations in beta-catenin. Nat Genet. 1999; 21:410–13. [PubMed: 10192393]

27. Chase K, Carrier DR, Adler FR, Jarvik T, Ostrander EA, et al. Genetic basis for systems of skeletal quantitative traits: principal component analysis of the canid skeleton. Proc Natl Acad Sci USA. 2002; 99:9930–35. [PubMed: 12114542]

28. Chase K, Sargan D, Miller K, Ostrander EA, Lark KG. Understanding the genetics of autoimmune disease: two loci that regulate late onset Addison's disease in Portuguese Water Dogs. Int J Immunogenet. 2006; 33:179–84. [PubMed: 16712648]

29. Chen WK, Swartz JD, Rush LJ, Alvarez CE. Mapping DNA structural variation in dogs. Genome Res. 2009; 19:500–9. [PubMed: 19015322]

30. Chen X, Johnson GS, Schnabel RD, Taylor JF, Johnson GC, et al. A neonatal encephalopathy with seizures in standard poodle dogs with a missense mutation in the canine ortholog of ATF2. Neurogenetics. 2008; 9:41–49. [PubMed: 18074159]

31. Clark LA, Wahl JM, Rees CA, Murphy KE. Retrotransposon insertion in SILV is responsible for merle patterning of the domestic dog. Proc Natl Acad Sci USA. 2006; 103:1376–81. [PubMed: 16407134]

32. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006; 127:469–80. [PubMed: 17081971]

33. Cruz F, Vila C, Webster MT. The legacy of domestication: accumulation of deleterious mutations in the dog genome. Mol Biol Evol. 2008; 25:2331–36. [PubMed: 18689870]

34. de Bie P, van de Sluis B, Klomp L, Wijmenga C. The many faces of the copper metabolism protein MURR1/COMMD1. J Hered. 2005; 96:803–11. [PubMed: 16267171]

35. Derrien T, Theze J, Vaysse A, Andre C, Ostrander EA, et al. Revisiting the missing protein-coding gene catalog of the domestic dog. BMC Genomics. 2009; 10:62. [PubMed: 19193219]

36. Dodman NH, Karlsson EK, Moon-Fanelli A, Galdzicka M, Perloski M, et al. A canine chromosome 7 locus confers compulsive disorder susceptibility. Mol Psychiatry. 2010; 15:8–10. [PubMed: 20029408]

37. Drogemuller C, Karlsson EK, Hytonen MK, Perloski M, Dolf G, et al. A mutation in hairless dogs implicates FOXI3 in ectodermal development. Science. 2008; 321:1462. [PubMed: 18787161]

38. Drogemuller C, Philipp U, Haase B, Gunzel-Apel AR, Leeb T. A noncoding melanophilin gene (MLPH) SNP at the splice donor of exon 1 represents a candidate causal mutation for coat color dilution in dogs. J Hered. 2007; 98:468–73. [PubMed: 17519392]

39. Eizirik E, Murphy WJ, Koepfli KP, Johnson WE, Dragoo JW, et al. Pattern and timing of diversification of the mammalian order Carnivora inferred from multiple nuclear gene sequences. Mol Phylogenet Evol. 2010; 56(1):49–63. [PubMed: 20138220]

40. Engenvall A, Bonnett BN, Ohagen P, Olson P, Hedhammer A, von Euler H. Incidence of and survival after mammary tumors in a population of over 80,000 insured female dogs in Sweden from 1995 to 2002. Prev Vet Med. 2005; 69:109–27. [PubMed: 15899300]

41. Evans JP, Brinkhous KM, Brayer GD, Reisner HM, High KA. Canine hemophilia B resulting from a point mutation with unusual consequences. Proc Natl Acad Sci USA. 1989; 86:10095–99. [PubMed: 2481310]

42. Everts RE, Rothuizen J, van Oost BA. Identification of a premature stop codon in the melanocyte-stimulating hormone receptor gene (MC1R) in labrador and golden retrievers with yellow coat colour. Anim Genet. 2000; 31:194–99. [PubMed: 10895310]

43. Fondon JW 3rd, Garner HR. Molecular origins of rapid and continuous morphological evolution. Proc Natl Acad Sci USA. 2004; 101:18058–63. [PubMed: 15596718]

44. Forman OP, Boursnell ME, Dunmore BJ, Stendall N, Van Den Sluis B, et al. Characterization of the COMMD1 (MURR1) mutation causing copper toxicosis in Bedlington terriers. Anim Genet. 2005; 36:497–501. [PubMed: 16293123]

45. Galibert F, Andre C. The canine genome: alternative model for the functional analysis of mammalian genes. Bull Acad Natl Med. 2002; 186:1489–99. discussion 99–502. [PubMed: 12669364]

46. Garcia M. Ichnologie generale de la grotte chauvet. Bull Soc Prehistoique Fr. 2005; 102:102–8.

47. Germonpre M, Sablin MV, Stevens RE, Hedges REM, Hofreiter M, et al. Fossil dogs and wolves from Palaeolithic sites in Belgium, the Ukraine and Russia: osteometry, ancient DNA and stable isotopes. J Archaeol Sci. 2009; 36:473–90.

48. Glickman LT, Raghavan M, Knapp DW, Bonney PL, Dawson MH. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. J Am Vet Med Assoc. 2004; 224:1290–97. [PubMed: 15112777]

49. Goldstein O, Zangerl B, Pearce-Kelling S, Sidjanin DJ, Kijas JW, et al. Linkage disequilibrium mapping in domestic dog breeds narrows the progressive rod-cone degeneration interval and identifies ancestral disease-transmitting chromosome. Genomics. 2006; 88:541–50. [PubMed: 16859891]

50. Gray MM, Granka JM, Bustamante CD, Sutter NB, Boyko AR, et al. Linkage disequilibrium and demographic history of wild and domestic canids. Genetics. 2009; 181:1493–505. [PubMed: 19189949]

51. Gray MM, Sutter NB, Ostrander EA, Wayne RK. The IGF1 small dog haplotype is derived from Middle Eastern gray wolves. BMC Biol. 2010; 8:16. [PubMed: 20181231]

52. Hare B, Brown M, Williamson C, Tomasello M. The domestication of social cognition in dogs. Science. 2002; 298:1634–36. [PubMed: 12446914]

53. Hart, BL.; Hart, L. Breed-specific profiles of canine (*Canis familiaris*) behavior. In: Mills, D.; Levine, E.; Landsberg, G.; Horwitz, D.; Duxbury, M., et al., editors. Current Issues and Research in Veterinary Behavioral Medicine. West Lafayette, IN: Purdue University Press; 2005. p. 107-12.

54. Hart BL, Miller MF. Behavioral profiles of dog breeds. J Am Vet Med Assoc. 1985; 186:1175–80. [PubMed: 3839221]

55. He Q, Fyfe JC, Schaffer AA, Kilkenney A, Werner P, et al. Canine Imerslund-Grasbeck syndrome maps to a region orthologous to HSA14q. Mamm Genome. 2003; 14:758–64. [PubMed: 14722725]

56. He Q, Madsen M, Kilkenney A, Gregory B, Christensen EI, et al. Amnionless function is required for cubilin brush-border expression and intrinsic factor-cobalamin (vitamin B12) absorption in vivo. Blood. 2005; 106:1447–53. [PubMed: 15845892]

57. Hejjas K, Kubinyi E, Ronai Z, Szekely A, Vas J, et al. Molecular and behavioral analysis of the intron 2 repeat polymorphism in the canine dopamine D4 receptor gene. Genes Brain Behav. 2009; 8:330–36. [PubMed: 19382953]

58. Hejjas K, Vas J, Kubinyi E, Sasvari-Szekely M, Miklosi A, Ronai Z. Novel repeat polymorphisms of the dopaminergic neurotransmitter genes among dogs and wolves. Mamm Genome. 2007; 18:871–79. [PubMed: 18049838]

59. Hejjas K, Vas J, Topal J, Szantai E, Ronai Z, et al. Association of polymorphisms in the dopamine D4 receptor gene and the activity-impulsivity endophenotype in dogs. Anim Genet. 2007; 38:629–33. [PubMed: 17986156]

60. Henthorn PS, Liu J, Gidalevich T, Fang J, Casal ML, et al. Canine cystinuria: polymorphism in the canine SLC3A1 gene and identification of a nonsense mutation in cystinuric Newfoundland dogs. Hum Genet. 2000; 107:295–303. [PubMed: 11129328]

61. Henthorn PS, Somberg RL, Fimiani VM, Puck JM, Patterson DF, Felsburg PJ. IL-2R gamma gene microdeletion demonstrates that canine X-linked severe combined immunodeficiency is a homologue of the human disease. Genomics. 1994; 23:69–74. [PubMed: 7829104]

62. Hillbertz NH, Andersson G. Autosomal dominant mutation causing the dorsal ridge predisposes for dermoid sinus in Rhodesian ridgeback dogs. J Small Anim Pract. 2006; 47:184–88. [PubMed: 16573760]

63. Hitte C, Madeoy J, Kirkness EF, Priat C, Lorentzen TD, et al. Facilitating genome navigation: survey sequencing and dense radiation-hybrid gene mapping. Nat Rev Genet. 2005; 6:643–48. [PubMed: 16012527]

64. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell. 1987; 51:919–28. [PubMed: 3319190]

65. Housley DJ, Venta PJ. The long and the short of it: evidence that FGF5 is a major determinant of canine "hair"-itability. Anim Genet. 2006; 37:309–15. [PubMed: 16879338]

66. Howell JM, Fletcher S, Kakulas BA, O'Hara M, Lochmuller H, Karpati G. Use of the dog model for Duchenne muscular dystrophy in gene therapy trials. Neuromuscul Disord. 1997; 7:325–28. [PubMed: 9267846]

67. Hungs M, Fan J, Lin L, Lin X, Maki RA, Mignot E. Identification and functional analysis of mutations in the hypocretin (orexin) genes of narcoleptic canines. Genome Res. 2001; 11:531–39. [PubMed: 11282968]

68. Huson HJ, Parker HG, Runstadler J, Ostrander EA. A genetic dissection of breed composition and performance enhancement in the Alaskan sled dog. BMC Genetics. 2010; 11:62. [PubMed: 20609214]

69. Jackson IJ. Molecular and developmental genetics of mouse coat color. Annu Rev Genet. 1994; 28:189–217. [PubMed: 7893123]

70. John J, Wu MF, Maidment NT, Lam HA, Boehmer LN, et al. Developmental changes in CSF hypocretin-1 (orexin-A) levels in normal and genetically narcoleptic doberman pinschers. J Physiol. 2004; 560:587–92. [PubMed: 15308685]

71. Jones P, Chase K, Martin A, Ostrander EA, Lark KG. Single-nucleotide-polymorphism-based association mapping of dog stereotypes. Genetics. 2008; 179:1033–44. [PubMed: 18505865]

72. Kanetsky PA, Swoyer J, Panossian S, Holmes R, Guerry D, Rebbeck TR. A polymorphism in the agouti signaling protein gene is associated with human pigmentation. Am J Hum Genet. 2002; 70:770–75. [PubMed: 11833005]

73. Karlsson EK, Baranowska I, Wade CM, Salmon Hillbertz NH, Zody MC, et al. Efficient mapping of mendelian traits in dogs through genome-wide association. Nat Genet. 2007; 39:1321–28. [PubMed: 17906626]

74. Katz ML, Khan S, Awano T, Shahid SA, Siakotos AN, Johnson GS. A mutation in the CLN8 gene in English Setter dogs with neuronal ceroid-lipofuscinosis. Biochem Biophys Res Commun. 2005; 327:541–47. [PubMed: 15629147]

75. Kazanskaya O, Glinka A, del Barco Barrantes I, Stannek P, Niehrs C, Wu W. R-Spondin2 is a secreted activator of Wnt/beta-catenin signaling and is required for xenopus myogenesis. Dev Cell. 2004; 7:525–34. [PubMed: 15469841]

76. Kerns J, Cargill E, Clark LA, Candille S, Berryere T, et al. Linkage and segregation analysis of black and brindle coat color in domestic dogs. Genetics. 2007; 176:1679–89. [PubMed: 17483404]

77. Kerns JA, Newton J, Berryere TG, Rubin EM, Cheng JF, et al. Characterization of the dog Agouti gene and a nonagoutimutation in German Shepherd Dogs. Mamm Genome. 2004; 15:798–808. [PubMed: 15520882]

78. Kerns JA, Olivier M, Lust G, Barsh GS. Exclusion of melanocortin-1 receptor (mc1r) and agouti as candidates for dominant black in dogs. J Hered. 2003; 94:75–79. [PubMed: 12692166]

79. Kijas J, Cideciyan A, Aleman T, Pianta M, Pearce-Kelling S, et al. Naturally-occurring *rhodopsin* mutation in the dog causes retinal dysfunction and degeneration mimicking human dominant retinitis pigmentosa. Proc Natl Acad Sci USA. 2002; 99:6328–33. [PubMed: 11972042]

80. Kijas JM, Bauer TR Jr, Gafvert S, Marklund S, Trowald-Wigh G, et al. A missense mutation in the beta-2 integrin gene (ITGB2) causes canine leukocyte adhesion deficiency. Genomics. 1999; 61:101–7. [PubMed: 10512685]

81. Kirkness, EF. SINEs of canine genomic diversity. In: Ostrander, EA.; Giger, U.; Lindblad-Toh, K., editors. The Dog and Its Genome. Cold Spring Harbor, NY: Cold Spring Harbor Press; 2005. p. 209-19.

82. Kirkness EF, Bafna V, Halpern AL, Levy S, Remington K, et al. The dog genome: survey sequencing and comparative analysis. Science. 2003; 301:1898–903. [PubMed: 14512627]

83. Kramer JW, Venta PJ, Klein SR, Cao Y, Schall WD, Yuzbasiyan-Gurkan V. A von Willebrand's factor genomic nucleotide variant and polymerase chain reaction diagnostic test associated with inheritable type-2 von Willebrand's disease in a line of German shorthaired pointer dogs. Vet Pathol. 2004; 41:221–28. [PubMed: 15133170]

84. Kukekova AV, Goldstein O, Johnson JL, Richardson MA, Pearce-Kelling SE, et al. Canine RD3 mutation establishes rod-cone dysplasia type 2 (rcd2) as ortholog of human and murine rd3. Mamm Genome. 2009; 20:109–23. [PubMed: 19130129]

85. Laidlaw J, Gelfand Y, Ng KW, Garner HR, Ranganathan R, et al. Elevated basal slippage mutation rates among the Canidae. J Hered. 2007; 98:452–60. [PubMed: 17437958]

86. Leonard JA, Wayne RK, Wheeler J, Valadez R, Guillen S, Vila C. Ancient DNA evidence for Old World origin of New World dogs. Science. 2002; 298:1613–16. [PubMed: 12446908]

87. Lin L, Faraco J, Li R, Kadotani H, Rogers W, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell. 1999; 98:365–76. [PubMed: 10458611]

88. Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 2005; 438:803–19. [PubMed: 16341006]

89. Lingaas F, Aarskaug T, Sletten M, Bjerkas I, Grimholt U, et al. Genetic markers linked to neuronal ceroid lipofuscinosis in English setter dogs. Anim Genet. 1998; 29:371–76. [PubMed: 9800325]

90. Lingaas F, Comstock KE, Kirkness EF, Sorensen A, Aarskaug T, et al. A mutation in the canine BHD gene is associated with hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis in the German Shepherd dog. Hum Mol Genet. 2003; 12:3043–53. [PubMed: 14532326]

91. Lipscomb DL, Bourne C, Boudreaux MK. Two genetic defects in alphaIIb are associated with type I Glanzmann's thrombasthenia in a Great Pyrenees dog: a 14-base insertion in exon 13 and a splicing defect of intron 13. Vet Pathol. 2000; 37:581–88. [PubMed: 11105947]

92. Lohi H, Young EJ, Fitzmaurice SN, Rusbridge C, Chan EM, et al. Expanded repeat in canine epilepsy. Science. 2005; 307:81. [PubMed: 15637270]

93. Lowe JK, Kukekova AV, Kirkness EF, Langlois MC, Aguirre GD, et al. Linkage mapping of the primary disease locus for Collie eye anomaly. Genomics. 2003; 82:86–95. [PubMed: 12809679]

94. Lozier JN, Dutra A, Pak E, Zhou N, Zheng Z, et al. The Chapel Hill hemophilia A dog colony exhibits a factor VIII gene inversion. Proc Natl Acad Sci USA. 2002; 99:12991–96. [PubMed: 12242334]

95. Malmstrom H, Vila C, Gilbert MT, Stora J, Willerslev E, et al. Barking up the wrong tree: modern northern European dogs fail to explain their origin. BMC Evol Biol. 2008; 8:71. [PubMed: 18307773]

96. Mamedov IZ, Arzumanyan ES, Amosova AL, Lebedev YB, Sverdlov ED. Whole-genome experimental identification of insertion/deletion polymorphisms of interspersed repeats by a new general approach. Nucleic Acids Res. 2005; 33:e16. [PubMed: 15673711]

97. Mealey KL, Bentjen SA, Gay JM, Cantor GH. Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. Pharmacogenetics. 2001; 11:727–33. [PubMed: 11692082]

98. Mellersh CS, Graves KT, McLaughlin B, Ennis RB, Pettitt L, et al. Mutation in HSF4 associated with early but not late-onset hereditary cataract in the Boston Terrier. J Hered. 2007; 98:531–33. [PubMed: 17611257]

99. Mellersh CS, Pettitt L, Forman OP, Vaudin M, Barnett KC. Identification of mutations in HSF4 in dogs of three different breeds with hereditary cataracts. Vet Ophthalmol. 2006; 9:369–78. [PubMed: 16939467]

100. Meuten, DJ., editor. Tumors in Domestic Animals. Ames, Iowa: Blackwell Publ; 2002. p. 788

101. Minnick MF, Stillwell LC, Heineman JM, Stiegler GL. A highly repetitive DNA sequence possibly unique to canids. Gene. 1992; 110:235–38. [PubMed: 1537560]

102. Moon-Fanelli AA, Dodman NH. Description and development of compulsive tail chasing in terriers and response to clomipramine treatment. J Am Vet Med Assoc. 1998; 212:1252–57. [PubMed: 9569164]

103. Mosher DS, Quignon P, Bustamante CD, Sutter NB, Parker HG, et al. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. PLoS Genet. 2007; 3:e79. [PubMed: 17530926]

104. Mueller F, Fuchs B, Kaser-Hotz B. Comparative biology of human and canine osteosarcoma. Anticancer Res. 2007; 27:155–64. [PubMed: 17352227]

105. Nadon NL, Duncan ID, Hudson LD. A point mutation in the proteolipid protein gene of the "shaking pup" interrupts oligodendrocyte development. Development. 1990; 110:529–37. [PubMed: 1723945]

106. Newton JM, Wilkie AL, He L, Jordan SA, Metallinos DL, et al. Melanocortin 1 receptor variation in the domestic dog. Mamm Genome. 2000; 11:24–30. [PubMed: 10602988]

107. Nicholas TJ, Cheng Z, Ventura M, Mealey K, Eichler EE, Akey JM. The genomic architecture of segmental duplications and associated copy number variants in dogs. Genome Res. 2009; 19:491–99. [PubMed: 19129542]

108. Niimi Y, Inoue-Murayama M, Murayama Y, Ito S, Iwasaki T. Allelic variation of the D4 dopamine receptor polymorphic region in two dog breeds, golden retriever and shiba. J Vet Med Sci. 1999; 61:1281–86. [PubMed: 10651047]

109. Ostrander EA, Giniger E. Semper fidelis: what man's best friend can teach us about human biology and disease. Am J Hum Genet. 1997; 61:475–80. [PubMed: 9326310]

110. Ostrander EA, Kruglyak L. Unleashing the canine genome. Genome Res. 2000; 10:1271–74. [PubMed: 10984444]

111. Ostrander, EA.; Lindblad-Toh, K.; Giger, U. The Dog and Its Genome. Cold Spring Harbor: Cold Spring Harbor Press; 2006. p. 584

112. Ostrander EA, Wayne RK. The canine genome. Genome Res. 2005; 15:1706–16. [PubMed: 16339369]

113. Overall KL. Natural animal models of human psychiatric conditions: assessment of mechanism and validity. Prog Neuropsychopharmacol Biol Psychiatry. 2000; 24:727–76. [PubMed: 11191711]

114. Pang JF, Kluetsch C, Zou XJ, Zhang AB, Luo LY, et al. mtDNA data indicate a single origin for dogs south of Yangtze River, less than 16,300 years ago, from numerous wolves. Mol Biol Evol. 2009; 26:2849–64. [PubMed: 19723671]

115. Paoloni MC, Khanna C. Comparative oncology today. Vet Clin North Am Small Anim Pract. 2007; 37:1023–32. v. [PubMed: 17950880]

116. Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, et al. Genetic structure of the purebred domestic dog. Science. 2004; 304:1160–64. [PubMed: 15155949]

117. Parker HG, Kukekova AV, Akey DT, Goldstein O, Kirkness EF, et al. Breed relationships facilitate fine-mapping studies: a 7.8-kb deletion cosegregates with Collie eye anomaly across multiple dog breeds. Genome Res. 2007; 17:1562–71. [PubMed: 17916641]

118. Parker HG, VonHoldt BM, Quignon P, Margulies EH, Shao S, et al. An expressed fgf4 retrogene is associated with breed-defining chondrodysplasia in domestic dogs. Science. 2009; 325:995–98. [PubMed: 19608863]

119. Patterson D. Companion animal medicine in the age of medical genetics. J Vet Internal Med. 2000; 14:1–9. [PubMed: 10668810]

120. Patterson EE, Minor KM, Tchernatynskaia AV, Taylor SM, Shelton GD, et al. A canine DNM1 mutation is highly associated with the syndrome of exercise-induced collapse. Nat Genet. 2008; 40:1235–39. [PubMed: 18806795]

121. Pele M, Tiret L, Kessler JL, Blot S, Panthier JJ. SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. Hum Mol Genet. 2005; 14:1417–27. [PubMed: 15829503]

122. Petersen-Jones SM. A review of research to elucidate the causes of the generalized progressive retinal atrophies [see comments]. Vet J. 1998; 155:5–18. [PubMed: 9455155]

123. Petersen-Jones SM, Entz DD, Sargan DR. cGMP phosphodiesterase-alpha mutation causes progressive retinal atrophy in the Cardigan Welsh corgi dog. Invest Ophthalmol Vis Sci. 1999; 40:1637–44. [PubMed: 10393029]

124. Philipp U, Hamann H, Mecklenburg L, Nishino S, Mignot E, et al. Polymorphisms within the canine MLPH gene are associated with dilute coat color in dogs. BMC Genet. 2005; 6:34. [PubMed: 15960853]

125. Podberscek AL, Serpell JA. Aggressive behaviour in English cocker spaniels and the personality of their owners. Vet Rec. 1997; 141:73–76. [PubMed: 9257436]

126. Quignon P, Herbin L, Cadieu E, Kirkness EF, Hedan B, et al. Canine population structure: assessment and impact of intra-breed stratification on SNP-based association studies. PLoS ONE. 2007; 2:e1324. [PubMed: 18091995]

127. Reisner IR, Houpt KA, Shofer FS. National survey of owner-directed aggression in English springer spaniels. J Am Vet Med Assoc. 2005; 227:1594–603. [PubMed: 16313036]

128. Reisner IR, Mann JJ, Stanley M, Huang YY, Houpt KA. Comparison of cerebrospinal fluid monoamine metabolite levels in dominant-aggressive and non-aggressive dogs. Brain Res. 1996; 714:57–64. [PubMed: 8861609]

129. Rivera P, Melin M, Biagi T, Fall T, Haggstrom J, et al. Mammary tumor development in dogs is associated with BRCA1 and BRCA2. Cancer Res. 2009; 69:8770–74. [PubMed: 19887619]

130. Runkel F, Klaften M, Koch K, Bohnert V, Bussow H, et al. Morphologic and molecular characterization of two novel Krt71 (Krt2–6g) mutations: Krt71rco12 and Krt71rco13. Mamm Genome. 2006; 17:1172–82. [PubMed: 17143583]

131. Sablin MV, Khlopachev GA. The earliest ice age dogs: evidence from eliseevichi 1. Curr Anthropol. 2002; 43:795–98.

132. Saetre P, Strandberg E, Sundgren PE, Pettersson U, Jazin E, Bergstrom TF. The genetic contribution to canine personality. Genes Brain Behav. 2006; 5:240–48. [PubMed: 16594977]

133. Salmon Hillbertz NHC, Isaksson M, Karlsson EK, Hellmen E, Pielberg GR, et al. Duplication of FGF3, FGF4, FGF19 and ORAOV1 causes hair ridge and predisposition to dermoid sinus in Ridgeback dogs. Nat Genet. 2007; 39:1318–20. [PubMed: 17906623]

134. Savolainen P, Zhang YP, Luo J, Lundeberg J, Leitner T. Genetic evidence for an East Asian origin of domestic dogs. Science. 2002; 298:1610–13. [PubMed: 12446907]

135. Schmutz SM, Berryere TG, Ellinwood NM, Kerns JA, Barsh GS. MC1R studies in dogs with melanistic mask or brindle patterns. J Hered. 2003; 94:69–73. [PubMed: 12692165]

136. Schmutz SM, Berryere TG, Goldfinch AD. TYRP1 and MC1R genotypes and their effects on coat color in dogs. Mamm Genome. 2002; 13:380–87. [PubMed: 12140685]

137. Sharp NJ, Kornegay JN, Van Camp SD, Herbstreith MH, Secore SL, et al. An error in dystrophin mRNA processing in golden retriever muscular dystrophy, an animal homologue of Duchenne muscular dystrophy. Genomics. 1992; 13:115–21. [PubMed: 1577476]

138. Shearin AL, Ostrander EA. Leading the way: canine models of genomics and disease. Dis Model Mech. 2010; 3:27–34. [PubMed: 20075379]

139. Sidjanin DJ, Lowe JK, McElwee JL, Milne BS, Phippen TM, et al. Canine CNGB3 mutations establish cone degeneration as orthologous to the human achromatopsia locus ACHM3. Hum Mol Genet. 2002; 11:1823–33. [PubMed: 12140185]

140. Singh S, Ganesh S. Lafora progressive myoclonus epilepsy: a meta-analysis of reported mutations in the first decade following the discovery of the EPM2A and NHLRC1 genes. Hum Mutat. 2009; 30:715–23. [PubMed: 19267391]

141. Suber ML, Pittler SJ, Qin N, Wright GC, Holcombe V, et al. Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta-subunit gene. Proc Natl Acad Sci USA. 1993; 90:3968–72. [PubMed: 8387203]

142. Sulem P, Gudbjartsson DF, Stacey SN, Helgason A, Rafnar T, et al. Genetic determinants of hair, eye and skin pigmentation in Europeans. Nat Genet. 2007; 39:1443–52. [PubMed: 17952075]

143. Sutter NB, Bustamante CD, Chase K, Gray MM, Zhao K, et al. A single IGF1 allele is a major determinant of small size in dogs. Science. 2007; 316:112–15. [PubMed: 17412960]

144. Sutter NB, Eberle MA, Parker HG, Pullar BJ, Kirkness EF, et al. Extensive and breed-specific linkage disequilibrium in *Canis familiaris*. Genome Res. 2004; 14:2388–96. [PubMed: 15545498]

145. Takeuchi Y, Kaneko F, Hashizume C, Masuda K, Ogata N, et al. Association analysis between canine behavioural traits and genetic polymorphisms in the Shiba Inu breed. Anim Genet. 2009; 40:616–22. [PubMed: 19397510]

146. Takeuchi Y, Mori Y. A comparison of the behavioral profiles of purebred dogs in Japan to profiles of those in the United States and the United Kingdom. J Vet Med Sci/Japanese Soc Vet Sci. 2006; 68:789–96.

147. Tchernov E, Valla FF. Two new dogs, and other Natufian dogs, from the Southern Levant. J Archaeol Sci. 1996; 24:65–95.

148. Tiret L, Blot S, Kessler JL, Gaillot H, Breen M, Panthier JJ. The cnm locus, a canine homologue of human autosomal forms of centronuclear myopathy, maps to chromosome 2. Hum Genet. 2003; 113:297–306. [PubMed: 12884002]

149. Vage J, Wade C, Biagi T, Fatjo J, Amat M, et al. Association of dopamine- and serotonin-related genes with canine aggression. Genes Brain Behav. 2010; 9(4):372–78. [PubMed: 20132314]

150. Valverde P, Healy E, Jackson I, Rees JL, Thody AJ. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nat Genet. 1995; 11:328–30. [PubMed: 7581459]

151. van De Sluis B, Rothuizen J, Pearson PL, van Oost BA, Wijmenga C. Identification of a new copper metabolism gene by positional cloning in a purebred dog population. Hum Mol Genet. 2002; 11:165–73. [PubMed: 11809725]

152. Van Den Berg L, Vos-Loohuis M, Schilder MB, van Oost BA, Hazewinkel HA, et al. Evaluation of the serotonergic genes htr1A, htr1B, htr2A, and slc6a4 in aggressive behavior of golden retriever dogs. Behav Genet. 2008; 38:55–66. [PubMed: 18066658]

153. Venta PJ, Li J, Yuzbasiyan-Gurkan V, Brewer GJ, Schall WD. Mutation causing von Willebrand's disease in Scottish terriers. J Vet Intern Med. 2000; 14:10–19. [PubMed: 10668811]

154. Verginelli F, Capelli C, Coia V, Musiani M, Falchetti M, et al. Mitochondrial DNA from prehistoric canids highlights relationships between dogs and Southeast European wolves. Mol Biol Evol. 2005; 22:2541–51. [PubMed: 16120801]

155. Veske A, Nilsson SE, Narfstrom K, Gal A. Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. Genomics. 1999; 57:57–61. [PubMed: 10191083]

156. Vila C, Savolainen P, Maldonado JE, Amorim IR, Rice JE, et al. Multiple and ancient origins of the domestic dog. Science. 1997; 276:1687–89. [PubMed: 9180076]

157. Vila C, Seddon J, Ellegren H. Genes of domestic mammals augmented by backcrossing with wild ancestors. Trends Genet. 2005; 21:214–18. [PubMed: 15797616]

158. Vonholdt BM, Pollinger JP, Lohmueller KE, Han E, Parker HG, et al. Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. Nature. 2010; 464:898–902. [PubMed: 20237475]

159. Walmsley GL, Arechavala-Gomeza V, Fernandez-Fuente M, Burke MM, Nagel N, et al. A duchenne muscular dystrophy gene hot spot mutation in dystrophin-deficient cavalier king charles spaniels is amenable to exon 51 skipping. PLoS One. 2010; 5:e8647. [PubMed: 20072625]

160. Wang Z, Chamberlain JS, Tapscott SJ, Storb R. Gene therapy in large animal models of muscular dystrophy. ILAR J. 2009; 50:187–98. [PubMed: 19293461]

161. Wayne RK, Ostrander EA. Lessons learned from the dog genome. Trends Genet. 2007; 23:557–67. [PubMed: 17963975]

162. Wiik AC, Wade C, Biagi T, Ropstad EO, Bjerkas E, et al. A deletion in nephronophthisis 4 (NPHP4) is associated with recessive cone-rod dystrophy in standard wire-haired dachshund. Genome Res. 2008; 18:1415–21. [PubMed: 18687878]

163. Wilbe M, Jokinen P, Hermanrud C, Kennedy LJ, Strandberg E, et al. MHC class II polymorphism is associated with a canine SLE-related disease complex. Immunogenetics. 2009; 61:557–64. [PubMed: 19636550]

164. Yuzbasiyan-Gurkan V, Blanton SH, Cao V, Ferguson P, Li J, et al. Linkage of a microsatellite marker to the canine copper toxicosis locus in Bedlington terriers. Am J Vet Res. 1997; 58:23–27. [PubMed: 8989491]

165. Zangerl B, Goldstein O, Philp AR, Lindauer SJ, Pearce-Kelling SE, et al. Identical mutation in a novel retinal gene causes progressive rod-cone degeneration in dogs and retinitis pigmentosa in humans. Genomics. 2006; 26:26.

166. Zanna G, Fondevila D, Bardagi M, Docampo MJ, Bassols A, Ferrer L. Cutaneous mucinosis in shar-pei dogs is due to hyaluronic acid deposition and is associated with high levels of hyaluronic acid in serum. Vet Dermatol. 2008; 19:314–18. [PubMed: 18786151]

167. Zhang F, Gu W, Hurles ME, Lupski JR. Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet. 2009; 10:451–81. [PubMed: 19715442]

168. Zheng K, Thorner PS, Marrano P, Baumal R, McInnes RR. Canine X chromosome-linked hereditary nephritis: a genetic model for human X-linked hereditary nephritis resulting from a single base mutation in the gene encoding the alpha 5 chain of collagen type IV. Proc Natl Acad Sci USA. 1994; 91:3989–93. [PubMed: 8171024]

SUMMARY POINTS

1. An extraordinary level of variation is captured within the dog genome. Although we don't yet understand how such variation occurred, studies of dog breeds offer the opportunity to understand how combinations of variants create complex phenotypes.

2. Studies of canine skeletal variation promise to expand the vocabulary of genes associated with growth, and in turn, diseases of growth regulation.

3. The availability of the dog genome sequence has made possible studies of genome architecture that have expanded our understanding of linkage disequilibrium, repeat elements, and gene organization.

4. Canine behavioral genetics is in its infancy, but studies are initiating that will permit us to understand how genetics contributes to both normal and anomalous behaviors.

5. Canine and human diseases are remarkably similar in terms of phenotypic presentation and causative genes. As such, the dog serves as a system for understanding human diseases that have otherwise proven difficult to study.

FUTURE ISSUES

1. We need to develop or improve methodologies in genomics and behavioral phenotyping to make use of the canine genome project to identify genes controlling both anomalous and naturally occurring behaviors.

2. We must use our new found knowledge to find genes important in complex traits, particularly diseases that are common in the human population, such as cancer, diabetes, and heart disease.

3. We must work to connect the dots created by individual morphological studies in order to understand the ways in which genetic variants combine to produce the amazing variety observed in domestic dog breeds.

4. In addition to the genomic advances, we need to focus on assembling the tools needed to undertake studies aimed at understanding the role of epigenetic events in phenotypic variation.
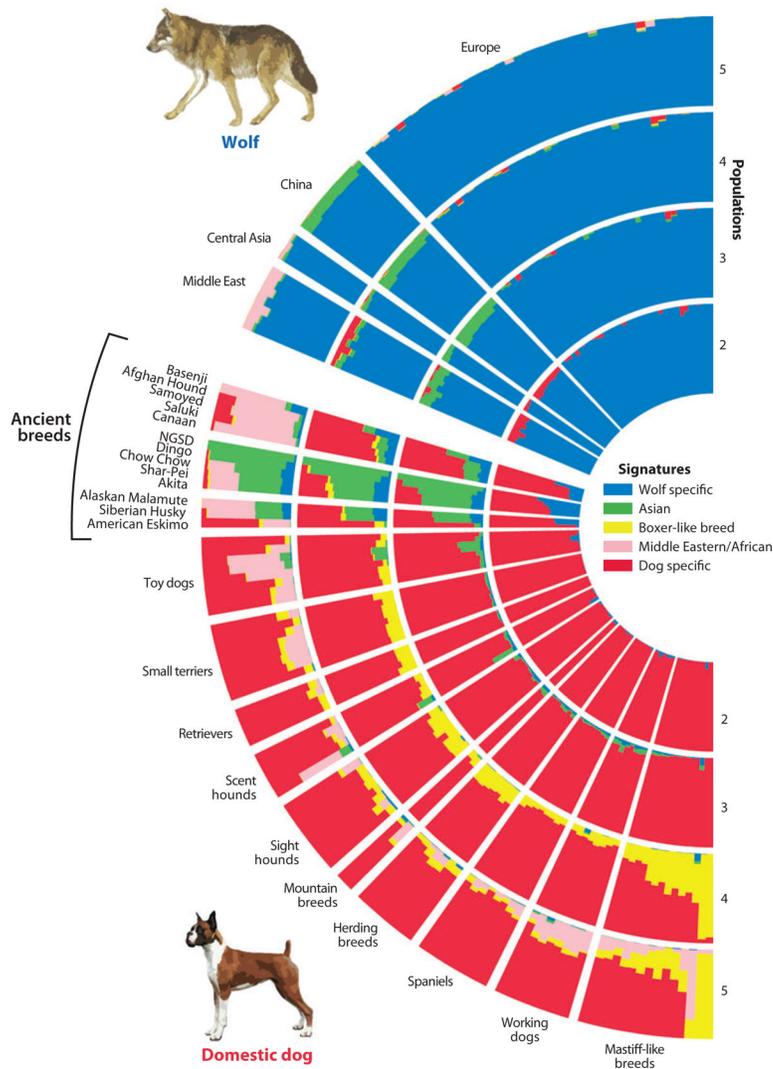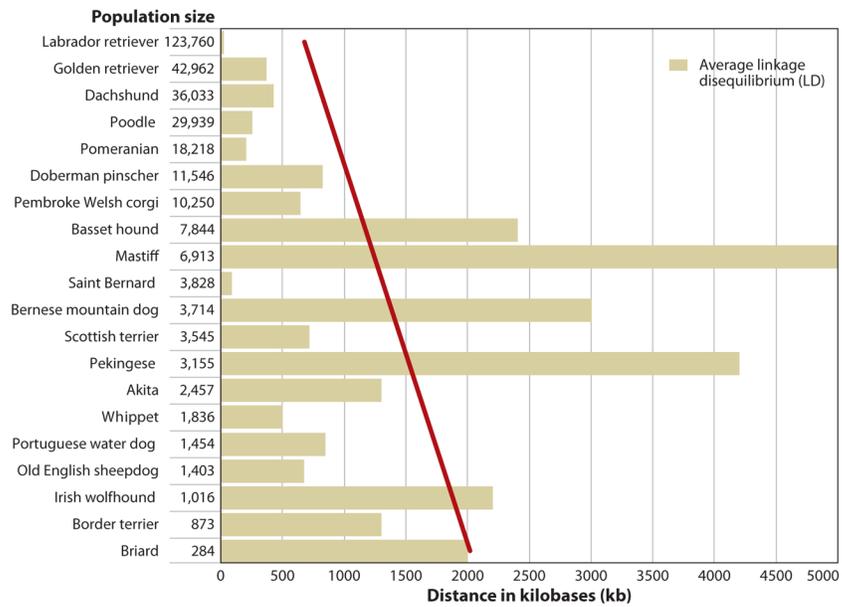
Figure 1.
An unsupervised cluster analysis of dogs and wolves. Using clustering algorithms with more than 43,000 single-nucleotide polymorphisms (SNPs), 85 dogs, representing 85 different breeds, along with 43 wolves from Europe and Asia, were assigned to 2–5 populations (*inner circle* to *outer circle*, respectively) based solely on genomic content. Each column represents a single individual divided into colors representing genomic populations. Blue indicates a wolf-specific signature, and red indicates a dog-specific signature. Note that the majority of crossover lies between ancient dog breeds and Chinese or Middle Eastern wolves. Figure originally published in *Nature* (158).
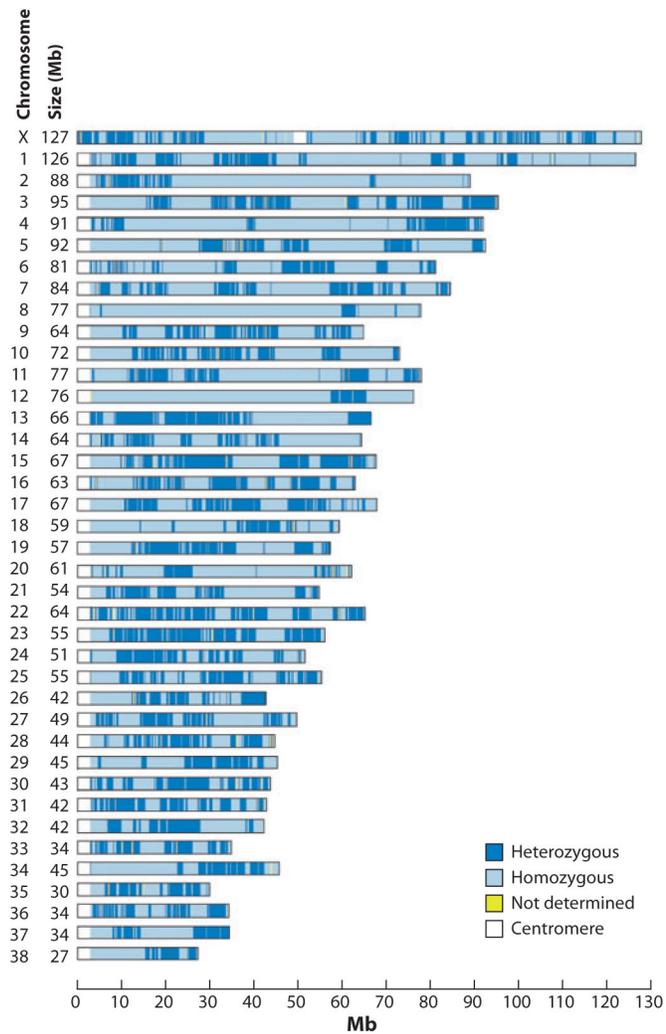
Figure 2.
Variation in dog breeds encompasses a large range of sizes, shapes, colors, and behaviors. Breeds (and behaviors) are listed from the upper left, clockwise: Border collie (herding: controlling the movement of livestock is one of the oldest described behaviors in dogs), Pug dog (companionship: one of the most prized traits a dog provides), Whippet (coursing: chasing small or large game, a natural forerunner to racing), Alaskan sled dogs (mushing: pulling a sled with driver as a member of a team for either sport or transportation), German shorthaired pointer (pointing: identifying the location of game), Foxhounds (tracking: working as a pack to follow scent of moving game and signal the hunter), Portuguese water dog (water retrieval: used on fishing boats to bring in nets and line, round up fish, and deliver messages between boats), Bernese mountain dog (drafting: pulling heavy loads individually or in pairs, usually to transport goods). Herding picture provided courtesy of paurian@flickr.
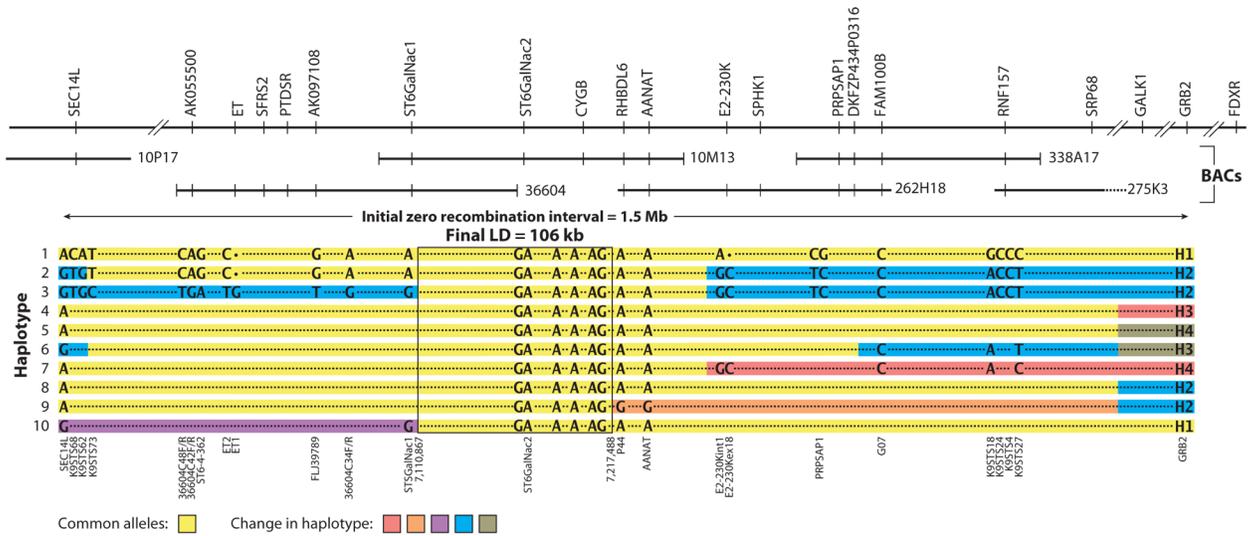
Figure 3.
Average linkage disequilibrium (LD) in 20 dog breeds sorted by breed population size. The breeds are listed at the left of the graph, followed by the number of dogs registered in the breed in 2009. LD was calculated at five unlinked loci by Sutter et al. and Gray et al. (50, 144). Linear trend line indicates overall tendency for LD to increase with a decrease in population size. Breeds that display considerable deviations from the trend likely have complex population histories involving multiple changes in population size, admixture, and/ or changing selective pressures.

Figure 4.
Regions of homozygosity and heterozygosity as found through direct sequencing of the boxer genome. All 38 autosomes and the X chromosome were sequenced from a female boxer to produce a 7.8x draft sequence. Heterozygous or homozygous state of each contig in the genome build was assessed though examination of approximately 770,000 single-nucleotide polymorphisms (SNPs) with heterozygous = 1 SNP in 1000 bps versus homozygous = 1 SNP in 20,000 bps. Contigs with the same state were merged to form blocks. Average size of the light-blue homozygous blocks is 6.9 Mb. Average size of the dark-blue heterozygous blocks is 1.1 Mb. White blocks indicate the centromeres. All canine chromosomes except the X are telocentric. Figure originally published in *Nature* (88).

Figure 5.

Haplotype shared among ten breeds of dog reduced the progressive rod-cone degeneration (prcd) locus from 1.5 Mb to 106 Kb. An identical haplotype spanning 106 Kb was found in miniature and toy poodles, English cocker spaniels, American cocker spaniels, Labrador retrievers, Portuguese water dogs, Chesapeake Bay retrievers, Nova Scotia duck tolling retrievers, Australian cattle dogs, and American Eskimo dogs with allelic forms of prcd. The causative mutation was identified in a novel gene, now called *PRCD*, located between *CYGB* and *ST6GALNAC2* (165). The top row shows a schematic of the chromosome region with genes named above. Below the chromosome are the bacterial artificial chromosomes (BACs) used to tile sequence across the interval. The haplotypes are shown with common alleles colored yellow, and a change in color indicates a recombination event or change in haplotype. Solid dots indicate deleted nucleotides. Names of tagging markers used for genotyping are given at the bottom of the haplotype chart. Figure originally published in *Genomics* (49) and reprinted with permission from Elsevier.

	Phenotype	FGF5	RSPO2	KRT71
a	Short	–	–	–
b	Wire	–	+	–
c	Wire and curly	–	+	+
d	Long	+	–	–
e	Long with furnishings	+	+	–
f	Curly	+	–	+
g	Curly with furnishings	+	+	+

a Basset hound **b** Australian terrier **c** Airedale terrier

d Golden retriever **e** Bearded collie

f Irish water spaniel **g** Bichon frisé

Figure 6.
Seven different coat phenotypes are created through the allelic variation at three genes. Protein altering mutations in *FGF5* and *KRT71* along with changes in expression levels of *RSPO2* combine to create the seven coat types displayed. The combinations of alleles are displayed to the right of the coat type. The coat types represented by each breed are as follows; (*a*) short, (*b*) wire, (*c*) curly-wire, (*d*) long, (*e*) long with furnishings, (*f*) long and curly, (*g*) long and curly with furnishings. Figure originally published in *Science* (20).

Table 1

Examples of molecular mechanisms associated with phenotypes in the dog

Class	Trait	Discovery method	Reference
Single base change: coding			
Morphology	Curly hair	GWAS	(20)
Morphology	Long hair	GWAS	(20)
Morphology	Brown coat[a]	Linkage	(136)
Morphology	Red/yellow coat	Candidate gene	(42, 106)
Morphology	Black mask	Candidate gene	(135)
Morphology	Recessive black coat	Candidate gene	(77)
Morphology	Fawn/sable coat[b]	Candidate gene	(11)
Disease	Degenerative myelopathy	GWAS	(7)
Disease	Renal cystadenocarcinoma and nodular dermatofibrosis	Linkage	(90)
Disease	Progressive rod-cone degeneration	Linkage	(165)
Disease	Neonatal encephalopathy	Linkage	(30)
Disease	Exercise-induced collapse	Linkage	(120)
Disease	Narcolepsy (DACH)[c]	Candidate gene	(67)
Disease	Cystinuria	Candidate gene	(60)
Disease	Cone degeneration (GSHP)[c]	Candidate gene	(139)
Disease	Dominant progressive retinal atrophy	Candidate gene	(79)
Disease	Shaking puppy (generalized tremor)	Candidate gene	(105)
Disease	Rod-cone dysplasia 1	Candidate gene	(141)
Disease	Glanzmann's thrombasthenia (OTTR)[c]	Candidate gene	(15)
Disease	Dystrophic epidermolysis bullosa	Candidate gene	(9)
Disease	Von Willebrand's type 2 (GWHP)[c]	Candidate gene	(83)
Disease	Hemophilia B	Candidate gene	(41)
Disease	Leukocyte adhesion deficiency	Candidate gene	(80)
Disease	Alport syndrome	Candidate gene	(168)
Single base change: noncoding			
Morphology	Dilute pigment	Linkage	(38, 124)
Disease	X-linked ectodermal dysplasia	Linkage	(23)
Disease	Narcolepsy (LAB)[c]	Candidate gene	(87)
Disease	Muscular dystrophy (GOLD and CKCS)[c,d]	Candidate gene	(137, 159)
SINE insertion: coding			
Disease	Centronuclear myopathy	Linkage	(121, 148)
SINE insertion: noncoding			
Morphology	Merle (patches of dilute pigment)	GWAS	(31)
Disease	Narcolepsy (DOBP)[c]	Linkage	(87)

Class	Trait	Discovery method	Reference
Insertion or deletion (not SINE): coding			
Morphology	Hairless	GWAS	(37)
Morphology	Dominant black coat	Linkage	(21)
Morphology	Brown coat[a]	Candidate gene	(136)
Morphology	Myofiber hyperplasia "Bully whippet"	Candidate gene	(103)
Disease	Copper toxicosis	Linkage	(44, 164)
Disease	Neuronal ceroid-lipofuscinosis	Linkage	(74, 89)
Disease	Cone degeneration (AMAL)[c]	Linkage	(139)
Disease	Imerslund-Grasbeck disorder	Linkage	(55, 56)
Disease	Cone-rod dystrophy (MLHD and SWHD)[c,e]	Linkage	(99, 162)
Disease	Rod-cone dysplasia type 2	Linkage	(84)
Disease	X-linked severe combined immunodeficiency	Candidate gene	(61)
Disease	von Willibrand's disease type 3 (SCOT)[c]	Candidate gene	(153)
Disease	Ivermectin sensitivity	Candidate gene	(97)
Disease	Hereditary cataracts (STAF)[c,f]	Candidate gene	(99)
Disease	Early onset cataracts (BOST)[c,f]	Candidate gene	(98)
Disease	Congenital night blindness	Candidate gene	(155)
Disease	Recessive progressive retinal atrophy	Candidate gene	(123)
Disease	Glanzmann's thrombasthenia (GPYR)[c]	Candidate gene	(91)
Disease	Cyclic hematopoiesis	Candidate gene	(10)
Insertion or deletion (not SINE): noncoding			
Morphology	Furnishings	GWAS	(20)
Disease	Collie eye anomaly	Linkage	(93, 117)
Disease	Glanzmann's thrombasthenia (GPYR)[c]	Candidate gene	(91)
Disease	Junctional epidermolysis bullosa	Candidate gene	(22)
Expanded repeat: coding			
Disease	Myoclonus epilepsy	Linkage	(92)
Gene or segmental duplication/ rearrangement			
Morphology	Ridgeback	GWAS	(133)
Morphology	Chondrodysplasia	GWAS	(118)
Disease	Hemophilia A	Candidate gene	(94)
Disease	Chronic myelogenous leukemia	Candidate gene[g]	(19)

SINE, short interspersed nuclear element; GWAS, genome wide association study.

[a]One deletion and two SNPs have been associated with brown coat color.

[b]Two SNPs in complete linkage disequilibrium are associated with fawn/sable coat color.

[c]Breed abbreviation key: AMAL-Alaskan malamute, BOST-Boston terrier, CKCS-Cavalier King Charles spaniel, DACH-dachshund, DOBP-Doberman pinscher, GOLD-golden retriever, GPYR-great Pyrenees, GSHP-German short-haired pointer, LAB-Labrador retriever, MLHD-

miniature long-haired dachshund, OTTR-otterhound, SCOT-Scottish terrier, STAF-Staffordshire bull terrier, SWHD-standard wire-haired dachshund.

[d]Different SNPs in the same gene are associated with muscular dystrophy in the GOLD and CKCS.

[e]Insertions in two different genes are responsible for cone-rod dystrophy; *RPGRIP1* in the MLHD and *NPHP4* in the SWHD.

[f]The same mutation was found associated with early onset cataracts in the BOST and hereditary cataracts in the STAF.

[g]Fluorescent in situ hybridization with probes from a candidate chromosomal region was used to identify the rearrangement that causes CML.