THE CANID GENOME: BEHAVIORAL GENETICISTS' BEST FRIEND?

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Abstract

We review a range of studies on the genetic contribution to behavior in canid spp. We begin by identifying factors that make canids a promising model in behavioral genetics and proceed to review research over the last decade that has used canids to identify genetic contributions to behavior. We first review studies that have selectively bred dogs to identify genetic contributions to behavior and then review studies that estimate heritability from populations of non-laboratory bred dogs. We subsequently review studies that used molecular genetics to identify gene-behavior associations and note associations that have been uncovered. We then note challenges in canid behavioral genetics research that require further consideration. We finish by suggesting alternative phenotyping methods and identify areas in which canids may have as yet unexploited advantages, such as in gene environment interaction studies (GXE) where genetic factors are found to moderate the effects of environmental variables.

The Canid Genome: Geneticists' best friend?

Advantages of a Canid Model for Behavioral Genetics

Animal models have been essential to the development of behavioral genetics and genomics. The central importance of the canid genome is exemplified by the fact that the dog was the fourth mammal to have its genome sequenced, preceded only by the human, mouse, and rat (O'Brien & Murphy, 2003). The priority given to sequencing the genome of the dog was based on several advantages of the dog as a model for basic genetics research and genetics research on human diseases (Kirkness et al., 2003; O'Brien & Murphy, 2003, see the white paper by Ostrander et al., http://www.genome.gov/Pages/%20Research/Sequencing/SeqProposals/%20CanineSEQedited.pdf)

One benefit of using canines is the structure of the canid genome. Linkage disequilibrium (LD, the deviation in the frequency of haplotypes in a population from the frequency expected if the alleles at different loci are associated at random; Griffiths et al., 2008) is higher in dogs than humans (Lindblad-Toh et al., 2005; Sutter et al., 2004a). Sutter et al. (2004a) reported average linkage within dog breeds range in the megabases, whereas linkage in human sub-populations average in the kilobases (Reich et al., 2001), meaning that haplotypes are much longer in dogs. Although different breeds vary in LD depending on the history of the breed and phenomena such as founder and bottleneck effects (Parker et al., 2010), several researchers have hypothesized that the overall higher LD of dogs implies that fewer than 30,000 single nucleotide polymorphisms (SNPs) are necessary for genome wide association studies (GWAS), compared to 200,000 or more SNPs necessary for human GWAS studies (International HapMap Consortium, 2003;

Spady & Ostrander, 2008; Sutter et al., 2004a). Karlsson et al. (2007) demonstrated effective use of a set of 27,000 SNPs in dogs. Thus GWAS studies in dogs are more economical than in humans (Karlsson et al. 2007; Parker et al., 2010; Shearin & Ostrander, 2010; Sutter et al., 2004a).

In addition, many haplotypes are shared across dog breeds, likely resulting from the genetic bottleneck of domestication (Lindblad-Toh et al., 2005; Parker et al., 2010). Together, these facts suggest that a single larger genome wide SNP map appropriate for use on all breeds could be developed (Spady & Ostrander, 2008; Wayne & Ostrander, 2007). It is important to note, however, that recent work in humans suggests that rare polymorphisms (minor allele frequency <0.5%) may have significant, population specific, effects on phenotypes (Nelson et al., 2012; Tennessen et al., 2012). Thus, rare genetic variation within breeds may have important effects on phenotypes of interest.

Another benefit of using canines is the resemblance of many clinical syndromes between dog and man. Of the more than 573 diseases that have been documented in dogs, over 277 resemble human diseases (Online Mendelian Inheritance in Animals, OMIA, http://omia.angis.org.au). Models of inheritance in dogs have allowed the isolation and identification of the causal genes for diverse biomedical disorders (Boyko, 2011; Karlsson & Lindblad-Toh, 2008; Ostrander et al., 2000a; Parker et al., 2010; Parker & Ostrander, 2005; Sutter & Ostrander, 2004b; Tsai, Clark & Murphy, 2007, Wayne & Ostrander, 2007). Two databases for inherited disorders in dogs are an important resource for research on inherited diseases in dogs: Online Mendelian Inheritance in Animals (OMIA, http://omia.angis.org.au) and Inherited Diseases in Dogs (http://www.vet.cam.ac.uk/idid: Sargan, 2004).

Mendelian inherited diseases can lead to neuropathologies and behavioral abnormalities. Single gene mutations can lead to ataxia, seizures, cerebellar cortical degradation, encephalopathies and other neurological disorders (Chen et al., 2008; Olby et al., 2004; Penderis et al., 2007). These single gene mutations show high penetrance (the proportion of individuals with a specific genotype that manifest that genotype at the phenotype level, Griffiths et al., 2008) and have profound effects on behavior. Through the analysis of dog pedigrees, search for causal genes can be focused and inform research on the relevant human disorders.

An additional benefit of using dogs is their great morphological and behavioral diversity (Jones et al., 2008; Karlsson & Lindblad-Toh, 2008; Ostrander, Galibert & Patterson, 2000a; Ostrander & Kruglyak, 2000b; Ostrander & Wayne, 2005; Parker & Ostrander, 2005; Parker et al., 2010; Shearin & Ostrander, 2010; Spady & Ostrander, 2008; Sutter & Ostrander, 2004b; Wayne & Ostrander, 2007). For example, the American Kennel Club (AKC) recognizes over 170 phenotypically distinct dog breeds (www.akc.org/breeds/complete_breed_list.cfm). Each breed is a genetically isolated population, with a unique set of behavioral and morphological characteristics.

Variance in dog behavior is analogous to that observed in the normal human population (Stein, Dodman, Borchelt & Hollander, 1994). Dogs show differences in temperament, compulsive disorders, anxiety level, social behavior, aggression, and more (for reviews see Jones & Gosling, 2005; Overall, 2000; Overall, Hamilton & Chang, 2006; Stein et al., 1994). Researchers have investigated complex behavioral temperaments in dogs such as general “sociability” or “confidence” (for a review see Jones & Gosling, 2005). Others have investigated conditions that may be analogous to

human psychiatric conditions (e.g., Canine-Compulsive Disorder may be analogous to human Obsessive-Compulsive disorder: Moon-Faelli, Dodman, Famula & Cottam, 2011; Overall, 2000).

Dogs are not the only members of the family *Canidae* that have served in behavioral genetic research. Silver foxes (a morph of the Red fox, *Vulpes vulpes*) have been bred for over 50 years at the Institute for Cytology and Genetics (ICG) in Novosibirsk, Russia. Starting in 1959, Dmitry Belyaev selectively bred foxes for tame behavior towards humans (for reviews see Kukekova et al., 2008b; Spady & Ostrander, 2007; Trut, 1999). Within two decades it was clear his experiment was a success, and now the ICG possess a strain of foxes that show high levels of sociable behavior towards humans, as well as a strain that is highly aggressive towards people. Foxes at the ICG are raised under controlled conditions allowing for genetic comparison to be made between domesticated foxes, aggressive foxes, F1 hybrids and backcrosses (Kukekova et al., 2008b). From this population of foxes, much has been learned about the morphological changes that can occur under behavioral selection pressures, and current work is aimed at identifying the molecular changes associated with domestication (Kukekova et al., 2010).

Behavioral Genetics: Identifying Heritability

Laboratory experiments in selective breeding

Scott and Fuller (1965) conducted over a decade of research identifying heritable differences in behavior and cognition on what they termed a “veritable genetic gold mine,” - the dog (p. 4). Scott and Fuller studied Basenjis, Beagles, Cocker Spaniels, Shetland Sheepdogs and Fox Terriers and their crosses on a battery of tests assessing problem solving, social behavior, leash training, fearfulness, timidity, and behavioral

development (for reviews see Dewsbury, 2011; Feuerbacher & Wynne, 2011). Rearing conditions were uniform across litters through the use of cross-fostering and other experimental manipulations of the environment.

Scott and Fuller noted that genetics did not control behavior in any "ironclad way" (p. 426). Unlike responses to single behavioral tests, general behavioral phenotypes such as problem solving, that predict performance on numerous related behavioral tests that would today be termed "cognitive," did not demonstrate strong genetic effects. This led Scott and Fuller to posit that most genes act on specific traits, such as the heart rate response to novel stimuli. They did not believe there were general pleitropic genes (genes that influence numerous phenotypes) responsible for large numbers of behaviors that might comprise a category such as "problem solving" "general intelligence," or "personality". Scott and Fuller also emphasized the importance of the environment, especially early environments, on later learning and behavior.

Another early large-scale study on selective breeding utilized the naturally occurring nervous strain of Arkansas Pointer Dogs. The nervous strain (E strain) was selectively bred to be more timid than the normal strain (A strain: for a review, see Dykman et al., 1979). Compared to the A strain the E strain dogs displayed an apprehension and catatonic like freezing in the presence of people, and heart arrhythmia, but no differences were noted in basal cortisol or ACTH levels (for cortisol measurements see Klein, Tomai & Uhde, 1990; Murphree, Peters & Dykman, 1967). Murphree, Peters and Dykman (1969) tested approach to and avoidance of people in the E strain, A strain, and crossbred dogs with tasks designed by Scott and Fuller (1965).

Crossbred dogs and nervous dogs all avoided humans and showed reduced exploration in comparison to the stable strain.

Murphree and Newton (1971) attempted to reduce the E strain's timidity through special handling. They gave half of each of the E strain's litters special human interaction and handling for 40 sessions over six months. This reduced their timidity but did not render them normal in behavior. To further distinguish genetic effects from any maternal effects, Murphree and Newton bred reciprocal crosses (E mother X A father and A mother X E father). No differences were seen in the reciprocal crosses indicating maternal effects did not contribute to timidity in the E strain. One potential biological mechanism for these strain differences may be differential serum concentrations of insulin-like growth factor 1 (IGF-I; Uhde, Malloy & Slate, 1992). The severity of fear in nervous dogs was significantly associated with IGF-I, with nervous dogs showing lower serum concentrations compared to normal dogs (Uhde et al., 1992).

The selection for tame behavior in Belyaev foxes led to numerous physiological changes, such as piebald coats, floppy ears, body size, and curly tails, although there was no explicit selection for these traits (Trut, 1999). Selection for tame behavior also produced changes in the sensitive period for socialization as measured by the onset of the fear response (Belyaev, Plyusnina & Trut, 1985). Plyusnina, Oskina & Trut (1991) compared exploratory responses in a novel situation and basal cortisol levels in foxes selected for tame behavior and foxes selected for increased aggression. At 40 days of age, aggressive foxes showed a significant increase in basal cortisol levels, whereas no such peak occurred in tame foxes. When the cortisol peak was abolished by the experimental injection of an inhibitor of the HPA axis, choloditane, the aggressive foxes

fear response in a novel cage was attenuated, but the foxes remained aggressive (Plyusnina, Oskina & Trut, 1991). When aggressive foxes were injected with l-tryptophan (a pre-cursor to serotonin) beginning at 45 days of age, aggression scores were significantly attenuated as adults. Thus, the selection pressure for reduced fear to humans or increased aggression can produce marked changes in important developmental periods.

Heritability of Behavior in Dog Populations Outside the Laboratory

Rather than raising and breeding dogs for experimental purposes, more recent studies have assessed heritability of behavior in working or pet dog populations. Goddard and Beilharz (1985), for example, tested fearful reactions to different stimuli in four breeds of guide dogs and their respective crosses. Factor analysis identified 12 principle components that were reduced to three discriminant functions related to fearfulness. These functions were identified to have genetic components with a high degree of genetic variance within breeds. The authors thus concluded that a selective breeding program would be useful (Goddard & Beilharz, 1985).

Other researchers have utilized larger data sets to identify heritability of various traits in dogs (Hsu & Serpell, 2003; Saetre, Sandberg, Sundgren, Pettersson, Jazin & Bergstöm, 2006). The Swedish Dog Mentality Assessment (DMA) was initiated in 1989 as a tool for selective breeding in working dogs (Saetre et al., 2006). The DMA has been applied to over 24,000 dogs (Saetre, et al., 2006). Using this data set and the pedigrees of the tested dogs, Saetre et al. (2006) noted that the genetic correlation of the score on one test was dependent on the score on another test. Contrasting with the hypothesis of Scott and Fuller (1965) that genetic effects act on *specific* behavioral traits, Saetre et al. (2006)

identified “shyness-boldness” as a generalized trait underlying many behavioral scores with a heritability of .25-.27. “Aggression” was the only other identified trait distinct from “shyness-boldness” (Saetre et al., 2006).

Also utilizing a sample of working dogs, van der Waaij, Wilsson and Strandberg (2008) assessed genetic correlations among components of a different behavioral test conducted by the Swedish Dog Training Center on two different breeds: German Shepherd Dogs and Labrador Retrievers. To allow comparisons of the genetic parameters across breeds, the same linear regression model was applied to both data sets. The heritabilities of the studied behaviors for Labrador Retrievers and German Shepherd Dogs ranged from .03 - .56. The genetic correlations between behavioral tests also differed between the two breeds. For example, a negative (-.67) correlation between the traits “hardness” and “cooperation” was observed for German Shepherd Dogs, whereas a positive (.28) correlation was observed for the same traits in Labrador Retrievers. This implies that a selective breeding program for specific traits may have differential effects depending on the breed. It is also important to note that the behavioral scores indicated substantial environmental contributions to the variation in phenotypes.

A similar study in Switzerland, also using German Shepherd Dogs, investigated the heritability of various behaviors derived from the Swiss German Shepherd Breeding Dog Club behavioral test (Ruefenacht, Gebhardt-Henrich, Miyake & Gaillard, 2002). On a sample of nearly 3,500 dogs Ruefenacht et al. (2002) found heritability of the behavioral traits to range from .09 - .23, with “sharpness” showing only a .09 heritability and reaction to gunfire showing a heritability of .23. Other traits such as “self-confidence,” “hardness,” and “temperament” showed intermediate heritabilities.

Schmutz and Schmutz (1998) utilized the data collected by the North American Versatile Hunting Dog Association (NAVHDA) from their natural ability test to calculate the heritability of various hunting-related behaviors in five breeds of hunting dogs. The natural ability test attempted to identify a dog's natural working ability (i.e. prior to training) on tasks such as pointing, nose work, retrieval, tracking, cooperation search, and desire to work. Heritabilities for a few abilities exceeded .40 (tracking for German Shorthaired Pointers); however, heritabilities varied greatly across breeds and tasks. For example, the heritability of tracking for Griffons was .13, whereas heritability of pointing for German Shorthaired Pointers was .25. Most heritabilities were low and did not reach statistical significance.

Other studies have also identified similar modest levels of heritability for hunting behaviors using the hunting behavior test of the Swedish Flatcoated Retriever Club (Lindberg, Standberg & Swenson, 2004), and hunting tests conducted in Norway (Brenøe, Larsgard, Johannessen & Uldal, 2002). Overall, the detailed testing and record keeping of hunting and breeding clubs has allowed researchers to identify the heritability of numerous behaviors in different breeding populations.

Given the millions of people bitten by dogs every year (Gilchrist, Sacks, White & Kresnow, 2008), the genetic underpinning of aggression is an important line of investigation (Houpt, 2007). One way to phenotype for aggression is behavioral observation. Saetre et al. (2006) identified aggression as a separate trait from "shyness-boldness" using the DMA. However, aggression may be too heterogeneous to function as a single classification (van den Berg, Schilder & Knol, 2003). Van den Berg et al. (2003), using a modified behavioral aggression test developed by Netto and Planta (1997),

presented Golden Retrievers with various subtests differing in stimulus conditions designed to elicit aggression. Van den Berg et al. correlated owner reports of aggression with the behaviorally assessed aggression scores and found the aggression score correlated best with owner reports for dogs reported with conspecific *and* owner directed aggression. Van den Berg et al. also noted that owner-reported aggressive dogs comprised a heterogeneous group of animals showing aggression towards conspecifics, people or both conspecifics and people. Thus, van den Berg et al. suggested that more homogenous groups of dogs based on their aggression type would be more amenable to genetic analysis.

Surveys of owners have been utilized to characterize dogs' behavior and reactions to stimuli (Duffy, Hsu & Serpell, 2008; Hsu & Serpell, 2003; Liinamo et al., 2007; Våge et al., 2008; van den Berg, Schilder, de Vries, Leegwater & van Oos, 2006). These methods provide a faster way of phenotyping dogs compared to the completion of a standardized behavioral test. Duffy et al. (2008) identified breed differences in aggression using the Canine Behavioral Assessment and Research Questionnaire (CBARQ; Hsu & Serpell, 2003). Differences were seen in whether the aggression was directed toward familiar people, unfamiliar people, or dogs (Duffy et al., 2008). Van den Berg et al. (2006) administered the CBARQ to the owners of the cohort of dogs previously tested on the modified Netto and Planta (1997) aggression test summarized above. Van den Berg et al. (2008) noted that a factor analysis from the aggression test yielded two factors similar to the CBARQ categories: "dog-directed aggression" and "stranger-directed aggression." In addition, Liinamo et al. (2007) noted a low correlation between these two forms of aggression, indicating that these concepts are probably partially genetically independent.

These data support the original conclusion of van den Berg et al. (2003) that dog directed and human directed aggression may be best studied as independent phenotypes.

Aggression phenotypes have been associated with coat color in English Cocker Spaniels (Podberscek & Serpell, 1996; Våge et al., 2008). Solid coat English Cocker Spaniels tend to be more aggressive than parti-colored dogs in general, but there also appear to be differences in coat color and the *type* of aggression (Podberscek & Serpell, 1996). Behavioral differences correlated with coat color have also been observed in Korean native Jindo dogs. White colored Jindo dogs are more fearful, more submissive, and scent mark less than fawn colored ones (Kim et al, 2010).

Molecular Approaches to Behavioral Genetics in the Dog

Before genetic polymorphisms can be associated with behaviors, the polymorphisms must first be identified. The publication of the 7.5x draft of the Boxer genome has greatly facilitated the identification of SNPs in the canine genome and has led to a comprehensive linkage map (Lindblad-Toh et al., 2005; Parker et al, 2010; Wong et al., 2010). In addition, researchers have sequenced important candidate genes to identify SNPs, copy number variants (CNVs), and variable number tandem repeats (VNTRs) within breeds, across breeds, and across species (Gronek et al., 2008; Hashizume et al., 2005; Hejjas et al., 2009; Irion et al., 2003; Ito et al., 2004; Jörn & Frode, 2008; Nara et al., 2005; Nicholas et al., 2009; Niimi et al., 2001; Switonski, Sczcerbal & Nowacka-Woszuk, 2009; Takeuchi et al., 2005; Våge & Lingaas, 2008; van den Berg et al., 2004; van den Berg, Kwant, Hestand, van Oost & Leegwater, 2005). These polymorphisms have been identified in putative candidate genes relating to serotonin (van den Berg et al., 2004; van den Berg et al., 2005), dopamine (Niimi et al.,

2001; Hejjas et al., 2009 DRD4), Tyrosine hydroxylase (Takeuchi et al., 2005) and others.

Candidate Gene Approach

As noted above, Liinamo et al., (2007) reported the heritability of human-directed aggression to be .81 in Golden Retrievers. Given this high heritability as a starting point, van den Berg et al. (2008) studied associations between human-directed aggression in this breed with SNPs in the serotonin receptor genes (1A, 1B, 2A), and a SNP in the serotonin transporter gene (*slc*6A4). Relationships between gene polymorphisms and owner-reported human-directed aggression were sought through linkage analysis, an association study, and a quantitative genetic analysis using CBARQ. Despite the use of multiple methods, no associations between human-directed aggression and any of the genotypes were found (see Table 2 for an overview of the Candidate Gene Studies). Although the most parsimonious conclusion is that there is no association between the candidate genes and aggression, many other factors may explain the failure to detect a genetic association, and these have received extensive treatment in the human literature (e.g., Colhoun, McKeigue & Smith, 2003; Cordell & Clayton, 2005). Two of the many possible reasons for the lack of association could be that the study was underpowered to detect a very small genetic effect, or the owner reports may not have provided a sufficiently precise phenotype to appropriately reflect the genetic effect (see Miguel et al., 2005 for a commentary on phenotyping issues using Obsessive-Compulsive Disorder as an example).

In English Cocker Spaniels, Våge et al. (2010) tested associations between 16 neurotransmitter-related genes and owner reported human-directed aggression.

Associations were found for the dopamine receptor D1, serotonin receptors 1D and 2C, and solute carrier family 6 (*slc*6A1; a neurotransmitter transporter). In the Shiba Inu, Takeuchi et al. (2009b) carried out a factor analysis on an owner survey of characteristic behaviors. They utilized the derived Factor 1, "stranger-directed aggression" as the phenotype to be associated with polymorphisms in nine neurotransmitter related genes. An association with the *slc*1A2 (a glutamate transporter) was identified; dogs with the CC genotype were significantly less likely to be aggressive.

Takeuchi et al., (2009a) phenotyped Labrador Retriever guide dogs through a factor analysis of the recorded notes of dog trainers. They attempted to associate the factor identified as "activity level" with polymorphisms in nine neurotransmitters and found it to be significantly associated with a TT polymorphism in the *slc*1A2 gene and with the *COMT* gene (Takeuchi et al., 2009a). Together, Takeuchi et al. (2009a) and Takeuchi et al. (2009b), have associated two *slc*1A2 polymorphisms with behavior: Shiba Inu dogs with the CC polymorphism were more likely to be reported with stranger directed aggression and Labrador Retrievers with a TT polymorphism were more likely to be reported as more active.

Konno, Inoue-Murayama and Hasegawa (2011) associated variable number tandem repeats (VNTR) in the Androgen receptor (AR) in Japanese Akita Inu dogs with aggression scores derived from owner responses to a questionnaire. Using an across breed mapping strategy, Ito et al. (2004) identified an association between a VNTR in exon 3 of the DRD4 gene and breeds that were rated as more "aggressive" and less "reactive" by a group of 191 dog experts. It should be noted, however, that the sample of breeds was not large enough to correct for possible population stratifications based on

geographical origins of the breeds (Ito et al., 2004), and population stratifications may lead to spurious genetic associations (Chang et al., 2009; Quignon et al., 2007).

Some studies have found an association between DRD4 and behavior within a breed (Hejjas et al., 2007a; 2007b; 2009). A VNTR in exon 1 of DRD4 was associated with owner reports of dogs' activity/impulsivity on a questionnaire (Hejjas et al., 2007a). In a population of German Shepherd Dogs, VNTRs in exon 3 of DRD4 were associated with owner reported activity/impulsivity ratings in police dogs but not pet dogs (Hejjas et al., 2007b). This differential effect, dependent on whether the dogs were kept as pets or police dogs, could indicate a gene by environment interaction. Unfortunately, the sample size was too small to detect such an effect (Hejjas et al. 2007b). A third study utilized a component of the DMA behavioral test to assess social impulsivity and identified a polymorphism in intron 2 and exon 3 of DRD4 that had an additive effect on impulsivity in German Shepherd Dogs (Hejjas et al., 2009).

Genome Wide Association Studies (GWAS)

GWAS identify associations between genes and behaviors using SNPs spaced across the entire genome. This contrasts with the candidate gene approach, which targets polymorphisms in a limited number of target genes. Dodman et al. (2010) used a GWAS approach on 92 Doberman Pinchers diagnosed with a Canine-Compulsive disorder (CCD) (Dodman et al. 2010). These Doberman Pinchers would compulsively suck their flanks or a blanket. Dodman et al. (2010) searched for genetic differences across the 92 affected dogs and the 68 control Doberman Pinchers. They found a SNP within *CDH2* (a widely expressed gene related to neuronal adhesion) on chromosome 7 that associated significantly with CCD (Dodman et al., 2010). In addition, the proportion of the

population with CCD associated genotypes (the TT or TC genotype) increased when more severe forms of CCD behaviors were considered (Dodman et al. 2010).

Other breeds have also been documented with high incidences of compulsive disorders. Bull Terriers show a high incidence rate of compulsive tail chasing (Moon-Fanelli et al., 2011; Tiira et al., 2011; Tiira et al., 2012) and German Shepherd Dogs may also be susceptible (Tiira et al., 2011; Tiira et al., 2012). A preliminary candidate gene study looking for an association between tail chasing and the chromosome 7 locus reported to be associated with compulsive flank sucking by Dodman et al., found no significant association with tail chasing (Tiira et al., 2011; Tiira et al., 2012). In addition, no significant genetic associations were found with tail chasing in a genome wide study; however the sample size included only 24 cases and 24 controls, and thus may have been under-powered (Tiira et al., 2011). Tiira et al. (2012) expanded this study focusing on *CDH2* with dogs of three different breeds, but with a limited sample size, and found no association.

Across breed mapping is a different approach to identifying genetic associations with behaviors unique to dog breeds, rather than individual dogs (Chase, Jones, Martin, Ostrander & Lark, 2009; Jones et al., 2008). With this approach, researchers compare a large number of breeds with a common set of informative SNPs. The breed of dog is used as a "meta-phenotype" to identify multiple fixed phenotypes within the breed (e.g. size, height, etc.). These phenotypes are then compared to identify correlated genetic differences across breeds. Jones et al. (2008) and Chase et al. (2009) used this approach on the same data set for both morphological and behavioral features. In these studies behavioral features were determined for each breed by a single experienced rater who

assigned a qualitative score for each of the 148 breeds for pointing, boldness, trainability and herding (Jones et al., 2008). This across breed mapping approach identified 10 putative loci for behavioral associations, of which five candidate genes were proposed. Chase et al. identified IGF-1 as a possible candidate gene for boldness. This is interesting in light of the finding by Uhde et al. (1992), that nervous Pointers have lower serum IGF-1 concentrations. DRD1 was also identified as a possible candidate gene for boldness. DRD1 has been associated with aggression in English Cocker Spaniels (Våge et al., 2010). Chase et al. (2009) and Jones et al. (2008) identified other potential candidate genes for pointing (CNIH – implicated in cranial nerve development), herding (MC2R – melocortin receptor activated by adrenocorticotropic hormone, C18orf1 – implicated in schizophrenia) and boldness (PCDH9 – encodes a cadherin-related neuronal receptor).

Across-breed mapping is a unique approach outside of a traditional GWAS study to identify putative candidate genes in dogs. This approach contrasts the approaches reviewed above (except Ito et al., 2004) by utilizing fixed traits in a breed instead of using the variance of phenotypes within a breed to identify genetic associations. Importantly, across breed comparisons may find spurious associations arising from population structures (Chase et al., 2009; Hamer & Sirota, 2000; Hejjas et al., 2007b; Ito et al., 2004; Jones et al, 2008). The associations identified with this method require further study using breeds in which the putative polymorphism is still segregating. We are unaware of any such follow-up studies. In addition, it is important to note that a single expert rater determined the behavioral traits that were fixed in each breed for Jones et al. (2008) and Chase et al. (2009). This contrasts the method of Ito et al. (2004) that utilized the opinion of 191 experts.

Foxes

The similarity of the fox genome to that of the dog (Kukekova et al., 2007; Spady & Ostrander, 2007) allowed Kukekova et al. (2007) to adapt dog microsatellite markers to the fox to create a meiotic linkage map. Kukekova et al. (2008a) then linked the markers to objectively measured behaviors from the tame, aggressive and unselected strains of Belyaev foxes as well as tame x aggressive F1 hybrids and a backcross of the F1s to tame foxes. Over three hundred fox behaviors and locations within the cage were coded from video using a binary scale. Kukekova et al. (2008a) utilized principle component analysis to reduce the original 311 behavioral codes to 50 significant behaviors which could be useful for a quantitative genetic analysis (Kukekova et al. 2008a).

In a subsequent study, Kukekova et al. (2010) used principle component analysis on the behavioral test in Kukekova et al. (2008a) to identify quantitative phenotypes that could be associated with genetic markers. PC1, which explained 48% of the variance and distinguished domesticated from non-domesticated foxes, was linked to the region VVU12. Kukekova et al. (2010) reported that this region is orthologous to a region that vonHoldt et al. (2010) identified as a locus for domestication in dogs. In addition, PC2 was also linked to VVU12. PC2 was similar to the previously described "shyness-boldness" factor; however, Kukekova et al. (2010) noted that although PC2 is independent from PC1 by definition, they are not unrelated. Aggressive foxes that attack (i.e. are more bold) are also more aggressive than foxes that do not approach the human (i.e. are more shy). Furthermore, tame foxes that approach humans (i.e. are more bold)

are tamer than foxes that remain in the back of the cage (i.e. are more shy). Thus, the observed "shyness-boldness" trait may be context dependent (Kukekova et al., 2010).

Challenges of a Canid Model for Behavioral Genetics

Defining a Behavioral Phenotype

Before any gene-behavioral phenotype associations can be identified, behavioral phenotypes must first be defined. Many human studies have utilized DSM categorizations to define behavioral phenotypes of interest. However, no such manual of behavior exists for the dog.

Overall (2000) developed one approach to address this problem by identifying behavioral, neurochemical and anatomical parallels between human psychiatric conditions and analogous dog behavioral syndromes such as CCD and Panic Disorder. Thus pathological cases can be thoroughly investigated and compared to control dogs (e.g. Tiira et al., 2011).

Other researchers have utilized factor analysis or principle component analysis on a battery of behavioral tests to define a behavioral phenotype of interest. Although in humans the definition of behavioral syndromes through DSM has a long history, there are ways in which the factor analytic approach may be preferable. Factor analysis is an objective method to identify correlated variables in a hypothesis free manner (Scott & Fuller, 1965). This contrasts with the more subjective way symptoms are combined in the DSM. In laboratory animal models, factor analyses from a battery of tests have been successfully utilized as phenotypes (e.g. Cook et al., 2002; Henderson et al., 2004; Holmes et al., 2003). For example, after giving a battery of tests including an open field test and an elevated plus maze, Henderson et al. (2004) identified anxiety-like factors in

mice that were then utilized as phenotypes to identify genetic associations with the different anxiety factors (Henderson et al., 2004).

The factor analytic method, however, also has limitations that are often overlooked. One basic assumption in genetic association studies is that behaviors that factor together (are highly correlated) have a common genetic underpinning. While this may be true, it is not necessarily the case. A complex behavioral phenotype may have multiple causal pathways (equifinality; Gottlieb, Wahlsten, Lickliter, 2007, Skinner, 1953). Furthermore, factor analysis does not discriminate common genetic elements from common environmental factors; thus, behavioral tests that factor together may not arise from a common genetic underpinning, but rather from common environmental stimuli across tests.

The factor analysis and principle component methods reduce a large number of behaviors assessed from a battery of behavioral tests to a more tractable smaller set of factors that are used as phenotypes. Although this offers attractive phenotypes for gene association studies, it is unclear how gene-factor associations translate back to gene-behavior associations.

Instead of searching for the genetic underpinnings to complex phenotypes such as "shyness-boldness" or "intelligence," researchers could identify associations between specific behavioral responses and genotypes. Such an approach may provide a clearer understanding of the effects of a gene at the behavioral, rather than the factor, level. Phenotyping using a simple behavioral response does not necessarily imply that only a single response to a single test is recorded. The behavioral response could be repeatedly assessed across different test parameters until consistent data is observed for each

individual, reducing noise variability. For example, a delayed discounting task has been used to assess impulsivity in different rat strains (e.g. Wilhelm & Mitchell, 2009) and in humans (e.g. Eisenberg et al., 2007). In this task, the individual is given a choice between a smaller immediate reward, and a larger reward following a delay. To determine their characteristic preference for immediacy or "impulsivity" subjects are given this choice with various parameters of the smaller reward and delays to the larger reward, Using a delayed discounting procedure in humans, Eisenberg et al. (2007) identified an association between impulsivity and a polymorphism in DRD4 and DRD2. Interestingly, this effect was not apparent in self-report measures of impulsivity (Eisenberg et al., 2007). An approach similar to that taken in rats could be profitably deployed in dogs.

Another approach may be to phenotype behaviors on the basis of their behavioral functions rather than their structural or topographic similarity. Here we are defining 'function' in behavior-analytic terms as the reinforcer of that behavior; i.e. the consequence that increase the probability the behavior will be emitted in the future. For example, van den Berg et al. (2003) utilized the phenotype of 'aggression' with limited success, and recommended breaking it into sub-categories based on the structure of the aggression (e.g., 'stranger-directed aggression'). Although all aggression towards humans may share a similar topography (growling, lunging, biting, etc.), the *function* of the aggression may vary across dogs (i.e. the reinforcer). In different subjects, different aspects of the environment may reinforce aggression, even when a similar topography is observed across the subjects. For example, some dogs may snarl because in the past such snarling has allowed the dog to escape an undesired situation such as grooming (an 'escape' function; Skinner, 1953). Others may snarl because this behavior has produced

high levels of attention (albeit disapproving attention) from the owner (an 'attention' function; Skinner, 1953). Finally, some dogs may snarl at an owner to access food he or she would have otherwise have withheld (a 'tangible' function). Behavioral functional analysis is the experimental assessment of the reinforcers that maintain a behavior by measuring the effects of removing and providing putative reinforcers (Iwata, Dorsey, Slifer, Bauman & Richman, 1982/1994). Numerous studies in humans and animals testify to its utility (e.g., Dorey, Rosales-Ruiz, Smith & Lovelace, 2009; Iwata et al., 1994; Iwata, Dorsey, Slifer, Bauman & Richman, 1982/1994; Martin, Bloomsmith, Kelley, Marr & Maple, 2011).

Behavioral functional analysis can be used to include the environmental variable maintaining a behavior in a genetic association study (see Figure 1). This approach segregates behaviors such as aggression by the environmental variable that maintains them (i.e. the reinforcer: such as escape from aversive stimuli, owner attention, a tangible item, etc.). Potentially, a gene may influence susceptibility to the reinforcer motivating the attack, and not necessarily the object to which the aggression is directed (e.g. stranger-directed, owner-directed, dog-directed aggression).

Measuring a Behavioral Phenotype

Many behavioral-genetic studies have phenotyped dog behavior with owner reports. While some of these studies have successfully associated candidate genes with behavioral phenotypes so defined it is surely noteworthy that the actual behavior of the subject is never directly assessed (see Baumeister, Vohs & Funder, 2007 for a discussion on surveys and behavioral measurement). Numerous variables other than the dog's behavior may influence owner reporting of dog behavior, including expectations of

typical dog behavior, owner temperament, and recent but untypical interactions with the dog. Caution should therefore be exercised when extending gene associations from owners' reported dog behavior to actual dog behavior. Eisenberg et al. (2007) is an interesting example of a failure to obtain correlations between individual's self-reports of behavior and genotype, while a significant association between genes and measured impulsivity was observed. Similarly, owners' reports based on recollection of their dog's behavior may not be as powerful as direct behavioral observation.

In across breed mapping, the assumption that a breed of dog possesses a characteristic behavioral phenotype is itself open to question. Unlike many morphological features, behavioral phenotypes such as pointing, chasing or herding may not apply equally to a whole breed. Rather, the behavioral phenotypes of individuals may vary as much within breeds as across the breeds utilized to detect the associations. Although the idea of breeds possessing common patterns of behavior is widely held, systematic objective studies supporting this belief are sparse (Coppinger & Coppinger, 2002). Researchers should validate meta-phenotypes by phenotyping individual subjects of the breeds of interest and assessing behavioral variability both within- and across-breeds. Behaviors demonstrating higher across breed variability but low within breed variability would then be good candidate behaviors for this approach. Across-breed genome wide associations are a novel approach that may prove useful in detecting gene-behavior associations. However, without data demonstrating the validity of the meta-phenotype and without subsequent successful replications within a single breed, the associations identified should be interpreted with caution.

Even within a breed, underlying population structure may lead to spurious gene-behavior associations (Chang et al., 2009; Quignon et al., 2007). Chang et al. (2009) analyzed four breeds, and found significant within-breed stratification in Border Collies. This stratification, if left uncontrolled, may lead to spurious gene-behavior associations (Chang et al., 2009). The underlying population structure may have arisen from geographical isolation or from different selection practices of different breeders (Chang et al., 2009) such as the creation of distinct "show" and "working" lines of a breed.

Measuring the Environment

Most behavior genetic studies on dogs have not included measures of potentially related environmental conditions. To further advance our understanding of the causes of behavior we need an interdisciplinary approach that includes *both* environmental and genetic measures.

Although it is conceptually and statistically convenient to discuss "genetic contributions" as separate effects from "environmental contributions," biologically they are inseparable (Johnston & Edwards, 2002; Meaney, 2010). Thus, trying to detect genes that produce behavior independent of all environmental influences may not be effective or theoretically useful (Johnston & Edwards, 2002; Meaney, 2010, Turkheimer, 1998).

Heritability estimates have been utilized to identify the proportion of variance in a population attributed to "genetic" factors that are separate from environmental variables (Meaney, 2010). However, heritability estimates separate environmental and genetic effects statistically, but not necessarily biologically (Gottlieb, Wahlsten & Lickliter, 2007; Gottesman & Hanson, 2005; Lewontin, 1974; Meaney, 2010). DNA sequence alone may not be the only factor influencing the function of a gene. For example, early

rearing environments (maternal behaviors of rat mothers) appear to influence gene expression of rat pups through structural DNA modifications such as DNA methylation (for a review see Meaney & Szyf, 2005). These structural changes influence gene expression and later adult behavior of the pups. These epigenetic modifications, however, are not fixed. Instead, environmental influences such as cross-fostering manipulations or pharmacological manipulations can reverse the epigenetic modification and its effects on behavior, making genetic effects responsive to the environment in the absence of sequence changes. Environmental effects, like epigenetic effects carried on for multiple generations, would be masked in heritability estimates, and inflate heritability (Maher, 2008; Meaney, 2010). Thus, changes in DNA sequences do not necessarily have to account for all of the variance attributed to "genetics" in heritability estimates (Meaney, 2010).

Some studies have attempted to separate genetic and environmental effects by studying subjects in relatively uniform environments (e.g. pet dogs, working dogs, and laboratory reared dogs). However, these environments can still vary greatly, particularly with pet dogs living in diverse human homes. Recent genetic analyses on human subjects have taken a slightly different approach and measured both environmental variables and genetic factors. Some of these studies have noted that genes and environments interact statistically; thus, the genetic effects depended on the environment (GXE; see Figure 1, and for reviews, see Caspi & Moffit, 2006; Caspi et al., 2010). In humans, a growing body of literature has investigated how environmental factors can moderate genetic influences. In a landmark study, Caspi et al. (2003) reported that the effect of a 5-HTT polymorphism on depression depended on exposure to the risk factor, life stress, in that

the short allele increased risk only for individuals exposed to stressful life events. In mice, models of gene-environment interactions have also been developed (see Laviola et al., 2009 for a review). In dogs, Hejjas et al., 2007b, noted the potential for a similar GXE effect. These authors found that the effect of a DRD4 allele appeared to depend on the dogs' everyday environment (police or working dog); however, the sample was too small to statistically detect an interaction.

Behavioral function-based phenotyping, as defined in the previous section, is a slightly different approach to looking at gene environment interactions. With function-based phenotyping, the proximate function of the behavior (i.e. the reinforcer that maintains the behavior, such as attention, food, escape from aversive stimuli etc.) is experimentally determined and then included as a factor that may interact with genetic effects. This compares to the environmental exposure GXE approach, in which exposure to a risk or protective factor is measured instead of identifying the consequence of the behavior that maintains the behavior. To return to the example of aggression: In a behavioral-function-based phenotyping approach, the researcher would identify the reinforcer for aggression in each subject, whereas in the environmental exposure gene-environment interaction approach, the researcher would identify whether each subject was exposed to a risk factor of interest (e.g., being chained outside) and not necessarily what reinforces the behavior (see Figure 1).

Function-based phenotyping can also be applied to abnormal behavior. For example, repetitive behaviors such as tail chasing may have different sources of reinforcement for different animals and this may interact with genetic effects. Tail chasing may be maintained by social consequences for some dogs (see Bain & Fan,

2012), as owner attention is a common response to tail chasing (Burn, 2011). For other dogs, tail chasing may serve as self-stimulation and would persist in the absence of social consequences. The function-based approach would identify the function of tail chasing and look for potential interactions with genotypes. For example, a given genotype may only influence tail chasing that is maintained by non-social consequences. In comparison, the environmental exposure GXE approach would assess the presence or absence of environmental risk (or protective) factors and its interaction with genotypes. For tail chasing, dietary supplements may reduce risk (Tiira et al., 2012). With the environmental exposure approach, the presence and absence of dietary supplements would be assessed for each subject and tested for an interaction with genotype and may find that a given genotype may increase risk only when dietary supplements are not given.

Importantly, a behavioral function-based phenotyping approach would need to be validated. This would require identifying the function of the behavior for a large group of subjects and testing for a relationship between the genotype and the function of the behavior. Function-based phenotyping would only be useful if replicable associations between a genotype and behavior are found to depend on the environmental consequence maintaining the behavior.

Unrecognized benefits of dogs for behavioral genetic work

Although we have identified areas where we feel that canine behavioral genetics confronts challenges that have not yet been successfully overcome, there are also benefits of dogs as model animals in behavioral genetic research that have not yet been fully exploited. The sheer number of dogs in our society (77 million in the United States alone; APPA, www.americanpetproducts.org/press_industrytrends.asp), and the large number

for whom lineages over scores of generations are available is a resource which has to date been relatively little utilized. Furthermore, dogs lead diverse roles in human society: as laboratory animals, pets, and in a variety of working roles. This diversity of environments may allow dogs to be a useful model for gene environment interaction studies. For example, researchers could capitalize on the varied rearing conditions, housing conditions, or quantity and quality of social interactions dogs already experience as environmental variables that may be moderated by genetics. In addition, dogs' roles as pets and laboratory animals give the dog a special status relative to animals that are primarily studied in the laboratory. Using dogs, the effect of independent variables can be studied in the laboratory *and* outside the laboratory using larger correlational studies more typical of human studies. For example, the interaction between early rearing environments and genotype can be studied both in the laboratory under controlled conditions and in less controlled conditions by using the variance in rearing conditions of pet dogs in human homes.

Dogs can thus be used to test for laboratory-induced effects on genetic correlations. For example, small bouts of environmental enrichment have important genetic effects on developing mice (Arai & Feig, 2010). In addition, behavioral enrichment decreases ß-Amyloid load in several brain regions of aging laboratory beagle dogs and protects against cognitive decline associated with aging (Christie, Opii & Head, 2009; Cotman & Head, 2008; Pop et al., 2010). In considering the possible implications of these results for the human case, however, it is not clear to what extent these findings may be a product of the deprived conditions offered by the laboratory. Enrichment for a laboratory dog included two 20-minute walks per week, pair housing, and giving the dogs

toys – far less activity and interaction than would be typical of pet dogs. The existence of pet dogs offers an important model to test the generality of effects observed in the laboratory, and the existence of laboratory dogs allows for correlational data from pet dogs to be confirmed through controlled experiments.

Future studies should also explore the various methods of behaviorally phenotyping individuals. We have noted above potential concerns with various methods currently used for behaviorally phenotyping dogs (factor analysis, owner surveys, meta-phenotypes, response to a single behavioral test) and have suggested behavioral function-based phenotyping may be useful. Future studies will be needed to compare the various phenotyping methods using the same group of subjects and will likely provide useful information on the effective methods for behaviorally phenotyping dogs, and other species including humans, for genetic analysis.

Conclusions

Canids are a useful system for studying behavioral genetics for many reasons. Prior work has identified numerous gene associations with behaviors and has demonstrated the heritability of complex traits. Whereas dogs are unique for their morphological and behavioral variances and efficiency in genomic mapping, one as yet little-utilized unique quality is their exposure to many different environments. Including the influence of the environment in genetic analyses may improve our ability to identify how genes influence behavior. As research emphasis turns away from trying to separate genes from the environment, and turns toward understanding the roles of genes in the context of a specific environment, dogs may grow as a powerful animal model for humans.

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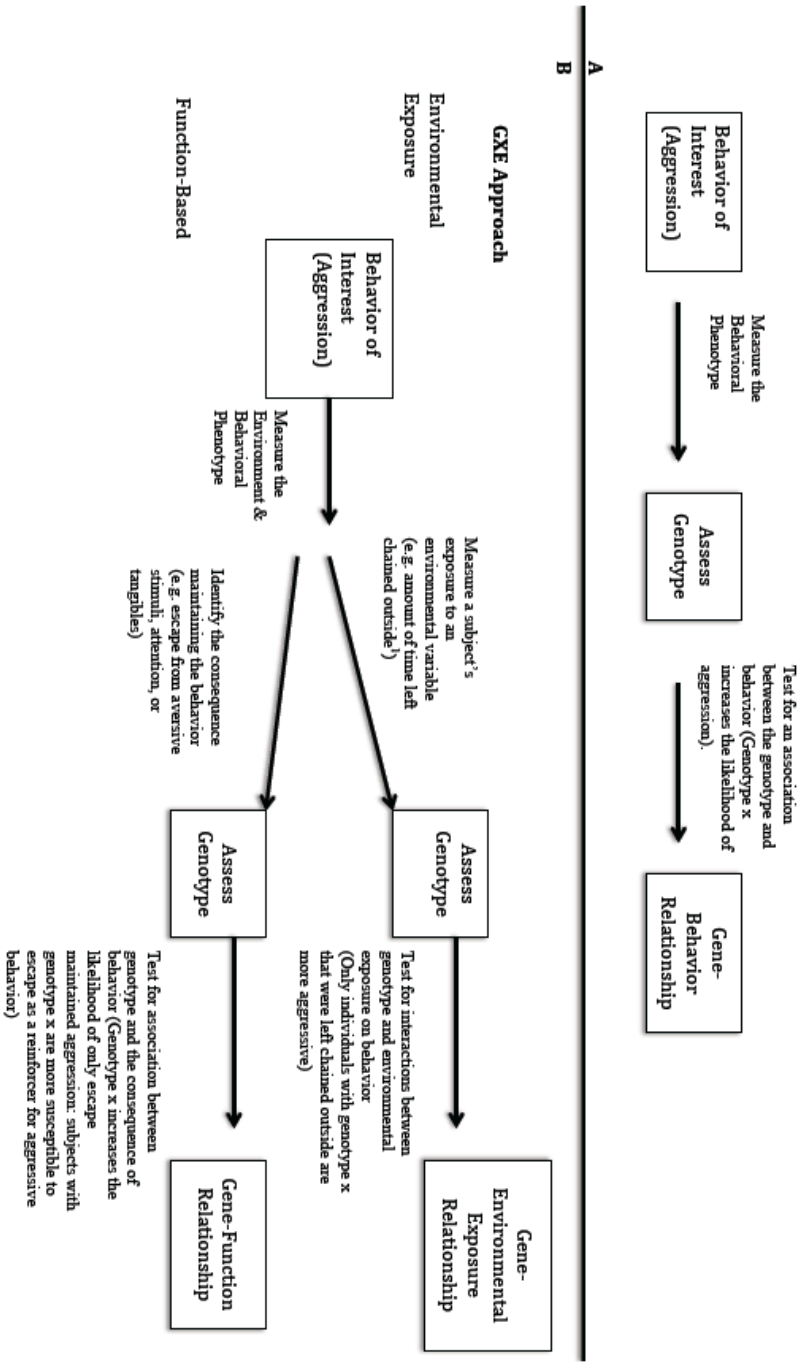

Figure 1. Comparison of different approaches. A: outlines the traditional genetic approach. B: outlines a GXE approach and differentiates between GXE studies that assess exposure to a risk or protective factor from the function-based approach proposed in the text. The upper path of Figure 1B diagrams the Environmental Exposure GXE studies, where an exposure to a potential environmental variable is included in the

analysis. The lower path of Figure 1B diagrams how a Function-based study may be conducted to test for relationships between genotype and the environmental consequences maintaining a behavior (i.e the reinforcer). The parentheses indicate a hypothetical example in which aggression is the behavior of interest. [1]Gershman, Sacks & Wright, 1994.

Genetic Approaches	**Description and Examples**
Heritability Estimates	Heritability of various complex traits of interests can be estimated with known pedigrees (Ruefenacht et al., 2002; Saetre et al., 2006; van der Waaij, Wilsson & Strandberg, 2008).
Linkage Analysis	Utilizes pedigree information from phenotyped subjects to trace the linkage of a marker to a trait (see van den Berg et al., 2008 for an example of linkage analysis using candidate genes).
Genome Wide Association (GWAS)	An association study that looks for gene-behavior associations with a large number of genetic polymorphisms across the genome (see Dodman et al. 2010 for an example of GWAS followed by a targeted gene analysis; see Tiira et al., 2011 for a GWAS and candidate gene study).
Candidate Gene	An association study between a few genes (candidate genes) and a phenotype (Hejjas et al., 2007b; Takeuchi et al., 2009b; Våge et al., 2010).
Across Breed Mapping	Utilizes fixed breed traits to identify associations between these traits and genetic polymorphisms (see Jones et al., 2008 and Chase et al., 2009 for a GWAS across numerous breeds).
Gene Environment Interaction (GXE)	A study that tests for an interaction between an environmental variable a and genetic polymorphism. For example, the effect of a gene may depend on the presence or absence of an environmental exposure (no known studies in dogs, see Caspi et al., 2003 for an example in humans: for a review see Caspi & Moffit, 2006).
Phenotyping Tools	
Owner Reports	The behavioral phenotype is determined by the owner's verbal report about the dog's behavior (Duffy, Hsu & Serpell, 2008; Hsu & Serpell, 2003; Takeuchi et al., 2009a; Våge et al., 2010)
Behavioral Test	Experimenter introduces a variable and records the dog's response as the phenotype. The phenotype could be the response to the first exposure of the test, could be the result of repeated testing or could be comprised from a combination of behavioral tests (Netto & Planta, 1997; Saetre et al., 2006).
Factor Analysis	Statistical methods to identify underlying "factors" that influence responses on multiple measured variables (Goddard & Beilharz, 1985; Takeuchi et al., 2009a)
Breed Fixed Traits	Traits that are no longer segregating within a breed. All members of the breed show the trait of interest (Chase et al., 2009; Ito et al., 2004; Jones et al., 2008).

Table 1. Tools and Approaches. The tools and approaches to genetic analysis reviewed or proposed in this article are summarized with citations of representative studies where possible.

Behavior	Citation	Sample Size	Breed	Approach	Genes Associated	Genes not Associated in Candidate approach
Activity/ Impulsivity						
Activity-Impulsivity	Hejjas et al (2007b)	189	German Shepherd Dogs	Candidate Gene	DRD4	-
Activity-Impulsivity	Hejjas et al (2007a)	59	Belgian Tervuren	Candidate Gene	DAT DBH DRD4	*TH*
Activity level	Takeuchi et al. (2009a)	81	Labrador Retrievers	Candidate Gene	slc1A2 COMT	*DRD2* *TH* *DBH* *htr1A* *ht21b* *DRD4* *MOAB*
Impulsivity	Hejjas et al. (2009)	96 behaviorally tested dogs	German Shepherd Dogs	Candidate Gene	DRD4	-
Aggression						
Human-directed	Våge et al. (2010)	50 aggressive, 81 controls	English Cocker Spaniels	Candidate Gene	*DRD1* *htr1d* *htr2c* *slc1A1*	*DRD2* *DRD3* *DBH* *htr*1A, *htr*1B, htr1D htr1F htr2A *htr*2B htr2C MAOA MAOB GAD1
Aggression	Takeuchi et al. (2009b	77	Shiba Inu	Candidate Gene	slc1A2	*DRD2* *TH* *DBH* *htr1A* *ht21b* *DRD4* *COMT* *MOAB*
Aggression	Konno et al. (2011)	100	Fawn colored Akita Inu	Candidate Gene	*AR*	-
Human-directed	van den berg et al. (2008)	49 aggressive, 49 controls	Golden Retriever	Candidate Gene	-	*htr*1B, *htr*1A, *htr*2A, *slc*6A4
Compulsive Behavior						
Compulsive	Dodman	92 affected,	Doberman	GWAS	CDH2	N/A

Disorder	et al. (2010)	68 controls	Pincher			
Tail Chasing	Tiira et al. (2011)	24 cases, 24 controls	Bull Terrier	Candidate Gene & GWAS	-	CDH2 (from Candidate Gene Study)
Tail Chasing	Tiira et al. (2012)	40 case, 28 control 11 case, 16 control 7 case, 5 control	Bull Terrier German Shepard Dog Staffordshire Bull Terriers	Candidate Gene	-	CDH2

Table 2. Outcomes of the molecular approach for within breed studies. Table summarizes studies using a molecular approach to traits segregating *within* a breed that are discussed in the text. – indicates the absence of any genes. N/A refers to the genes not identified in a GWAS study, as these would be too numerous to list.