Behavior Genetics
https://doi.org/10.1007/s10519-023-10166-7

ORIGINAL RESEARCH

Transposons in the Williams–Beuren Syndrome Critical Region are Associated with Social Behavior in Assistance Dogs

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Received: 6 May 2023 / Accepted: 8 November 2023

Abstract

A strong signature of selection in the domestic dog genome is found in a five-megabase region of chromosome six in which four structural variants derived from transposons have previously been associated with human-oriented social behavior, such as attentional bias to social stimuli and social interest in strangers. To explore these genetic associations in more phenotypic detail—as well as their role in training success in a specialized assistance dog program—we genotyped 1001 assistance dogs from Canine Companions for Independence®, including both successful graduates and dogs released from the training program for behaviors incompatible with their working role. We collected phenotypes on each dog using puppy-raiser questionnaires, trainer questionnaires, and both cognitive and behavioral tests. Using Bayesian mixed models, we found strong associations (95% credibility intervals excluding zero) between genotypes and certain behavioral measures, including separation-related problems, aggression when challenged or corrected, and reactivity to other dogs. Furthermore, we found moderate differences in the genotypes of dogs who graduated versus those who did not; insertions in *GTF2I* showed the strongest association with training success ($\beta = 0.23$, $CI_{95\%} = -0.04, 0.49$), translating to an odds-ratio of 1.25 for one insertion. Our results provide insight into the role of each of these four transposons in canine sociability and may inform breeding and training practices for working dog organizations. Furthermore, the observed importance of the gene *GTF2I* supports the emerging consensus that variation in *GTF2I* genotypes and expression have important consequences for social behavior broadly.

Keywords Behavioral genetics · Canine · Cognition · Working dog · Hypersociability · C-BARQ

Introduction

Understanding how individual differences are generated and maintained within a population has long been a goal of quantitative genetics. The rise of genomics has opened new avenues of study and led to some answers; however, aside from certain Mendelian disease and morphological traits, it remains difficult to predict most phenotypes from genomic information. This is especially true for behavioral traits, likely due to the complexity and plasticity of behavior and the extreme polygenicity of most behavioral traits (Hoekstra and Robinson 2022). Thus, the development and evolution of consistent individual differences in behavior (i.e., animal personality) remain central to questions in biology, psychology, and neuroscience (Réale et al. 2010; York 2018; Sih et al. 2019; Niepoth and Bendesky 2020; Ashton 2023).

It is increasingly clear that behavioral and cognitive traits are highly polygenic (Wehner et al. 2001; Chabris

Handling Editor: Theodore Garland.

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et al. 2015; Hoekstra and Robinson 2022; Morrill et al. 2022), and domestic dogs (*Canis lupus familiaris*) represent an ideal model system for exploring the interaction of genetic and environmental variation that shape such complex behavioral phenotypes (Wayne and Ostrander 2007). Dogs have a relatively long, complex evolutionary and demographic history as the first domesticated animal, with millennia of selective breeding and increasingly strict closed breeding pools (Parker et al. 2004, 2017; vonHoldt et al. 2010) that have created hundreds of breeds with standardized morphology (Boyko et al. 2010) and—to a lesser extent—breed-specific behavioral tendencies (reviewed in Spady and Ostrander 2008; Mehrkam and Wynne 2014; Rigterink 2014). Several genome-wide association studies (GWAS) across breeds have identified associations between single-nucleotide polymorphisms (SNPs) and complex traits such as boldness (Vaysse et al. 2011); fear and aggression (Zapata et al. 2016); inhibitory control, sensitivity to human gestural communication, memory, and physical reasoning (Gnanadesikan et al. 2020a); herding, predation, temperament, and trainability (Shan et al. 2021); as well as multiple owner-perceived personality traits and "problem" behaviors (MacLean et al. 2019). A smaller number of studies have also begun to explore genome-wide associations with owner-perceived traits within single breeds (Ilska et al. 2017; Friedrich et al. 2019). Using estimates of narrow-sense heritability, multiple studies have explored the proportion of behavioral variation in dogs that can be explained by breed or other genetic factors (e.g. MacLean et al. 2019; Gnanadesikan et al. 2020b; Morrill et al. 2022). In fact, studies that have leveraged among-breed approaches with breed-average genomic data found a wide range of narrow-sense heritability estimates for various behavioral and cognitive phenotypes ($h^2 = 0.17 - 0.77$) (MacLean et al. 2019; Gnanadesikan et al. 2020b). A study at the individual level reported moderate heritability estimates ($h^2 > 0.25$), although only 9% of the behavioral variation was attributable to breed membership (Morrill et al. 2022). These findings are congruent with those across species reporting that behavioral variation is partially genetic, but to different degrees across behaviors (Hoekstra and Robinson 2022).

While much of the focus of behavioral genetics research to date has been on SNPs, the importance of structural variation—deletions, insertions, inversions, translocations, and duplications—has recently become evident. For example, structural variants in *Drosophila*, cichlids, salmon, and yaks appear to have contributed to rapid evolutionary change and are enriched in or near genes regulating behavior (Rech et al. 2019; Bertolotti et al. 2020; Penso-Dolfin et al. 2020; Zhang et al. 2021). Transposable elements (TEs) are short fragments of DNA that can mobilize and replicate in a genome, acting as a common source of structural and regulatory variation, and contributing to genomic evolution (Bourque 2009; Fambrini et al. 2020). While thousands of structural variants have been documented across dog breeds (Kim et al. 2019), minimal work has explored their associations with behavior. However, one genomic region with structural variation has previously been associated with both dog-wolf and individual-level behavioral differences: a five-megabase region on canine chromosome six. In canines, this region was first identified as showing a strong signature of selection in dog-wolf comparisons, with a single candidate gene (*WBSCR17*) tagged by the low-density genetic marker panel (vonHoldt et al. 2010). In the homologous region of the human genome, a hemizygous deletion of 28–30 genes is responsible for Williams–Beuren Syndrome (WBS) (Schubert 2009)—a congenital neurodevelopmental condition characterized by hypersociability and certain cognitive deficits, in addition to altered physical traits. Individuals with WBS are verbally fluent and highly sociable, with significant reductions in stranger anxiety, while having impaired visuospatial cognition, learning difficulties, and often generalized anxiety (Meyer-Lindenberg et al. 2006). Based on homology with the human and mouse genomes—and despite its larger size and reorganization (Fig. 1)—the five-megabase region of canine chromosome six is designated the "WBS critical region" (Merla et al. 2002; Segura-Puimedon et al. 2014; vonHoldt et al. 2017).

Motivated by potential similarities between the hypersociability observed in human WBS and changes in human-oriented sociability in dogs through the domestication process, research has focused on elucidating the role, nature, and molecular properties of the TEs in the canine WBS critical region. First, over 80 structural variants were identified in this region (vonHoldt et al. 2017). Using a "solvable" puzzle task and a "sociability" task across a small number of dogs (n = 16) and wolves (n = 8), four of these TEs were identified as polymorphic insertions demonstrating significant associations with "attentional bias to social stimuli" and "social interest in strangers" (vonHoldt et al. 2017). A transcriptome analysis of whole blood from wild gray wolves further associated these TEs with altered gene expression in at least six genes that are also implicated in human WBS (vonHoldt et al. 2018). Although these genes are intact in dogs, rather than the hemizygous deletion seen in human WBS, their altered expression may result in related social phenotypes in a dosage-dependent manner. Thus, a plausible hypothesis is that positive selection in this genomic region contributed to behavioral evolution during dog domestication, reducing fear and increasing tolerance of humans, and thereby promoting interspecific sociality (Gácsi et al. 2005, 2009; Hare and Tomasello 2005; Hare et al. 2010). However, testing this hypothesis requires deeper examinations of these phenotypes, genotypes, and their associations.

A subsequent study examined these four loci in both pet and medical assistance dogs, finding additional associations

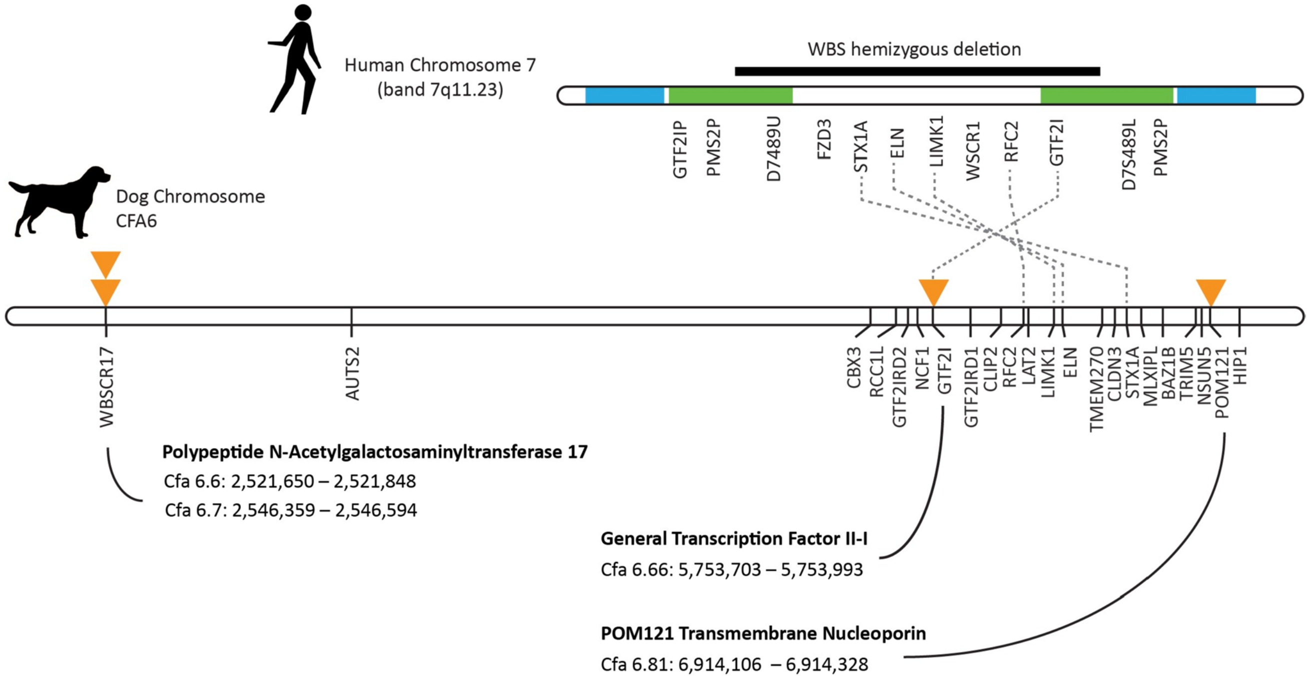

Fig. 1 The human WBS region (top), with a black bar outlining the ~1.8 Mb region that exhibits a hemizygous deletion in cases of Williams–Beuren Syndrome. The blue and green bars denote duplicated regions that are important for the erroneous recombination event that leads to the deletion. The ~5 Mb area around the homologous region of dog chromosome CFA six (bottom), known as the "canine WBS critical region", with some of the genes connected by dashed gray lines to highlight the reorganization of the region. The transposable elements studied here are indicated with orange triangles, and the information of the genes that they are within or closest to is annotated below, including genome coordinates from the CanFam 3.1 assembly. These transposons are, however, expected to have pleiotropic effects both within and potentially outside of this region

between genotypes and behavior as assessed by an owner survey (n = 228), as well as population differences in insertion frequencies between pet and assistance dogs (Tandon et al. 2019). In that study, locus Cfa6.6 had the greatest predictive power for items assessing attention-seeking and separation-related problems, as well as attachment; furthermore, a single population of German Shepherd medical assistance dogs (n = 49) carried more insertions and decreased heterozygosity at locus Cfa6.6 compared to pet dogs (n = 69) (Tandon et al. 2019). These findings suggest that genotype data might be informative for determining the eligibility of individual dogs for specific tiers of specialized training and assistance roles. Here, we explored this potential in a large population bred over many generations for the functional roles of assistance dogs by assessing the ability of these genotypes to predict behavioral phenotypes and success in working dog training programs.

Working dogs perform a wide variety of jobs in our society, including livestock herding and guarding, assisting people with disabilities, bomb and drug detection, and search and rescue. This study focused on candidate working dogs in training at Canine Companions for Independence® (CCI), the largest provider of assistance dogs in the United States. CCI trains their dogs to perform a variety of tasks for specific service roles (e.g., turning on light switches, opening and closing doors and drawers, retrieving dropped items, and pulling wheelchairs) and places the dogs—free of charge—with individuals with disabilities so they can lead more independent lives. This pedigreed dog population has been selectively bred over approximately 35 years for success as an assistance dog within this training program, as well as for certain health measures, although outside breeders are brought into the population periodically to maintain genetic diversity. Although the selection criteria have been less formalized and implemented much less strongly than in experiments such as the Siberian Farm Fox experiment (Trut et al. 2009), there has been sustained selection in this population for low reactivity to environmental stressors, as well as for high sociability and trainability. Thus, it is plausible that this population is phenotypically different from the average pet retriever.

Most large working dog organizations, including CCI, have graduation (training success) rates of approximately 50%, and the waiting time for an assistance dog is usually over a year. Thus, any predictive information that could help organizations make programmatic decisions ranging from breeding to training would have a significant impact on their ability to provide dogs for a variety of working roles (reviewed in Cobb et al. 2015; Bray et al. 2021c). There have been several attempts to predict working dogs' success from a variety of behavioral measures; these predictions improve on null models but are not yet highly accurate (MacLean and Hare 2018; Bray et al. 2019; Hare and Ferrans 2021; Lazarowski et al. 2021). Genotyping is a potentially powerful tool to aid in prediction, as specific genetic loci can be assessed across an entire population relatively inexpensively and early in the lifespan, prior to behavioral evaluations that typically occur at or after 1 year of age. However, developing such a tool requires strong, well-understood associations between genotypes and training success. So far, such assessments have been complicated by breed differences, population founder effects, and diverse reasons for training failure with insufficient data on these behaviors. We were therefore interested in exploring the contribution of structural variation in the WBS critical region of canine chromosome six to both specific behavioral and cognitive phenotypes and success as a working dog. In this study, we focus on the four previously implicated TEs in this region (CFA6:2-7 Mb; Fig. 1) and explore associations with a variety of behavioral and cognitive phenotypes with a social component, as well as outcomes of the training program (see Fig. 2 for overview), in 1,001 dogs from the CCI population.

Methods

Study Population

Our study population consisted of dogs from the training and breeding programs at Canine Companions for Independence® (CCI). The CCI breeding program produces approximately 900 puppies a year, all of which are Labrador retrievers, golden retrievers, or mostly crosses between the two. Most dogs are bred within driving distance of headquarters, located in Northern California; after being raised by volunteer puppy raisers, all dogs then return for professional training to one of six regional campuses, located in Northern California, Southern California, Texas, Ohio, Florida, and New York. Throughout their time in puppy-raiser homes and in training, CCI collects data on each dog's behavior, some of which we utilize here (Fig. 2).

Sampling and Transposon Genotyping

We selected 1,001 dogs for this study from the larger CCI population based on the following criteria: (1) individuals with adult cognitive phenotypes measured in past and ongoing studies, regardless of training outcome ($n = 408$), and (2) graduates and behavioral releases for whom we had Canine Behavioral Assessment and Research Questionnaire

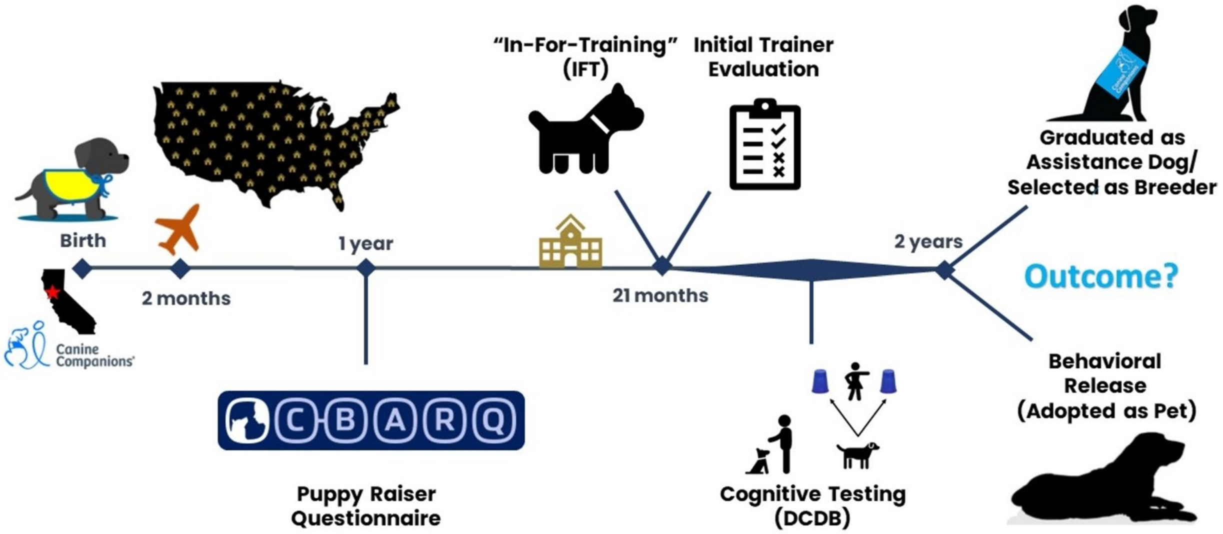

Fig. 2 Overview of key points in each dog's early life and our data collection. Ages shown are all approximate; details are given in the methods. Most Canine Companions for Independence (CCI) dogs are born in northern California, at or near CCI national headquarters. At about 2 months old, puppies are sent to individual volunteer puppy-raiser homes around the U.S. The puppy raisers fill out Canine Behavioral Assessment and Research Questionnaires (C-BARQs) when the dog is about 1 year of age. Soon after the dogs return to regional campuses for professional training, the In-For-Training (IFT) tests and Initial Trainer Evaluations are conducted by CCI staff. On average, dogs are in professional training for 6 months. These phenotypes are available for the majority, but not all, of our 1001 study subjects (see Table 1 for details). Around 2 years of age, on average, we conducted cognitive testing on a subset of individuals in our sample ($n = 408$). Finally, dogs graduate from the training program and are placed as assistance dogs or selected as breeders for the breeding program (both considered successful outcomes), or those with behavioral issues are released from the program and adopted out as pets

(C-BARQ) data, chosen to capture variability in sociable phenotypes ($n = 593$). For all participants, we obtained banked whole blood or buffy coat samples from either our own collections or from CCI's biobank. We extracted and purified genomic DNA using the DNAeasy Blood and Tissue Kit (Qiagen 69504 and 69506) following the manufacturer's protocol. We then amplified the four loci with TEs previously found to be significantly associated with human-directed canine hypersociability, Cfa6.6, Cfa6.7, Cfa6.66, and Cfa6.83 (Fig. 1), following the genotyping protocol described in vonHoldt et al. (2017). We visualized and resolved co-dominant TE genotypes on a 2% agarose gel. Linkage disequilibrium (LD) between loci was estimated using PLINK v1.90b3i (Chang et al. 2015).

Behavioral data

In order to explore genotypic associations with a wide variety of behavioral and cognitive traits, we collected data using both questionnaires—completed by people who lived or worked closely with the dog—and experiments with standardized quantitative scoring. For an overview of the data collection with respect to key points in the dog's life and training program, see Fig. 2.

Canine Behavioral Assessment and Research Questionnaire (C-BARQ)

The C-BARQ is a widely used and extensively validated questionnaire (Hsu and Serpell 2003; Duffy and Serpell 2008, 2012; Nagasawa et al. 2011) focused on various types of "problem" behaviors seen commonly in pet and working dogs. Many of the behavioral traits measured by the C-BARQ show high among-breed heritability using breed-average genetic data (MacLean et al. 2019) and moderate within-breed heritability in Labrador retrievers using pedigree-based relatedness (Ilska et al. 2017). In the present study, C-BARQs were completed when the dog was approximately 1 year old (range = 41–99 weeks; mean = 59 weeks; median = 55 weeks). At CCI's request, the questionnaire was completed by each individual's puppy raiser, the volunteer who raised and socialized CCI dogs in their homes between 2-months old and 1.5-years old (Fig. 2). In its current full-length form, the C-BARQ consists of 100 questions with Likert-style responses that are scored to contribute to 14 subscales (Duffy and Serpell 2012). For this study, we chose to focus on the nine subscales most explicitly related to aspects of sociability: attachment and attention-seeking, dog-directed aggression, dog-directed fear, dog rivalry (i.e., aggression towards family dogs in the same household), owner-directed aggression, separation-related behavior, stranger-directed aggression, stranger-directed fear, and trainability. For more information on the questions and the scoring of these subscales, see Duffy and Serpell (2012). For most of these subscales, scores higher than 0 were relatively rare; therefore, the scores were ultimately discretized into binary categories for 'no issues' (score of 0 on that factor) or 'some issues' (score > 0), as discussed in Sect. "Bayesian Linear Mixed Models". Distributions of the raw trait values from the C-BARQ are presented in Figure S7.

Internal CCI Behavioral Evaluations: In-For-Training Test & Initial Trainer Evaluation

We also used behavioral data from two internal CCI evaluations that their staff conduct on almost all dogs: the In-For-Training Test and the Initial Trainer Evaluation. The In-For-Training (IFT) test is a behavioral assessment conducted with most dogs at the beginning of professional training, approximately a week after matriculation (Duffy and Serpell 2008; Bray et al. 2019). The exact age of matriculation in the program varies across individuals for logistical reasons, leading to variability at age of phenotyping (range = 69–113 weeks; mean and median = 90 weeks). The IFT evaluates reactivity by presenting the dog with a variety of potentially stressful, standardized situations that are modeled on events that a dog might experience while working. We focused on scores from the two explicitly social scenarios: "unfamiliar dog reaction" and "threatening stranger reaction." In the unfamiliar dog scenario, the subject is led by a handler to approach a life-sized stuffed animal that looks like a realistic large-breed dog. In the threatening stranger scenario, the subject is led toward a hooded figure who is hunched over, striking a cane against the ground, and yelling. In each scenario, the dog's reaction is scored along multiple dimensions: the severity of their initial reaction, their recovery and approach after the stranger stops being threatening or their recall to the handler after the unfamiliar dog encounter, as well as additional binary variables such as presence of raised hackles or barking/growling. For this study, we conducted principal components analysis (PCA) on the measures within each of these scenarios, using the *psych* package in R (Revelle 2022), to reduce the data to a single measure for each scenario. Ultimately the continuous component scores were discretized into binary variables (< 0 vs. ≥ 0), as discussed in Sect. "Bayesian Linear Mixed Models". See Online Resource 1 for a full description of the IFT process, quantification, and PCA.

The Initial Trainer Evaluation also occurs early in training, usually a few weeks after the IFT (range = 69–119 weeks; mean and median = 93 weeks). The evaluation involves each dog's primary CCI trainer reflecting on their experiences with the dog and answering Likert-style questions relating to the dog's perceived suitability for a working role and related behaviors. This evaluation is modeled on the more widely used Behavior Checklist (BCL) from the International Working Dog Registry (2019). Again, we focused on the items with clear connections to social behaviors and/or overall success in the training program: aggression in various contexts (dog-directed, stranger-directed, and trainer-directed when challenged or corrected), attachment, distraction by dogs, distraction by other animals or objects, fear in two contexts (dog-directed, stranger-directed), separation anxiety, and "responsiveness" to handling score. For more information on these measures, see Online Resource 1. For many of these measures, as with the C-BARQ, scores higher than 0 were relatively rare; therefore, the scores on these items were ultimately discretized into binary categories for 'no issues' (score of 0 on that factor) or 'some issues' (score > 0), as discussed in Sect. "Bayesian Linear Mixed Models". Distributions of the raw trait values for the Initial Trainer Evaluation are presented in Figure S7.

Dog Cognitive Development Battery (DCDB)

For a subset of our sample ($n = 408$ dogs), we measured a variety of cognitive and behavioral phenotypes using the Dog Cognitive Development Battery (DCDB), which is composed of experimental cognitive and behavioral tasks designed to measure human-oriented social cognition, independent problem solving, working memory, inhibitory control, perceptual discriminations, and temperamental measures (e.g., neophobia and recovery when exposed to novel objects and startling events). These measures have already been reported in both puppies and adults with a subset of the individuals included in this study (Bray et al. 2020, 2021a, b) and were mostly based on measures that have been associated with training outcomes in previous studies of assistance and explosive detection dogs (Bray et al. 2017a, b; MacLean and Hare 2018). Most of these individuals were tested as puppies and then again as adults early in training, while some individuals were tested only once as adults. For this study, we chose to focus on phenotypes at the adult timepoint (range = 0.99–10.8 years; mean = 2.65 years; median = 2.01 years); while most dogs were tested around 2 years of age, a small number of breeders were tested at older ages. Out of all the DCDB measures, we focused here on the more temperamental and human-oriented social phenotypes, specifically: duration of gaze to a human experimenter during an unsolvable task ("unsolvable gaze"), duration of gaze to a human experimenter who is talking to the dog ("human interest gaze"), and duration of physical contact afterwards ("human interest contact"), engagement in a ball retrieval game with a human experimenter ("retrieval"), usage of human gestural communication using both a conventional pointing cue ("pointing") and a novel cue using a wooden block ("communicative marker"), reaction to being left alone for two minutes with a robotic cat ("novel object"), and reaction to a series of potentially startling scenarios ("surprising events"). In the case of the last two tasks, the metric used is a principal component reduction of behavioral ethogram coding, resulting in a principal component interpreted as shyness-boldness, with positive scores reflecting bolder reactions to the novel object or surprising event (Figure S2). For more information on the DCDB experimental methods and metric quantification, see Online Resource 1 and Gnanadesikan et al. (2023); for example videos of each task, please consult the electronic supplementary information in Bray et al. (2021b).

Training Outcomes

Only a subset of dogs that begin professional training ultimately graduate from the program, usually ≤ 50%. This determination is made by professional trainers based on their direct experiences working with the dog. It should be noted that the scores on the measures described above, which are collected predominantly for research purposes, do not currently affect this graduation decision directly (although the initial trainer evaluation is considered during the process of breeder selection). For the purposes of this paper, training outcomes are considered binary: success or failure (i.e., behavioral release). Analyses of training outcomes were conducted on two overlapping sets of individuals: (1) all behavioral releases and "successes" including both graduate assistance dogs and individuals chosen to be breeders in the population (mutually exclusive) ($n = 966$), and (2) all behavioral releases and "successes" including only graduates but excluding breeders ($n = 771$). Although individuals are usually chosen for breeding based on desired behavioral traits, a variety of other reasons also influence this decision, including health factors and genetic diversity. It should also be noted that some individuals included in the study due to their cognitive data are excluded from the outcome analyses because they were released from the program due to medical reasons ($n = 35$).

Statistical Analyses

Bayesian Linear Mixed Models

Following much recent quantitative genetics work, we have utilized a Bayesian approach for a variety of reasons: Both the results and uncertainty can be interpreted more intuitively, it provides more information about the extent to which a model is congruous with the data, and perhaps most importantly, modern implementations of Bayesian models (e.g., stan, brms) allow users to fit complex models—such as the ones we use here—with custom covariance structures and a variety of link functions and error distributions (Sorenson & Gianola 2002; de Villemereuil 2019). In addition, this sort of modeling framework is useful for organizations attempting to make data-informed breeding decisions (e.g., Miglior et al. 2007; Martínez-García et al. 2017). All linear modeling was conducted on a high-performance computing cluster using the *brms* package version 2.16.3 (Bürkner 2017, 2018, 2021) in R version 4.1.0 (R Core Team 2021). We used weakly regularizing priors for the beta coefficients, normally distributed with a mean of 0 and standard deviation of 1; this approach is inherently more conservative than frequentist approaches in the context of multiple hypotheses, with Type S (sign, i.e. positive vs. negative association) error rates typically < 2.5% when 95% credibility intervals are used (Gelman and Tuerlinckx 2000). Credibility intervals also more clearly reflect the uncertainty inherent in a given model and estimate, as opposed to a problematic reliance on binary significance (Hespanhol et al. 2019).

Phenotypic variables were scaled to have a mean of 0 and a standard deviation of 1. We initially fit all models without further statistical transformations of the phenotypic data, but we found that most phenotypes needed transformation for the models to reasonably fit the data; we performed posterior predictive checks, and for variables where the model predictions and observed data were dissimilar, we tried several transformations. In many cases, transforming the data using continuous transformations was still ineffective (Sect. 2.4 in Online Resource 1), so several phenotypes were discretized into binary variables, improving the model fit (examples in Online Resource 1, Figures S3–S6; raw distributions in Figure S7). The transformations and discretization methods used for each variable are indicated in Table 1, along with the corresponding model family type. Due to

Table 1 Overview of linear mixed models

Instrument	Measure	Transform	Family	N
C-BARQ	Attachment and attention-seeking	Z-Score	Gaussian	868
	Dog-directed aggression	Binary 0 or > 0	Bernoulli	856
	Dog-directed fear	Binary 0 or > 0	Bernoulli	842
	Dog rivalry “Family Dog Agg”	Binary 0 or > 0	Bernoulli	801
	Owner-directed aggression	Binary 0 or > 0	Bernoulli	877
	Separation-related behavior	Binary 0 or > 0	Bernoulli	874
	Stranger-directed aggression	Binary 0 or > 0	Bernoulli	872
	Stranger-directed fear	Binary 0 or > 0	Bernoulli	875
	Trainability	Z-Score	Gaussian	880
IFT	Threatening stranger reaction	PCA, Binary	Bernoulli	778
	Unfamiliar dog reaction	PCA, Binary	Bernoulli	781
Initial trainer eval	Aggression-challenged/corrected	Binary 0 or > 0	Bernoulli	965
	Aggression-dog	Binary 0 or > 0	Bernoulli	967
	Aggression-stranger	Binary 0 or > 0	Bernoulli	967
	Attachment score	Binary 0 or > 0	Bernoulli	964
	Distraction-dog	Binary 0 or > 0	Bernoulli	966
	Distraction-other	Binary 0 or > 0	Bernoulli	965
	Fear-dog	Binary 0 or > 0	Bernoulli	965
	Fear-stranger	Binary 0 or > 0	Bernoulli	964
	Responsiveness score	None	Cumulative	968
	Separation anxiety	None	Poisson	961
	Human interest contact	Rank normal	Gaussian	408
	Human interest gaze	Rank normal	Gaussian	408
	Communicative marker	N Incorrect	Poisson	406
	Novel object	PCA, Single component	Gaussian	408
	Pointing	N Incorrect	Poisson	407
	Retrieval	Z-Score	Gaussian	407
	Surprising events	PCA, Single component	Gaussian	406
	Unsolvable gaze	Rank normal	Gaussian	404
Training outcome	Outcome-excluding breeders	Binary	Bernoulli	771
	Outcome	Binary	Bernoulli	966

In some cases, due to the distribution of the observed data, we discretized measures into binary variables to represent no issues (0) or some issues (> 0). For more information on the measurement of each phenotype, see Online Resource 1. Full model results are available in Online Resource 2 incomplete data on individuals, sample sizes varied across models (Table 1). Binary variables were modeled using a Bernoulli distribution with a logit link function. Z-scored and rank-normalized variables were modeled using a Gaussian distribution. The two human gesture tasks (marker and pointing) were transformed to be the number of incorrect choices and then modeled using a Poisson distribution and the link function $\mu = \log$; the sign of the regression coefficients has been flipped in both the text and figure for a more intuitive visualization and interpretation of the associations between genotypes and task performance. Ordinal variables were recategorized to ensure sufficient sample size for each category and ordinal regression was performed using a cumulative logit link function.

We fit separate linear mixed models for each phenotype at each of the four loci. All models had approximately the same form: Phenotype ~ locus genotype + sex + breed + age at phenotyping + CCI training region + relatedness. Locus genotypes were modeled as 0, 1, or 2, reflecting the number of insertions at a given locus. Regarding breed, most dogs in this population are crosses between Labrador and golden retrievers, but they are not all first- or second-generation crosses that are easily categorized. We therefore used the entire population pedigree to calculate each individual's percent Labrador retriever ancestry and then constructed three binned breed categories: mostly golden ancestry (0–33.3% Labrador), relatively even breed composition (33.3–66.7% Labrador), and mostly Labrador ancestry (66.7–100% Labrador). This breed category was used as a covariate in all models and allowed us to explore the effect of breed. We chose this approach, instead of using percent Labrador ancestry as a continuous predictor, to accommodate potentially nonlinear effects of breed composition, such as crosses performing better than either golden or Labrador retrievers. The "age at phenotyping" was used for all phenotypes except training outcomes and was scaled to have a mean of 0 and standard deviation of 1. CCI training region refers to one of six regional campuses where a dog resides for the duration of professional training, as discussed above. We controlled for relatedness among individuals using an animal model and the population pedigree (Wilson et al. 2010). The animal model helps control for other genetic effects, which is especially important in this relatively inbred population; both relatedness and breed category are ways to accommodate population structure at different scales. To control for potential experimenter effects on DCDB phenotypes, the experimenter's identity was included as an additional covariate for those models; experimenter IDs associated with fewer than 15 observations were collapsed into an "other" factor level. We focus our interpretation of results on cases in which the 95% credibility interval did not overlap 0, but we occasionally comment on additional associations near this arbitrary threshold. Full model results and diagnostic values are provided in Online Resource 2; genotypic, phenotypic, and demographic data are provided in Online Resource 3; the genetic relatedness matrix is provided in Online Resource 4.

Haplotype Phasing and Modeling

We also explored whether phased transposon insertion alleles provided more information on behavior than analyses of single-locus alleles. We found some concordance with the locus-level results; however, in general, the categorical nature of the haplotypes made interpretation challenging. For information on the phasing, haplotype statistics, and haplotype linear model results, see Online Resource 1.

Results

Genotyping Results

We genotyped TE insertions at the four loci previously associated with human-directed hypersocial behavior (see Fig. 1) for 1001 dogs from CCI for whom we also had behavioral phenotypes. Concordant with previous findings, we found that the TE insertion at Cfa6.6 had the highest frequency of insertion ($f_{INS} = 0.706$); this insertion was present on average in at least a single copy ($mean \pm s.d. = 1.4 \pm 0.7$ insertions). Further, the high frequency of an insertion at Cfa6.6 manifested as a homozygous insertion in 52% of the dogs genotyped ($f_{homNI} = 0.108$, $f_{het} = 0.373$, $f_{homINS} = 0.520$). Other loci were more variable, often lacking the TE insertion (Cfa6.7 = 0.49 ± 0.6, $f_{INS} = 0.247$; Cfa6.66 = 0.55 ± 0.6, $f_{INS} = 0.274$; Cfa6.83 = 0.59 ± 0.7, $f_{INS} = 0.294$; see Figure S1 in Online Resource 1). All loci met expectations of Hardy-Weinberg equilibrium ($p > 0.05$). The only detectable linkage disequilibrium between loci in our sample was between the two loci that were physically closest and within the same gene: Cfa6.6 and Cfa6.7 (r = – 0.37).

Behavioral and Cognitive Associations

We used Bayesian linear mixed models to assess associations between our phenotypes and genotypes at each of the four loci (see Table 1 for an overview of each model). A small number of phenotypes exhibited strong associations that met our credibility threshold. On the questionnaire measures, we found strong associations for dog distraction, aggression when challenged or corrected, and separation-related behavior (Fig. 3). Insertions at Cfa6.83 were associated with decreased dog distraction ($\beta = -0.43$, $CI_{95\%} = -0.74, -0.11$), but increased aggression when challenged or corrected ($\beta = 0.55$, $CI_{95\%} = 0.17, 0.93$). In contrast, insertions at Cfa6.6 ($\beta = -0.37$, $CI_{95\%} = -0.68, -0.05$) and Cfa6.66 ($\beta = -0.39$, $CI_{95\%} = -0.79, -0.02$) were

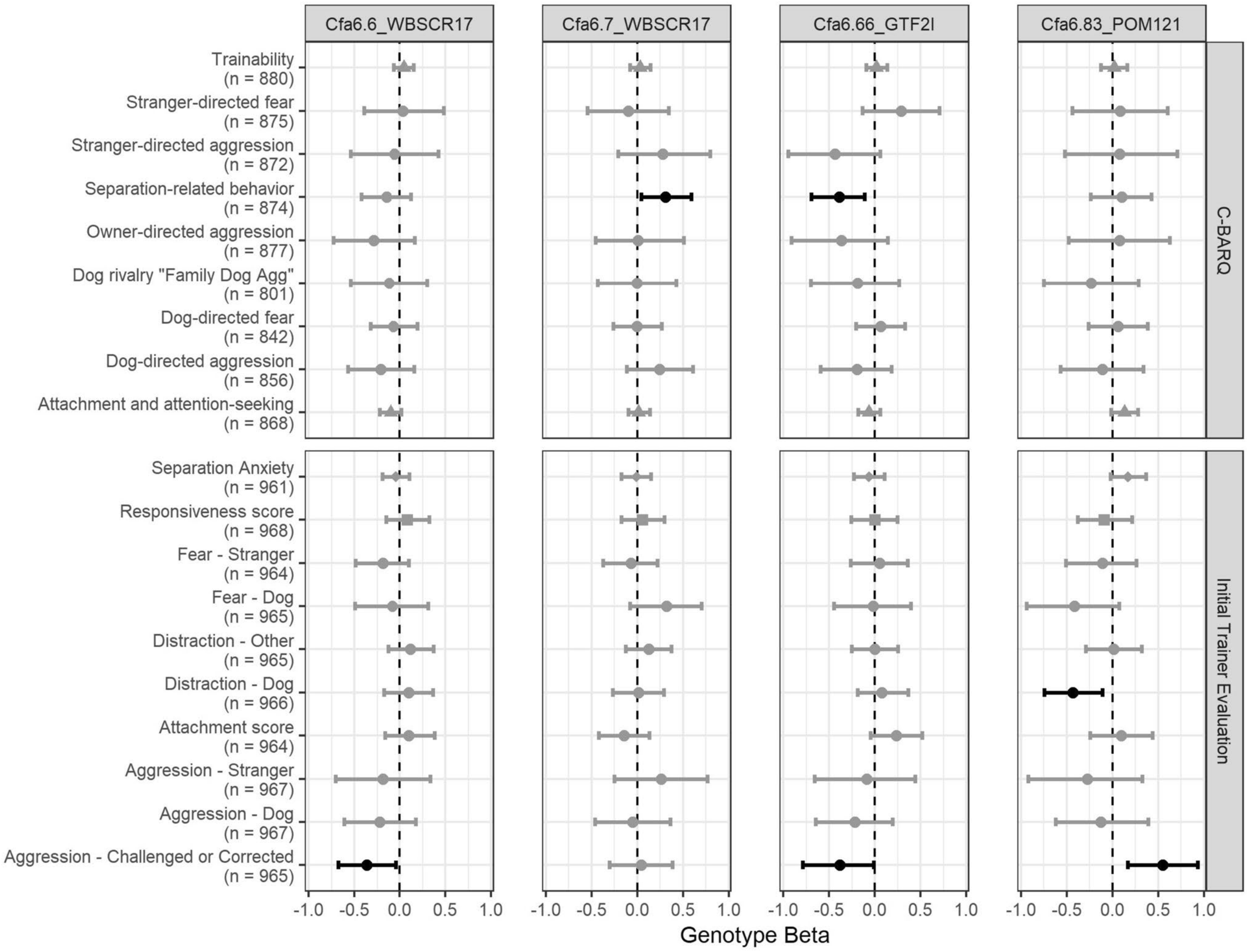

Fig. 3 Associations between genotypes and questionnaire-based phenotypes from the C-BARQ and Initial Trainer Evaluation. Credible associations were observed for C-BARQ separation-related behavior, dog distraction, and aggression when challenged or corrected. The points represent the means of the posterior distributions, and the error bars represent the 95% credible intervals. Associations that meet the 95% credible interval threshold are in black, while all others are grey. Point shape reflects the model type used (circle = Bernoulli, triangle = Gaussian, square = cumulative logit ordinal, diamond = Poisson, see Table 1 for model details)`

associated with decreased aggression when challenged or corrected. A higher incidence of separation-related behaviors on the C-BARQ was associated with more insertions at Cfa6.7 ($\beta = 0.31$, $CI_{95\%} = 0.04, 0.59$) and fewer insertions at Cfa6.66 ($\beta = -0.39$, $CI_{95\%} = -0.7, -0.11$). Although not rising to our credibility threshold, we found associations of a similar magnitude and direction as separation-related behavior for both stranger-directed and dog-directed aggression with both Cfa6.7 and Cfa6.66 (Fig. 3).

Among our experimental measures, the only phenotype with associations meeting our credibility threshold was the reaction to an unfamiliar dog (Fig. 4): insertions at Cfa6.66 were associated with less reactivity ($\beta = -0.37$, $CI_{95\%} = -0.65, -0.1$), while insertions at Cfa6.7 were associated with more reactivity ($\beta = 0.33$, $CI_{95\%} = 0.04, 0.62$).

None of the phenotypes from the Dog Cognitive Development Battery (DCDB) showed results that met our threshold (Fig. 4). However, insertions in Cfa6.66 were moderately associated with increased eye contact with a human experimenter during an unsolvable task ($\beta = 0.14$, $CI_{95\%} = -0.04, 0.32$), while insertions in Cfa6.7 were moderately associated with decreased performance on the communicative marker task ($\beta = -0.26$, $CI_{95\%} = -0.53, 0.01$).

We also found credible breed effects for some of our phenotypes, including multiple measures on the Initial Trainer Evaluation, human interest contact on the DCDB, and threatening stranger reaction on the IFT (Fig. 6, S8). Full model results and diagnostic values are provided in Online Resource 2.

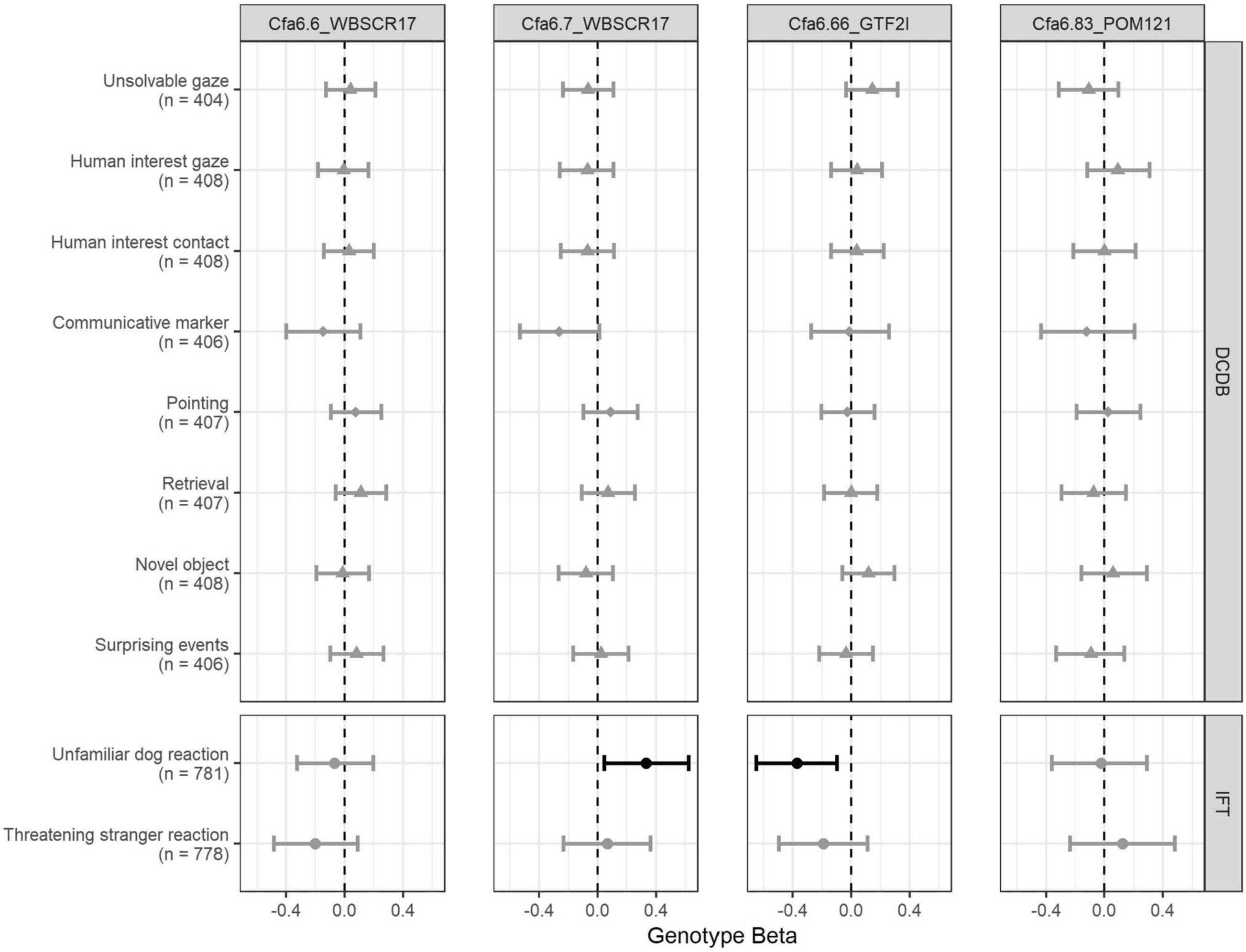

Fig. 4 Associations between genotypes and experimental phenotypes. Credible associations were observed only for the unfamiliar dog reaction, in Cfa6.7 and Cfa6.66. The plotted points represent the means of the posterior distributions, while the error bars represent the 95% credible intervals. Associations that meet the 95% credible interval threshold are in black, while all others are grey. Point shape reflects the model type used (circle = Bernoulli, triangle = Gaussian, square = cumulative logit ordinal, diamond = Poisson, see Table 1 for model details). Note: the communicative marker and pointing tasks required transformation to number of incorrect choices and have therefore been plotted with the sign of the beta values flipped for a more intuitive visualization and interpretation of the associations between genotypes and task performance

Training Outcomes

Association analyses between training outcomes and our four loci did not reveal any results that met our 95% credibility threshold. However, the strongest association was found for the polymorphic TE insertion at locus Cfa6.66 in the gene *GTF2I* ($\beta = 0.23$, $CI_{95\%} = -0.04, 0.49$, $LOR = 1.25$; Fig. 5), indicating that a single insertion at this locus was associated with a 25% increase in the odds of success in the training program. This result was robust to the exclusion of breeders from the analysis, although the credible intervals were wider with fewer individuals ($\beta = 0.24$, $CI_{95\%} = -0.14, 0.63$, $LOR = 1.27$; Fig. 5). Breed was strongly associated with success in the training program when breeders were excluded from the analyses; dogs with high-percent Labrador ancestry were the most likely to graduate (Fig. 6, S8).

Discussion

Previous work associated four TEs in the WBS critical region of the canine genome with human-directed hypersociability (vonHoldt et al. 2017) and found that assistance dogs differed from pet dogs in their patterns of TE insertions (Tandon et al. 2019). We explored the relationships between these four loci and a larger variety of behavioral and cognitive measures in a single large assistance dog population and report several specific genotype-phenotype

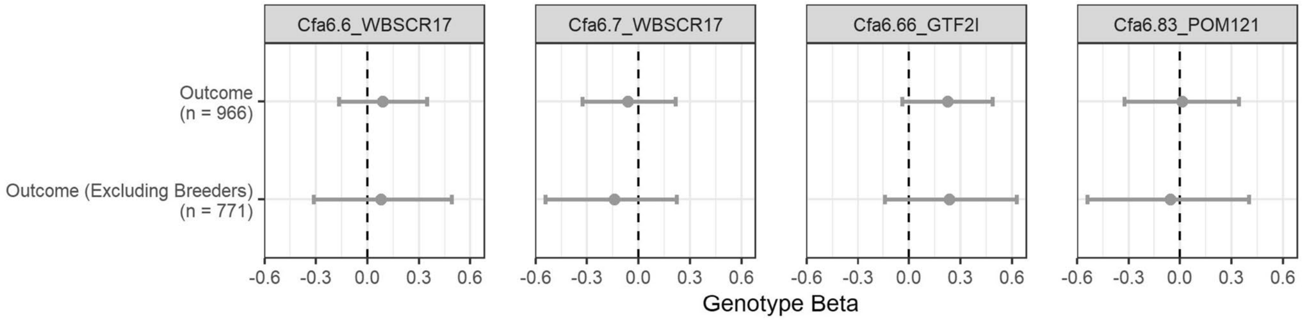

Fig. 5 Associations between genotypes and training outcomes. Results are provided for two sets of models: in the first, breeders (along with graduated assistance dogs) are considered 'successful' outcomes, while in the second, breeders are excluded from the model to provide a cleaner contrast (because breeder decisions also depend on other health and genetic diversity factors), although the sample size is then smaller. The points represent the mean of the posterior distribution, and the error bars represent the 95% credible intervals. While none of the associations meet the 95% credible interval threshold, the association for Cfa6.66 in *GTF2I* is relatively strong, with betas translating to an odds ratio of 1.25–1.27, with and without breeders included, indicating that insertions in *GTF2I* increase the chances of success in the training program

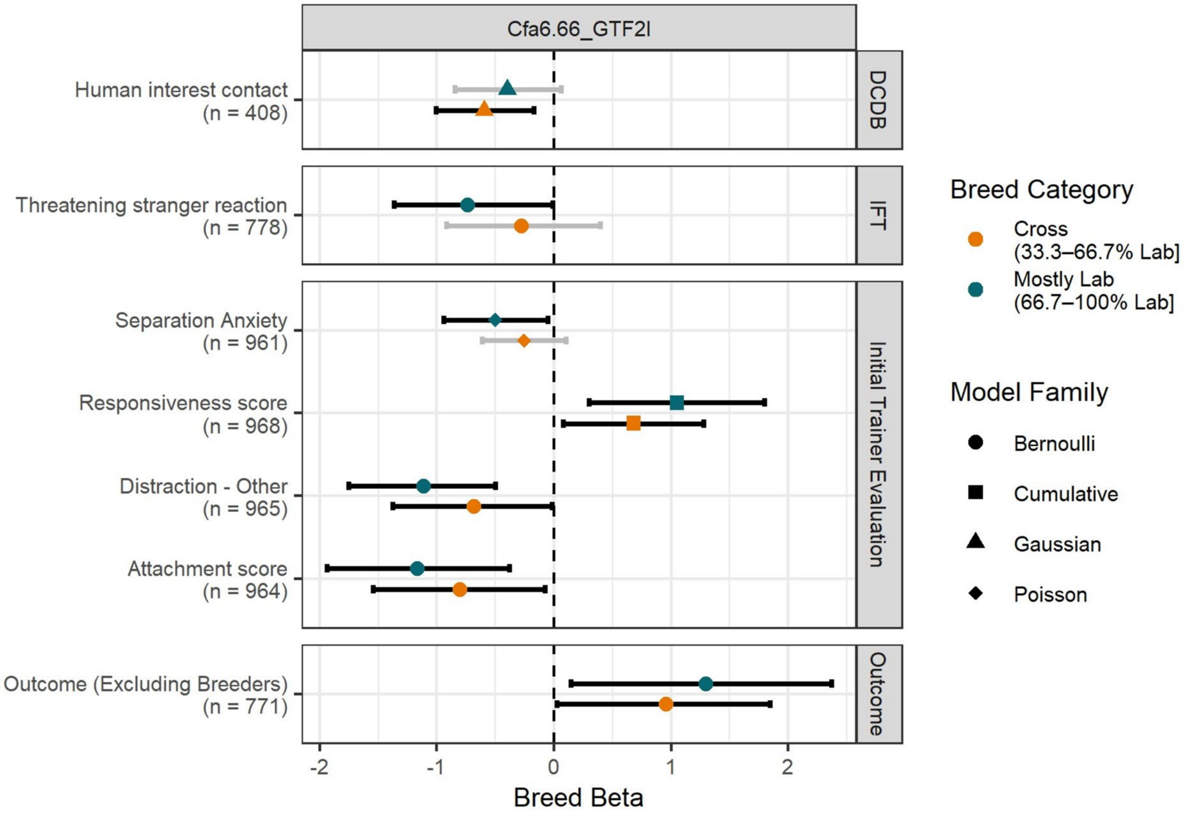

Fig. 6 Credible breed effects across phenotypes from the locus Cfa6.66 models. Only phenotypes with a credible effect (black error bars) for at least one breed category are shown. The plotted beta estimates are for the breed categories "Cross [33.3–66.7% Lab]" (orange points) and "Mostly Lab [66.7–100% Lab]" (teal points), with "Mostly Golden [0-33.3% Lab]" as the reference level and therefore not plotted. We show only the results for one locus here, but the pattern is similar across loci models, as these breed effects are for the phenotypes themselves and not primarily explained by these loci. For all phenotypes and loci, see Figure S7 in Online Resource 1

associations. We found strong genotype-phenotype associations for separation-related problems, aggression when challenged or corrected, and reactions to other dogs. We did not find any cognitive associations that met our credibility threshold, although it should be noted that the sample sizes for the cognitive testing were about half of the sample sizes for the other phenotypes, potentially contributing to the lack of credible associations.

We next assessed associations between insertions at each locus and outcomes in the CCI training program. The locus with the strongest association with training success was Cfa6.66 in *GTF2I*. Previous comparisons of assistance and pet dogs have relied on population level comparisons, which are prone to confounds, including founder effects; here, we have data on both successful and unsuccessful dogs in the same population, circumventing these issues. This may be one reason why some of our results differed from Tandon et al. (2019); specifically, they reported that insertions at locus Cfa6.6 were most important in terms of predictive power for sociability across pet and assistance dogs, and that German Shepherd assistance dogs had more insertions—and increased homozygosity—at this locus. Our results suggest that locus Cfa6.66 is more important in the CCI population. For example, where Tandon et al. (2019) found an association between insertions at Cfa6.6 and separation distress, we found associations for loci Cfa6.7 and Cfa6.66. Tandon et al. (2019) also found multiple strong associations for locus Cfa6.66, with specific questions of the C-BARQ, especially in their combined sample of pet and assistance dogs aged 1–5 years; however, one of their strongest results was a positive association for stranger-directed aggression, where our results suggest a negative association in this population.

Across our phenotypes, all four loci demonstrated at least one association that met our credibility threshold. However, Cfa6.66 in the gene *GTF2I* consistently emerged as the most important locus, with the largest number of strong phenotypic associations and the strongest association with training outcomes. To illustrate within one family the statistical associations found in the population more broadly, we present the pedigree of a single litter with the entire range of genotypic variation in the *GTF2I* locus, along with the phenotypes for the three traits credibly associated with this locus in the analyses reported above (Fig. 7). Our results emphasize the ability for structural variants to explain individual variation in social behavior.

The gene *GTF2I* encodes a transcription family II–I transcription factor that activates transcription in most cellular contexts, has multiple splice variants, and is implicated in several neurocognitive disorders, systemic lupus erythematosus, and cancer (Roy 2017). Further, mouse models have demonstrated that homozygous deletions of *GTF2I* are embryonic lethal due to defects in neural tube closure and exencephaly during gestation; mouse models generated to be heterozygous for this deletion have altered social behavior, while learning and memory remain mostly intact in non-social contexts (Sakurai et al. 2011). A selective *GTF2I* knockout in excitatory neurons within the embryonic mouse forebrain increases sociability and anxiety while decreasing myelination, suggesting that impaired axonal conductivity is the causative mechanism; these behavioral phenotypes in mice were rescued by the administration of drugs that restore myelination or improve axonal conductivity by selectively blocking potassium channels (Barak et al. 2019).

In humans, two SNPs in *GTF2I* have been associated with autism spectrum disorder (Malenfant et al. 2012), as well as both social anxiety and social-communication abilities in healthy populations (Crespi and Hurd 2014). Intriguingly, one of these *GTF2I* SNPs has also been associated with reactivity of salivary oxytocin—a neuropeptide implicated in affiliative social behaviors—after watching a video designed

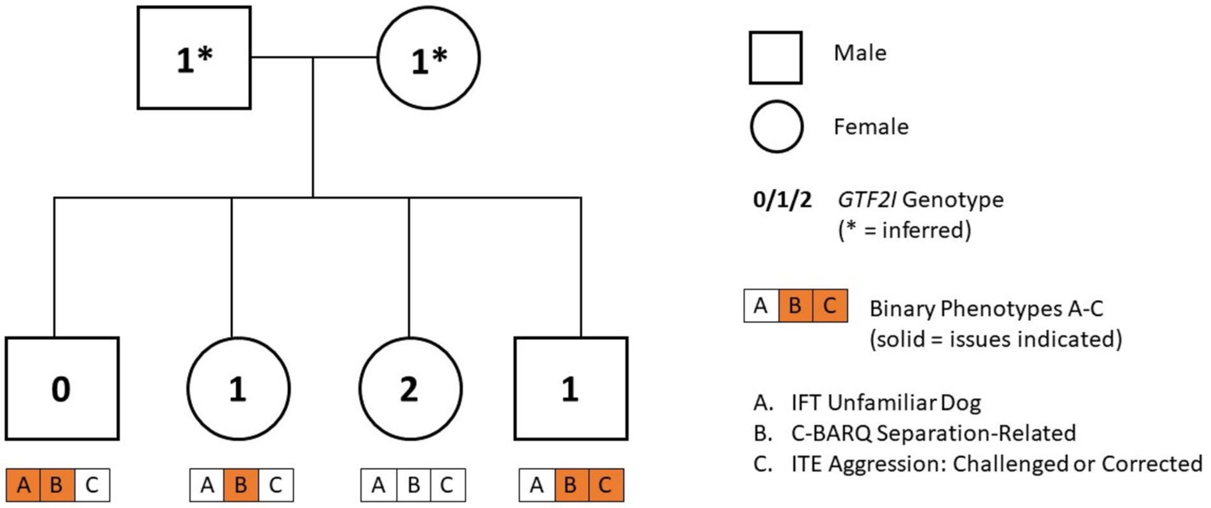

Fig. 7 A case of *GTF2I* variation among littermates. Individuals in this litter exhibited the entire range of genotypic variation, with corresponding variation in the three phenotypes identified as having credible associations with this locus. This illustrates within one family the statistical associations across the entire population that are captured in our linear mixed models

to evoke empathy (Procyshyn et al. 2017). Examination of individuals with only a partial deletion of the WBS critical region also suggests the importance of *GTF2I*—as well as the related *GTF2IRD1* and *GTF2IRD2*—for social cognition, including for social judgment and theory of mind tasks (Serrano-Juárez et al. 2021). Behavioral phenotypes seem to be dosage sensitive in both directions, affected not only by deletions, but also duplications. Experimental duplication of *GTF2I* causes increased separation anxiety in mouse pups, while in human children, duplication of the entire WBS critical region has been associated with increased risk of separation-anxiety disorder (Mervis et al. 2012). Our results in dogs add to these findings in humans and rodents and emphasize the importance of *GTF2I* in social behavior, with some concordance in specific phenotypes, such as separation-related behaviors. Insertions at this locus in *GTF2I* appear to be the ancestral state (i.e., much more common in wolves than dogs). This suggests that domestication may have resulted in hypersociability that is actually counterproductive for certain aspects of assistance work, such as separation-related behaviors.

Across both behavioral phenotypes and outcomes, we observed that breed was an important variable in some of our models (see Online Resource 1 for more on breed effects). Combined with the high heritability of some of these traits (Bray et al. 2021a), this finding points to the importance of other genetic factors not explored here. While the WBS critical region—and especially *GTF2I*—is clearly important for some of these behaviors, it is likely that these phenotypes are highly polygenic and further research will illuminate additional genetic contributions to these traits.

Dogs are frequently used for a variety of jobs in modern society, ranging from herding and guarding dogs for livestock handling and protection to service dogs for people with disabilities. Despite their importance for human livelihoods, working dog training programs are expensive and have low success rates, often ≤ 50% (Cobb et al. 2015; Bray et al. 2019, 2021c). On average, the cost to breed and train an assistance dog is estimated to be approximately $50,000 per dog, and many organizations have waiting lists of 1–2 years (Wirth and Rein 2008). Any information that could be used to more efficiently select, breed, and train dogs could thus have a large impact on the availability of dogs for people with disabilities. Such information could lead to targeted decisions about breeder selection and pairing, data-informed training strategies, or simply data-informed decisions at the program level to focus resources on the dogs most likely to succeed.

Many working dog organizations and dog breeders already conduct genetic screens for various health risk factors to reduce the risk of certain conditions in their populations. For example, there are simple genetic screens for exercise-induced collapse, ichthyosis, hereditary nasal parakeratosis, and progressive rod cone degeneration. However, no such screens have yet been implemented for behavioral traits. We were therefore interested in exploring the contribution of the WBS critical region to both specific behavioral and cognitive phenotypes and to success as a working dog. We suggest that this region of dog chromosome six could be used to screen for behavior, probably best in combination with behavioral phenotypes, although more data should be collected before implementation. It is also possible that these results will be useful to other organizations who breed and train working dogs for a variety of purposes. However, we caution that our results are potentially specific to the CCI population, and that different founding effects and breed compositions could result in population-specific effects. Furthermore, it is increasingly clear that there are differences between organizations in terms of what behavioral phenotypes are ideal for the dog's working role (Bray et al. 2021c). Thus, it will be important to collect more data in other populations before implementing genotypic prediction tools in the assistance dog sector.

Conclusion

Our results demonstrate the importance of the WBS critical region of dog chromosome six to both social behavior and assistance dog training success. While the downstream consequences of these TEs are not yet fully understood, it is known that all four loci affect transcription and chromatin interactions. Thus, although the genes themselves are still intact in dogs—unlike the hemizygous deletion in human Williams–Beuren Syndrome—the insertions likely have similar, although perhaps more subtle, dosage-dependent effects on social behavior. The current results indicate that genotype screening at an early age could help predict some behavioral phenotypes, which might be leveraged to improve the efficiency of training in this population, while breeding decisions informed by these genotypes might help the population achieve higher success rates, although more research is needed on both fronts. This research can also be expanded to other populations of working dogs to inform breeding and training decisions, although some differences across populations, breeds, and different working roles should be expected.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10519-023-10166-7.

Acknowledgements We would like to acknowledge the contributions of many individuals in collecting the phenotypic data in this study. First, we thank Canine Companions for their ongoing commitment to research and collaboration, especially Kerinne Levy, as well as all of the Canine Companions' early care and kennel technicians, veterinary and breeding team, and training staff; this work would not have been possible without their support and collaboration. We also acknowledge the following students, lab coordinators, and other volunteers who helped us conduct the cognitive testing and video coding: Carter Bradshaw, Cooper Bradshaw, Molly Byrne, Averill Cantwell, Richard Cawood, Christina Cawood, Amanda Chira, Alexzia Clark, Zachary Cohen, Laura Douglas, Abby Flyer, Kyla Guinon, Erin Hardin, Stephanie Hargrave, Theresa Hatcher, Victoria Holden, Daniel Horschler, Emily Humphrey, Camden Olson, Facundo Ortega, Alessandra Ostheimer, Camila Risueno-Pena, Amber Robello, Kerri Rodriguez, Ashley Ryan, Rachel Shaberman, Paige Smith, Holland Smith, Mia Wesselkamper, Kacey Bauer, Amelia Byrony, Elizabeth Carranza Acevedo, Leah Chaudoir, Victoria Coon, Alex Evans, Gabi Garcia, Jen Geary, Mia Hickey, Ada Lam, Jessica Nelson, Gianna Ossello, Ellenor Spencer, Lorelei Switzer, and Lily Tees.

Author Contributions GEG: conceptualization, methodology, formal analysis (linear models), investigation, data curation, visualization, writing—original draft, writing—review and editing, resources, funding acquisition, project administration. DT: conceptualization, formal analysis, writing—original draft, writing—review and editing. EEB: conceptualization, methodology (phenotyping), investigation (phenotyping), data curation, writing—review and editing, funding acquisition. BSK: investigation, writing—review and editing, resources, project administration. ST: formal analyses (haplotype network), visualization (haplotype network), writing—review and editing. ELM: conceptualization, methodology, writing—review and editing, supervision, resources, funding acquisition. BMH: conceptualization, methodology (genotyping), investigation (genotyping), writing—original draft, writing—review and editing, supervision, resources, funding acquisition.

Funding This study was supported by funding from Nestlé Purina (#90031348 to B.M.V.), the National Science Foundation Graduate Research Fellowship Program (DGE-1746060 to G.E.G.), the P.E.O Scholar Award (to G.E.G), the Office of Naval Research (ONR N00014-17-1-2380 and N00014-20-1-2545 to E.L.M.), and the American Kennel Club Canine Health Foundation (Grant No. 02518 to E.E.B and E.L.M.). Any opinions, findings, and conclusions or recommendations expressed in this paper are those of the authors and do not necessarily reflect the views of these institutions.

Declarations

Competing Interests Gitanjali E. Gnanadesikan, Dhriti Tandon, Emily E. Bray, Brenda S. Kennedy, Stavi R. Tennenbaum, Evan L. MacLean declare that they have no conflict of interest. Bridgett M. vonHoldt has filed a patent “Early genetic screening to aid in the selection of dogs for assistance training programs” (US20210047699A1).

Ethical Approval Blood draws and testing procedures for the DCDB were approved by the University of Arizona Institutional Animal Care and Use Committee (IACUC #16-175) and were collected in accordance with relevant guidelines and regulations. All other data was collected directly by Canine Companions as part of their training program and shared with the authors.

Human and Animal Rights and Informed Consent Participation in all cognitive and behavioral testing was voluntary for the dogs, using established opt-out criteria. There were no human subjects in this research, and thus informed consent is not applicable.

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