# The Genetics of Canine Skull Shape Variation

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**ABSTRACT** A dog's craniofacial diversity is the result of continual human intervention in natural selection, a process that began tens of thousands of years ago. To date, we know little of the genetic underpinnings and developmental mechanisms that make dog skulls so morphologically plastic. In this *Perspectives*, we discuss the origins of dog skull shapes in terms of history and biology and highlight recent advances in understanding the genetics of canine skull shapes. Of particular interest are those molecular genetic changes that are associated with the development of distinct breeds.

SOMETIME during the Paleolithic, a remarkable transformation occurred. Small numbers of gray wolves adopted a new pack master—humans. Through the process of domestication, the modern dog emerged. Today most dogs share little resemblance to their lupine ancestors. As a result of artificial selection, dogs radiated to fill niches in our lives, becoming our herders, guardians, hunters, rescuers, and companions (Wilcox and Walkowicz 1995). The range of sizes among dogs extends beyond that of wolves, giving dogs the distinction of being the most morphologically diverse terrestrial mammalian species known (Stockard 1941). Equally dramatic to humans' effect on scale, is the effect on the dog's facial features, particularly the skull (Figure 1).

Given its prominence as a hallmark for domestication and its indication of breed identity, we devote the remainder of this review to a discussion of our current understanding of canine skull diversity and its mechanistic underpinnings as they relate to domestication, genetics, and disease. Although every attempt is made to keep our discussion skull-centric, many of the topics broached are pertinent to other traits that distinguish different dog breeds, as are the genomic techniques currently employed to map and validate causal genetic variation underlying skull morphology. In such situations we have, of necessity, made our discussion broader to keep it inclusive.


doi: 10.1534/genetics.112.145284
Supporting information is available online at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.145284/-/DC1.
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## Variation in Skull Shape and Dog Domestication

Molecular clock estimates from mitochondrial DNA suggest domestication started as early as 135,000 years ago (Vilà *et al.* 1997). More conservative estimates are based on archaeological records, which indicate that dog domestication began somewhere between 15,000 and 36,000 years ago (see summary by Larson *et al.* 2012). Current archaeological estimates depend on carbon dating of bones, whose morphologies appear distinct from that of contemporary wolves. In many cases, these distinctions are pronounced in both the skull and its dentition. This suggests that, among incipient dogs, the skull was at the leading edge of several anatomical changes that would transform wolves. For example, prehistoric dog skulls excavated in Russia were from massive animals that had shortened snouts and widened palates (Sablin and Khlopachev 2002). Elsewhere, ancient dogs were smaller than wolves (Napierala and Uerpmann 2010).

Like other large domesticates, modern dogs exhibit an increased brain-to-body-size ratio (termed encephalization). Yet the overall size of dogs' brains relative to that of wolves has decreased by nearly 30% (Coppinger and Schneider 1995; Zeder 2012). This decrease is particularly acute in the limbic system, which is integral to fight or flight responses (Coppinger and Schneider 1995; Zeder 2012). Domestication may have, therefore, reduced areas of the wolf brain that enabled tolerance to human contact. It is likely that skull shape changed in response to changes in brain morphology. As proof of principle, during the 40 years it took to domesticate silver foxes, in a well-documented experiment performed in Novosibirsk, Russia, changes in cranial dimensions, among other morphologic features,

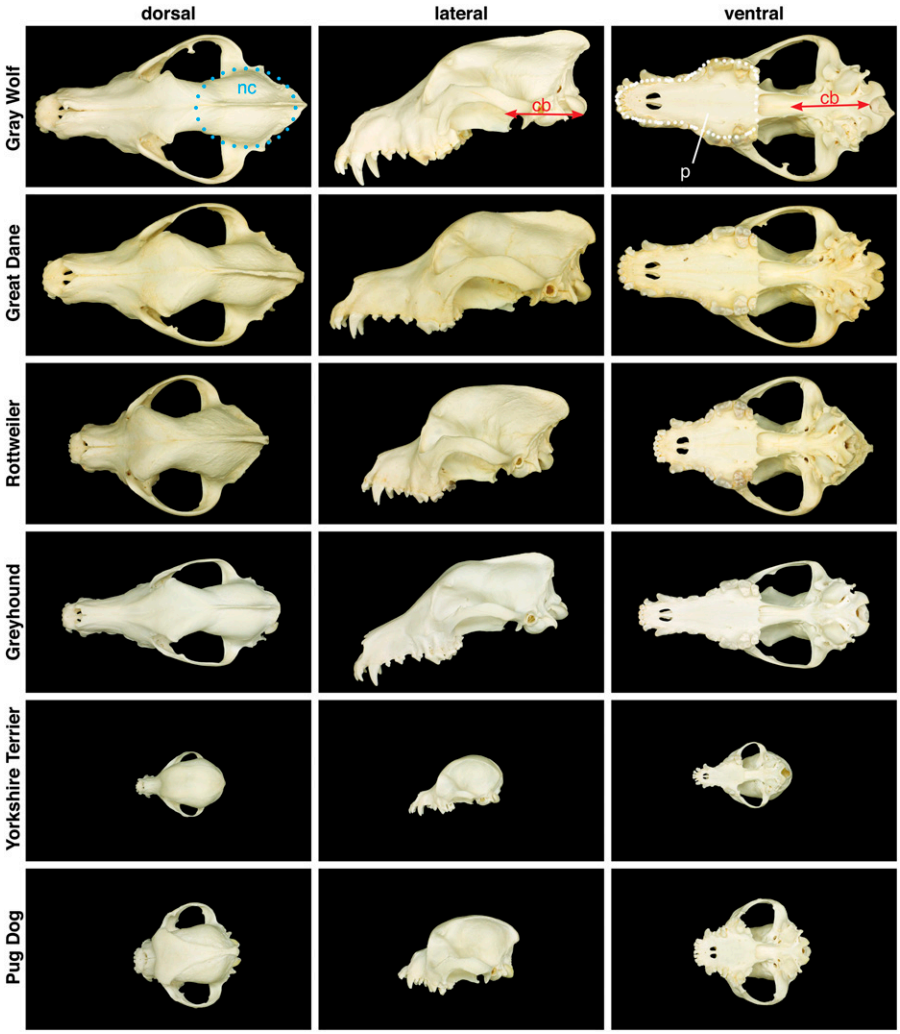


**Figure 1** A montage of canine craniofacial shape demonstrates the incredible morphologic diversity of *Canis familiaris*. Dorsal, lateral, and ventral perspectives of various breeds of dogs. Lateral views are articulated so that the skull base (red line, wolf) is approximately parallel between breeds. Prominent differences across breeds include palate shape (p, indicated by white dots), neurocranium shape (nc, enclosed by blue dots), cranial base length (cb, red line). Also note the angle of the palate relative to the cranial base.

were also noted as a correlate to tameness (Trut 1999; Zeder 2012).

Some have likened dogs to wolf pedomorphs, proposing that dogs are juvenilized wolves that are developmentally restrained in behavior and physical maturation (Gould 1977; Wayne 1986; Coppinger and Coppinger 2001; Drake and Klingenberg 2010; Drake 2011). Even in adulthood, a vast number of modern breeds display some sort of growth arrest, likened to wolf neoteny. With a few exceptions, modern dog breeds are smaller and have snouts and crania that are proportionally intermediate to wolf neonates and adults.

However, the idea that pedomorphism is a major driver of canine craniofacial variation is not without controversy. Certain skull characteristics, such as the angle between the palate and the neurocranium, appear static during wolf development, whereas in dogs the angle differs substantially from that of wolves' throughout development, leading to the conclusion that dog skull shape is neomorphic (Drake 2011). This debate can be settled only by developing a genetic understanding of the variation underlying canine skull diversity. Identification of causative genetic mechanisms will also enable inference of the evolution of dog skull shape and provide insight regarding the demographics of wolves or ancestral dogs from which this variation emerged.

## Emergence of Dog Breeds

By the 19th century, a new fad had swept across Europe that the British not only embraced, but also actively promoted. Dog fanciers began breeding and trading dogs that were "specialized" for both physical and behavioral traits. With the advent of the Kennel Club in 1873, lineages became standardized by appellation, bloodline, appearance, and behavior. Thus, to be recognized as a "bulldog," it was insufficient for a dog to be squat in stature and display a shortened snout. Rather, a purebred bulldog had to conform to the club's breed-specific standards, and, most importantly, the dog's parents had to be club-registered bulldogs, ensuring bloodline purity.

Dog fanciers quickly recognized that structured breeding could be harnessed to transmit desirable traits. But how such selection could work on a grand scale was not immediately appreciated and attempts to parse the genetic mechanisms that dictate how canine traits such as skull

shape are passed from parents to offspring were not formally studied until decades later. Most prominent were the studies conducted by Charles Stockard, whose detailed dog pedigrees include designed crosses and backcrosses of morphologically disparate breeds (Stockard 1941). On the basis of live observation and analysis of skeletal preparations of parents and progeny, Stockard concluded that breed-defining skull shape features, such as the bulldog's shortened rostrum, did not follow patterns of Mendelian inheritance. Nearly 70 years would pass until genome-wide association studies (GWAS) would confirm Stockard's predictions (Boyko *et al.* 2010; Schoenebeck *et al.* 2012).

Three factors were key to the acceptance of the dog as a system for studying genetics. First, geneticists recognized that different dog breeds were characterized by an enormous variety of genetically fixed morphologic traits whose genetic underpinnings were likely to enlighten our understanding of mammalian developmental biology. Second, it also became clear that the population structure of domestic breeds would allow geneticists to overcome many of the difficulties faced when doing either linkage or association studies in human populations. With >400 documented breeds worldwide, complex traits including morphologic traits such as body size, bone length and width, and skull shape, as well as disease susceptibility or even behavior could be disentangled by studying dog breeds, and the results would likely be applicable to other mammalian systems, including human (Karlsson and Lindblad-Toh 2008; Shearin and Ostrander 2010; Ostrander 2012). Finally, it became clear that the genomic methods under development for navigating the mouse and human genomes were readily transferable to studying the dog genome. The rapid development of both genetic (Mellersh *et al.* 2000) and physical (Guyon *et al.* 2003) maps of the dog, followed by a 7.5× sequence and draft assembly (Lindblad-Toh *et al.* 2005), put the dog on par with traditional model organisms for performing genetic studies.

## Modern Domestic Dog Breed Variation

Today, >400 breeds of dogs exist worldwide. Together, the morphological variation among these breeds is so diverse and readily discernible that, for many, skull shape is breed-defining (Figure 1). Some canine skull conformations are named after their resemblance to human craniosynostoses, such as brachycephaly and dolichocephaly (Figure 2). Brachycephalic breeds, such as the bulldog, pug, and Boston terrier, are easily recognized by their short "pushed-in" faces, underbite, and widely placed, shallow orbits. Brachycephaly, which means "short head," is a term borrowed from human medicine. In dogs, a number of craniofacial anomalies can contribute to brachycephaly, including a reduction in the length of bones that form the rostrum, chondrodysplasia of the cranial base, and changes in the palate position relative to the cranial base (Figure 2; Huber 1974; Nussbaumer 1978).

At the other end of the continuum is the dolichocephalic appearance of breeds such as the Saluki, Borzoi, and collie. These dogs tend to have narrow, sometimes elongated, snouts and orbitals that are less forward set. A dolichocephalic morphology is exactly what one would predict based on the relationship between morphology and ecology/hunting behavior of wild canids. Many dolichocephalic breeds such as Afghans and Salukis were originally bred for coursing small prey (Figure 2). Thus it make sense that these sighthounds would have a craniofacial configuration predicted to enhance horizon scanning (Miller and Murphy 1995; McGreevy *et al.* 2004), as is necessary for spotting prey.

Angulation between the skull base and hard palate also differs substantially between dog breeds. Klinorhynchy, the hallmark downward-pointing snout of bull terriers, is morphologically opposite to the rostrum angle observed in breeds such as the boxer and bullmastiff. These examples of brachycephalic breeds display extreme airorhynchy, meaning that their rostra angle dorsally. Thus, the rostrum angle of the bull terrier and the boxer represent opposing extremes of an interbreed continuum (Figure 3A; Nussbaumer 1982).

The neurocranium also bears discussion. Many small dogs from toy and teacup breeds feature brain cases reminiscent of human hydrocephalus. An extreme example is the Chihuahua, whose American Kennel Club breed standard describes the desirable skull as having an "apple dome" and also as permitting open fontanelles—holes within the cranium due to incomplete closure of the skull's sutures (American Kennel Club 2006). The neuroanatomy of brachycephalic dogs is also quite unusual as the brain is rotated with respect to its mediolateral axis (Roberts *et al.* 2010). Rotation of the brain in these breeds raises a question about cause and effect: Does the rotation of the brain influence cranial vault shape or vice versa?

Using geometric morphometric analysis, Drake and Klingenberg (2010) found evidence of modularity between the rostrum and the neurocranium of dogs, such that changes in rostrum shape are not strictly correlated with shape changes in the neurocranium. This observation raises the possibility that mixing and matching genetic variants that independently regulate development of either structure enrich canine skull diversity. In mammals and avians, the rostrum and neurocranium are primarily derived from neural crest and paraxial mesoderm, respectively (Noden and Trainor 2005). It is tempting to speculate that canine skull modularity was achieved through selection of genetic variants whose effects alter the development of one or both bone-forming tissues types (Nussbaumer 1976; Noden and Trainor 2005).

## Candidate Genes for Skull Variation

Although the morphological variation of canine skulls has been described extensively, the identification of underlying

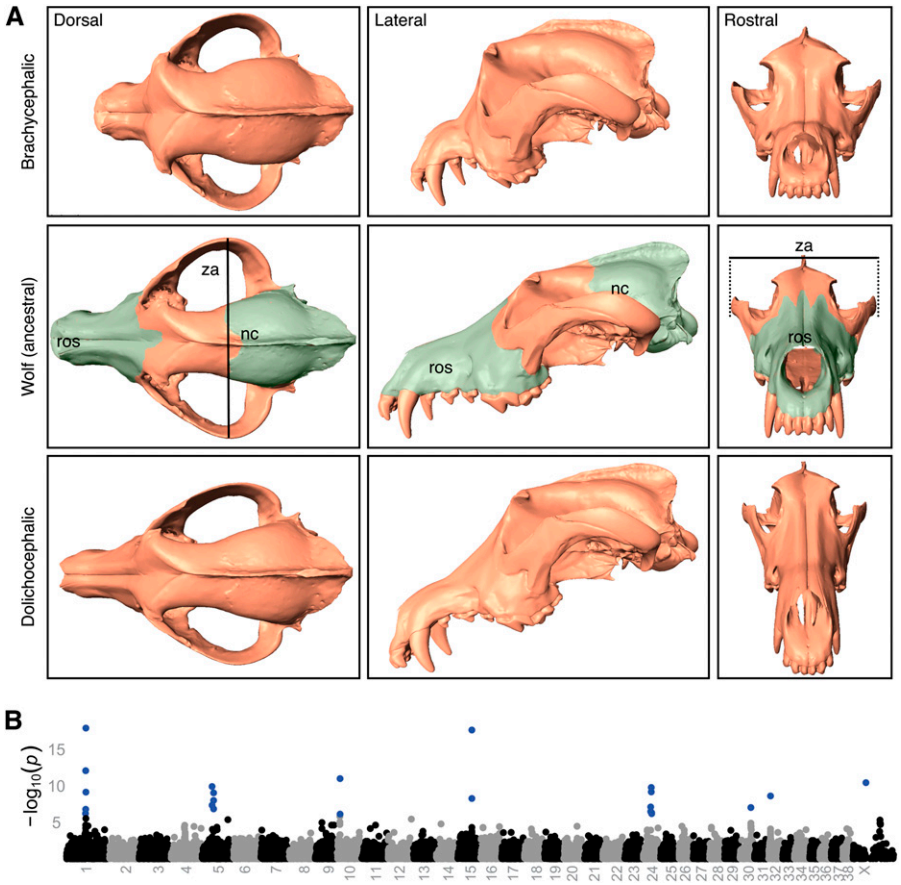


**Figure 2** Canine skull length is a complex trait. (A) Surface scans of a wolf skull morphed to illustrate the differences between brachycephalic, ancestral, and dolichocephalic skull states of canids are shown. Brachycephalic dog breeds have a shortened rostrum (ros), wide zygomatic arches (za), and a rounded neurocranium (nc). In a dolichocephalic dog, the width of the rostrum and zygomatic arches is reduced, and the rostrum tilts ventrally relative to the neurocranium. (B) GWAS of skull length demonstrates that multiple QTL are highly associated with face length. Each dot represents a single marker (a SNP). The *y*-axis represents the strength of the association [$-\log_{10}$(*P*-value)]. The *x*-axis lists marker location by chromosome. Statistically significant associations that exceed correction for multiple testing are indicated in blue. Figure is adapted with permission (Schoenebeck *et al.* 2012).

causal genetic variants has only recently become possible. To date, most efforts to identify the genetic underpinnings of canine skull shape have focused on brachycephaly. Early efforts at finding such causal variants have focused on candidates identified in humans.

Human brachycephaly is associated with morbidity and is diagnostic of many syndromes, including Apert's, Crouzon's, and Pfeiffer's syndromes (Miraoui and Marie 2010; Johnson and Wilkie 2011; Ursitti *et al.* 2011; Levi *et al.* 2012). In humans, brachycephaly occurs as a result of growth zone

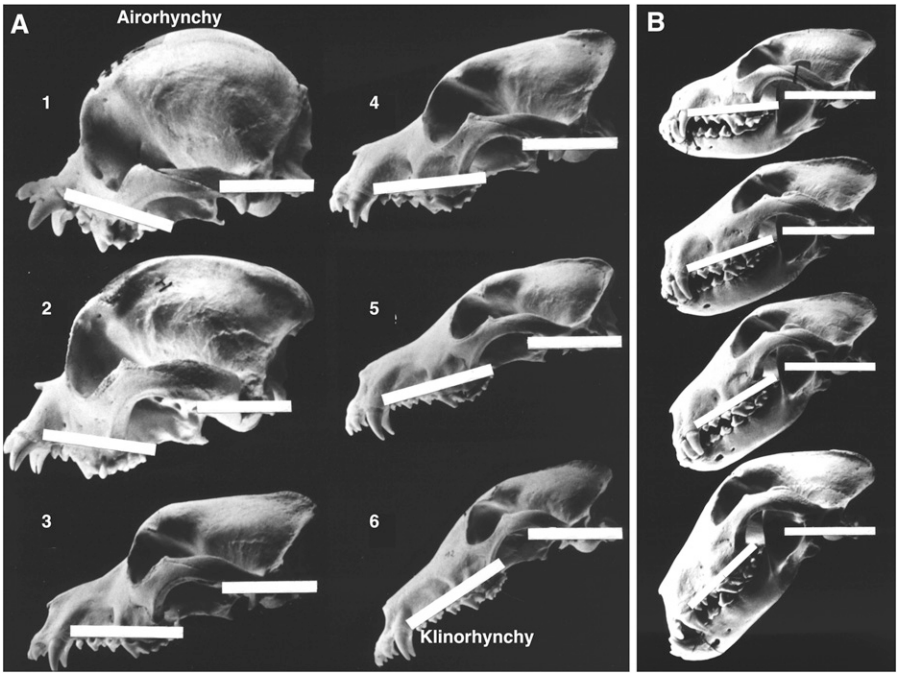


**Figure 3** Craniofacial diversity exists between and within breed dogs. White strips highlight the palate (left) and brainstem (right) in each skull example. (A) The continuum of airorhynchic and klinorhynchic dog breeds, arranged in order of severity. Examples include a Pekingese (1), French bulldog (2), Chow Chow (3), Bernese Mountain Dog (4), German Shepherd (5), and Borzoi (6). (B) Bull terrier skulls demonstrate the continual morphological evolution in breed dogs. Skulls are arranged chronologically from the oldest (top) to the most modern (bottom). Figure is adapted with permission (Nussbaumer 1982).

defects within the developing skull. Both premature fusion of the coronal suture (bilateral coronal synostosis) and defective endochondral ossification at the synchondroses of the skull base are contributors of brachycephaly (Cendekiawan *et al.* 2010).

Mutations that affect fibroblast growth factor (Fgf) signaling through compromised receptor activity feature prominently in brachycephalic-type craniosynostosis, as do mutations in genes encoding muscle segment homeobox2 (*MSX2*) and twist homolog 1 (*TWIST1*) transcription factors. Other genetic defects leading to craniosynostosis implicate ephrin-B1 (*EFNB1*), the ras-related protein *RAB23*, fibrillulin1 (*FNB1*), P450 (cytochrome), oxidorectortase (*POR*), transforming growth factor, beta receptor 1 (*TGFBR1*), and transforming growth factor, beta receptor 2 (*TGFBR2*). Extrapolating from this short list of candidates, it is clear that FGF and TGF-β signaling are integral for maintaining patency and growth at the sutures and synchondroses (supporting information, Table S1; Online Mendelian Inheritance in Man 2012).

Frontonasal dysplasias can adversely affect face shape in a manner consistent with canine brachycephaly. For example, mutation of Treacher Collins-Franschetti syndrome 1 (*TCOF1*), whose product normally facilitates ribosome production, results in hypoplasia of frontal and zygoma bones (Dixon *et al.* 2004; Valdez *et al.* 2004).

In dogs of all breeds, coronal synostosis is normally absent. However, the cranial base of brachycephalic breeds, as well as some dolichocephalic breeds, is disproportionate to overall body size (Stockard 1941; Lüps 1974). This suggests that regulation of growth at the synchondroses plays a role in the genesis of brachycephalic and dolichocephalic skull conformations.

Taking inspiration from human studies, Haworth *et al.* (2001) examined the coding sequence from *TCOF1* and *MSX2* in select dog breeds of varying head shapes. The authors reported a variant at the locus *TCOF1* that appeared to be correlated with head shape; however, interpretation of this finding was quickly disputed when additional breeds were examined (Hunemeier *et al.* 2009). Variation at *FGFR3* was examined for its role in canine chondrodysplasia; however, no variants were found when sequences were compared to the Boxer (a brachycephalic breed) reference genome (Smith *et al.* 2008).

## Genome-Wide Association Studies for Finding Loci of Interest

The reference assembly of the dog genome and development of single nucleotide polymorphism (SNP) chips enabled geneticists to undertake mapping studies of all types in the dog. Because linkage disequilibrium (LD) is extensive in dogs (Sutter *et al.* 2004; Lindblad-Toh *et al.* 2005), with alternating stretches of near homozygosity separated by regions of high heterozygosity, comparably fewer SNPs should be needed to identify associative loci in dogs than in humans, where high levels of heterozygosity are the norm. We and others, using just 100 unrelated cases and controls, showed that only 30,000 informative SNPs are needed to fully interrogate a single trait in the 2.4-Gb dog genome to achieve a 99% probability of successfully detecting an association (Sutter *et al.* 2004; Lindblad-Toh *et al.* 2005). In practice, the strong LD within dog breeds is a double-edged sword: GWAS are quite successful at finding genetic associations; however, the resulting critical intervals are often extensive LD blocks that can extend hundreds of kilobases or more. This makes it difficult to go from marker to variant of interest.

For a typical binary GWAS (*e.g.*, a case-control study), the allele frequency differences for one group with a trait are compared to another group without it. In its most basic form, allele frequencies are tested for statistically significant differences using a chi-square test on a marker-by-marker basis. For complex traits such as skull shape, the statistical power of GWAS decreases relative to binary studies. To compensate, we and others have utilized study designs that include multiple breeds with the same trait, which likely share a common ancestral mutation (Goldstein *et al.* 2006; Parker *et al.* 2007; Karlsson and Lindblad-Toh 2008). Aside from boosting statistical power, crossbreed comparisons benefit fine mapping, as interbreed-associated haplotypes are inevitably smaller than intrabreed-associated haplotypes.

GWAS test population-based allele frequencies for association to traits of interest. Used in conjunction with selective sweep mapping, it has been possible to detect breeder-selected genetic variation. Based on this approach, Bannasch *et al.* (2010) used a binary design in which SNP chip genotypes of dogs from nine brachycephalic breeds were compared to nine other breeds. This comparison resulted in detection of a large association on canine chromosome (CFA) 1, as well as a number of other associations that were not described further. Fine mapping on CFA1 defined a 296-kb critical interval that included thrombospondin2 (*THSB2*), next to sparc-related modular calcium binding 2 (*SMOC2*). While both remain potential candidates genes, no causal variants have been reported to date.

We also used GWAS to map canine traits, favoring treatment of skull shape as a quantitative trait (Boyko *et al.* 2010; Schoenebeck *et al.* 2012). Both of our studies relied on the CanMap dataset that entails ∼62,000 SNP profiles from 915 dogs, representing ∼80 breeds. However, the composition of genetic profiles and craniometric data used between the two studies differed. Skull traits profiled by Boyko *et al.* (2010) were based on linear measurements, while Schoenebeck *et al.* (2012) used geometric morphometry to quantify nonallometric skull shape. This latter approach demonstrated that principal component 1 (PC1) explained ∼76% of skull variance among skulls that were measured and described the continuum of morphological changes extending between brachycephalic and dolichocephalic dog breeds. Given the difficulties in collecting dog skull data, both studies made an assumption that is arguably

unique to dog population studies; given their morphological standardization, "breed average" metrics were used as quantitative traits.

We found an association on CFA1 (Schoenebeck *et al.* 2012), matching that previously reported (Bannasch *et al.* 2010; Boyko *et al.* 2010). Just as important, numerous other associations with snout length and cranium shape were described, including QTL on CFA -5, -24, -30, and -32 and the X chromosome, as well as other QTL that were unique to each study's design (Figure 2B). These findings begin to explain the complex genetic nature of brachycephaly that was originally predicted by Stockard (1941), as well as shed light on other craniofacial traits.

We fine-mapped the CFA32 QTL, resulting in identification of a phenyalanine → leucine mutation at a highly conserved position within the mature domain of bone morphogenetic protein 3 (*BMP3*) (Schoenebeck *et al.* 2012). The biological function of BMP3 is not well understood; however, a number of studies suggest that it can inhibit TGF-β signaling and that it restricts osteogenesis (Bahamonde and Lyons 2001). Postnatal expression in rats indicates that *Bmp3* is highly expressed at synchondroses, suggesting a role in chondrogenesis (Kettunen *et al.* 2006).

In dogs, the *BMP3* mutation was nearly fixed in small and medium brachycephalic breeds and was found among a number of smaller breed dogs whose rostrum length tends toward being brachycephalic. Reiterating the genetic complexity of brachycephaly, the mutation was absent from medium-to-large brachycephalic breeds including the boxer (medium), bullmastiff (giant), and Dogue de Bordeaux (giant). As one would expect, the *BMP3* mutation was absent from dolichocephalic breeds, with one exception. Curiously, Scottish terriers were also fixed for the mutation, despite their dolichocephalic skull conformation. Whether the effects of the *BMP3* mutation manifest permissively in the context of other genetic variation, another variant acts epistatically in Scottish terriers, or Scottish terriers actually do share some aspect of craniofacial shape that is similar to small brachycephalic breeds remains a topic for future investigation. A potentially telling clue is the observation that other asymmetrically chondrodysplastic (short-legged) breeds like Scottish terriers are also carriers of the *BMP3* mutation, although the allele frequency of the mutation among these breeds was found to be unfixed (Schoenebeck *et al.* 2012). This raises the possibility that the effects of the *BMP3* mutation extend beyond the skull. Unraveling such anomalies will clearly require the identities and characterization of the causal variation underlying the remaining skull shape QTL, including that on CFA1 described by all three GWAS, as well as consideration of postcranial skeletal traits (Bannasch *et al.* 2010; Boyko *et al.* 2010; Schoenebeck *et al.* 2012).

## Finding Additional Skull-Associated Genetic Variants

The associated genetic variation identified in the aforementioned GWAS is "old" since carrier breeds implicitly shared a common founder sometime in their history. Adding to breed relatedness are the remnants of genetic bottlenecks and the use of popular sires among breeders, resulting in genetic substructure and allele frequencies that often deviate from Hardy–Weinberg equilibrium. Such confounders make GWAS in dogs prone to generating false-positive associations. Permutation, population stratification, multiple test correction, and the use of mixed models (Kang *et al.* 2008; Zhang *et al.* 2010; Lipka *et al.* 2012; Zhou and Stephens 2012), which take into account genetic potential, fixed effects, and kinship, are essential tools for reducing incidence of false positives that are encountered on a genome-wide scale.

Fine mapping is also fraught with challenges. While comparing multiple breeds helps reduce the extent of LD at a locus of interest, typically the resulting critical interval is still too large and prohibitively expensive to exhaustively Sanger sequence. However, as the cost of targeted sequence capture and next-generation sequencing continue to decrease, fine-mapping regions of extensive LD should become less problematic, enabling unprecedented haplotype resolution and prioritization of genetic variation for further investigation.

While the extensive LD, relatedness, and cryptic genetic structure are potential confounders in nearly all dog GWAS studies, mapping skull traits is uniquely complicated phenotypically, largely because of interbreed differences in scale. Disentangling morphologically allometric variation (size-related) from nonallometric variation is a formidable challenge. Many skulls available for morphometric analysis lack postcranial skeletons, necessitating animal size estimation from the skull itself. Popular proxies of overall size such as the skull's centroid or cranial base length do not linearly correlate with size across all breeds. There is particular deviation among extreme brachycephalic, dolichocephalic, and some chondrodysplastic breeds (Lüps 1974; Nussbaumer 1976). In practical terms, cross-contamination by allometric variation can be minimized by regressing shape by size during skull quantification and, later, by including a size covariate in the linear regression used for the GWAS.

It is also important to note that morphometric approaches to quantifying shape variation have a strong bearing on what skull traits can be QTL-mapped. Geometric morphometrics offer a three-dimensional perspective of shape variation and are suitable for quantifying robust canine skull shapes such as brachycephaly and dolichocephaly. Other phenotypes such as rostrum angle are poorly captured by such methods because landmark data are typically rotated to determine best fit prior to shape analysis, which effectively removes palate–cranial base angle variation. Principal components analysis (PCA), which is commonly used to categorize shape across fitted datasets, also has shortcomings. As previously noted, PCA tends to bury subtle dog phenotypes by spreading variation across components and can lump similar types of variation together within the same component (Chase *et al.* 2002; Fondon and Garner 2007). Thus, the shape

diversity of dog skulls is probably best described using morphometric approaches that are contextually appropriate.

To date, our skull trait GWAS has relied on use of breed averages as quantitative traits. This approach was done out of necessity, as access to skull data from live, healthy subjects is expensive, scarce, and stressful on the dogs being measured. The breed average skull traits used in our studies were obtained predominantly from museum specimens within the United States and Europe. An unavoidable consequence of this approach is that direct phenotype–genotype relationships are broken. In addition, for traits like brachycephaly, we are unable to determine what percentage of the trait is accounted for by the loci that we discovered. Moreover, for the QTL that we do find, we have no way to determine the rank order of their contribution to the trait. As museums continue to add DNA preservation as a facet of their repositories and as sequencing technologies continue to improve, it is possible that dog morphology association studies using direct phenotype–genotype relationships will be possible. Even with direct phenotype–genotype data, modeling genetic effects could also benefit from outbred populations such as mixed-breed or village dogs (Boyko *et al.* 2010) to isolate the effects of individual QTL.

The most daunting hurdle of any genetic mapping study is proving genetic causality. For obvious reasons, testing putatively causal variants identified in dogs requires biological surrogate(s), making the effort that much more challenging. Currently, the diversity of commercially available canine cell lines for *in vitro* studies is almost negligible, lacking chondroblasts and osteoblasts that are ideal for characterizing variants that affect skull shape.

Animal models such as mice and zebrafish can provide means of testing variant functionality though transgenesis, exogenous overexpression, and knockdown. As one might expect, each model has its strengths and disadvantages. In addition to targeted transgenics, the similarity in mammalian genomic architecture makes interrogation of canine intergenic variation possible using mice. Also, owing to their more recent common ancestry, mice are arguably better suited for modeling the mechanistic impact that genetic variants exert on craniofacial morphology. By comparison, zebrafish offer rapid and accessible development and are amenable to gene overexpression, morpholino-mediated knockdown, and certain types of transgenic approaches. Despite interspecific differences in the mammalian and teleost head structures, the genetic pathways that regulate vertebrate craniofacial development are highly conserved across both species (Schilling 1997; Szabo-Rogers *et al.* 2010). When faced with needing to rapidly evaluate *BMP3* function, we turned to zebrafish. Our experiments revealed that Bmp3 plays an ancient role in craniofacial development, and overexpression indicated that functional differences were encoded in the variants that we identified in dogs (Schoenebeck *et al.* 2012).

Beyond understanding the mechanisms of dog craniofacial diversity, identification of causal genetics is necessary for understanding just what makes dog breeds so morphologically diverse in the first place. Future studies will need to address the origins of the genetic variation that underlies traits like brachycephaly to determine whether variants sprung forth following domestication or were consolidated from wild canids (Wayne and Vonholdt 2012). Also, despite genetic isolation from one another, skull shape continues to rapidly change within many breeds (Figure 3B). Previous studies suggested that hypermutable tandem repeats or SINE element activity could be drivers of dogs' continuously evolving morphology; however, uncertainty as to what extent such mechanisms actually contribute to morphological diversity remains (Fondon and Garner 2004; Wang and Kirkness 2005; Cordaux and Batzer 2006; Fondon and Garner 2007; Laidlaw *et al.* 2007). The ideas of Fondon and Garner (2004) are particularly interesting. They examined 37 tandem repeats located within coding regions of developmentally relevant transcription factors in 142 dogs from 92 breeds. Runt-related transcription factor 2 (*RUNX2*), which is considered to be a master regulator of osteoblast differentiation, was shown to have a modest correlation between its total allele length and alanine/glutamine ratio *vs.* physical traits such as dorsoventral rostrum bend and midface length (Fondon and Garner 2004). They note that inactivation of RUNX2 causes human cleidocranial cysplasia. Moreover, the authors suggest that slippage events that result in contraction and expansion of tandem repeats represent a novel mechanism of rapid evolution. Regardless of how continual morphological changes occur at the molecular level, mapping nascent causal variation will require new approaches. Whole-genome sequencing may provide the answer, although the analysis of such data is still enormously challenging and arguably less well suited to detect structural variants such as copy number variants and tandem repeats. As we delve deeper into the mysteries of skull morphology, it becomes increasingly apparent that complex and rare genetics are at play in dogs and defining their contributions will be far from routine.

## Conclusion

The dog model is young in human years, yet the remarkable insights gleaned from the eight years since its genome's public debut make it an old soul. Using household pets in biological research is unorthodox, yet it is this animal's symbiosis with humans that makes it uniquely suited to address the genetic basis of domestication, evolution, morphology, and disease. The pertinence of answers awaiting canine geneticists, we believe, reaches beyond the dog, as we have demonstrated by our discussion of canine craniofacial biology and genetics.

## Acknowledgments

We thank our many colleagues who have collaborated with us as well as worked independently to advance the field

canine skeletal genetics. We thank Maud Rimbault and Jonine Figueroa for helpful comments on this manuscript. We thank the many institutions that shared their canine skull collections with us. Finally, we thank the many pet owners, veterinarians, and breeders who have supplied us with DNA and information about their dogs for this and other studies. E.A.O. and J.J.S. gratefully acknowledge support from the Intramural Program of the National Human Genome Research Institute. J.J.S. was also supported by a National Institute of General Medical Sciences Pharmacology Research Associate fellowship.

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*Communicating editor: A. S. Wilkins*

GENETICS

**Supporting Information**
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.145284/-/DC1

# The Genetics of Canine Skull Shape Variation

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DOI: 10.1534/genetics.112.145284

**Table S1 A sampling of human syndromes with resemblance to dog skull morphologies.** Craniofacial phenotypes listed are not comprehensive and are limited to those aspects with potential relevance to dog skull traits. Information presented is compiled from OMIM (http://omim.org/).

| SYNDROME (OMIM #) | ALTERNATE DESIGNATIONS | LOCUS and GENE | CRANIOFACIAL DEFECTS |
|---|---|---|---|
| Achondrodysplasia (ACH, 100800) | -- | 4p16.3; FGF3 | Frontal bossing, midface hypoplasia |
| Apert (#101200) | Acrocephalosyndactyly, Type I | 10q26.13; *FGFR2*, | Brachycephaly |
| Axenfeld-Rieger Syndrome, Type 1 (RIEG1, #180500) | Rieger Syndrome, Type 1 | 4q25; *PITX2* | Maxillary hypoplasia, mild prognathism |
| Axenfeld-Rieger Syndrome, Type 2 (RIEG2, %601499) | Rieger Syndrome, Type 2 | 13q14 | Mild craniofacial dysmorphism, hydrocephalus |
| Axenfeld-Rieger Syndrome, Type 2 (RIEG3, #602482) | Axenfeld-Rieger Anomaly Anterior Segment Mesenchymal Dysgenesis Anterior Chamber Cleavage Syndrome Rieger Syndrome, Type 3 | 6p25.3; *FOXC1* | Hypertelorism |
| Chiari malformation, type I (CM1, %118420) | Chiari Malformation Type I with Syringomyelia, Included | -- | Hydrocephalus, occipital bone hypoplasia |
| Chiari malformation, type II (CM2, %207950) | Arnold-Chiari Malformation | -- | Hydrocephalus |
| Carpenter (#201000) | Acrocephalosyndactyly, Type II | 6p11.2; *RAB23* | Acrocephaly |
| Cleidocranial Dysplasia (CCD, #119600) | -- | 6p21.1; CBFA1/RUNX2 | Maxillofacial dysmorphogenesis, open fontanelles |
| Craniosynostosis, Type I (CRS1, #123100) | Craniosynostosis, Craniostenosis | 7p21.1, *TWIST1* | Scaphocephaly (dolichocephaly) |
| Craniosynostosis, Type II (CRS2, #604757) | Craniosynostosis, Boston-Type | 5q34-35; *MSX2* | Forehead retrusion, frontal bossing, turribrachycephaly, cloverleaf skull anomaly |
| Crouzon (#123500) | Craniofacial dysostosis | 10q26.13; *FGFR2*, | Brachycephaly |

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|---|---|---|---|
| Down (#190685) | Trisomy 21 | 21q22.3 | Midface hypoplasia, maxillofacial dysmorphogenesis |
| Greig cephalopolysyndactyly (#175700) | Polysyndactyly with Peculiar Skull Shape | 7p14.1; *GLI3* | Frontal bossing, hypertelorism, scaphocephaly (dolichocephaly) |
| Jackson-Weiss (#123150) | Craniosynostosis, Midfacial Hypoplasia, and Foot Abnormalities | 10q26.13; *FGFR2*<br>8p11.23-p11.22; *FGFR1* | Brachycephaly |
| Loeys-Dietz, Type1A (LDS1A; #609192) | Furlong Syndrome<br>Loeys-Dietz Aortic Aneurysm Syndrome | 9q22.23,*TGFBR1* | Craniosynostosis, hypertelorism, hydrocephalus |
| Loeys-Dietz, Type2A (LDS2A; #608967) | Aortic aneurysm, familial thoracic | 9q22.23;TGFBR1 | Frontal bossing, dolichocephaly, hypoplastic suprorbital margins |
| Marshall (MRSHS, #154780) | -- | 1p21.1; *COL11A1* | Flat or retracted midface |
| Muenke (MNKES, #602849) | Muenke Nonsyndromic Coronal Craniosynostosis | *4p16.3; FGFR3* | Similar to Saethre-Chotzen, but with mental delay, hearing loss, and other subtle facial features |
| Myopathy, Congenital, Compton-North (#612540) | -- | 12q12; *CNTN1* | Scaphocephaly (dolichocephaly) |
| Noonan Syndrome 1 (NS1, #163950) | Noonan Syndrome<br>Male Turner Syndrome<br>Female Pseudo-Turner Syndrome<br>Turner Phenotype with Normal Karyotype | 12q24.13; *PTPN11* | Hypertelorism, low-set ears |
| Pfeiffer (#101600) | Acrocephalosyndactyly, Type V<br>Noack Syndrome | 10q26.13; *FGFR2*<br>8p11.23-p11.22; *FGFR1* | Brachycephaly, midface hypoplasia |
| Pierre Robin (%261800) | Glossoptosis, Micrognathia, and Cleft Palate<br>Pierre Robin Sequence | -- | Maxillofacial dysmorphogenesis, micrognathia |

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|---|---|---|---|
| Saethre-Chotzen (SCS, #101400) | Acrocephalosyndactyly, Type III<br>Chotzen Syndrome<br>Acrocephaly, Skull Asymmetry, and Mild Syndactyly | 7p21.1; *TWIST1*<br><br>10q26.13; *FGFR2* | Craniosynostosis of the coronal suture, acrocephaly, intracranial hypertension |
| Scaphocephaly, Maxillary Retrusion, and Mental Retardation (#609579) | -- | 10q26.13; *FGFR2* | Scaphocephaly (dolichocephaly), macrocephaly, hypertelorism, and maxillary retrusion |
| Shprintzen-Goldberg Craniosynostosis (SGS, #182212) | Craniosynostosis with Arachnodactyly and Abdominal Hernias<br>Marfanoid Disorder with Craniosynostosis, Type I<br>Marfanoid Craniosynostosis Syndrome | 15q21.1; *FBN1* | Maxillary and mandibular hypoplasia, severe exophthalmos. Hypertelorism, midface hypoplasia, brachycephaly, scaphocephaly, reports of hydrocephalus (Chiari type I malformation). |
| Simpson-Golabi-Behmel, Type 1 (SGBS1; #312870) | Bulldog Syndrome<br>Dysplasia Gigantism Syndrome, X-linked<br>Golabi-Rosen Syndrome<br>Simpson Dysmorphia Syndrome | Xq26.2; *GPC3, GPC4?* | Coarse facial feaures, large protruding jaw, upturned nasal tip, widened nasal bridge, gigantism. |
| Stickler, Type I (STL1, #108300) | Stickler Syndrome, Vitreous Type 1<br>Stickler Syndrome, Membranous Vitreous Type (STL1)<br>Arthroophthalmopathy, Hereditary Progressive | 12q13.11; *COL2A1* | Flat midface |
| Stickler, Type II (STL2, #604841) | Stickler Syndrome, Vitreous Type 2<br>Stickler Syndrome, Beaded Vitreous Type | 1p21.1; *COL11A1* | Mild mid-facial and nasal hypoplasia, Pierre Robin sequence. |
| Stickler, Type III (STL3, #184840) | Stickler Syndrome, Nonocular Type | 6p21.32; *COL11A2* | Mild mid-facial and nasal hypoplasia, Pierre Robin sequence. |

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|---|---|---|---|
| Stickler, Type IV (STL4, #614134) | -- | 6p13; *COL9A1* | Mild mid-facial and nasal hypoplasia, Pierre Robin sequence. |
| Stickler, Type V (STL5, #614284) | -- | 1p34.2; *COL9A2* | Mild mid-facial and nasal hypoplasia, Pierre Robin sequence. |
| Treacher-Collins Type I (TCS1, #154500) | Treacher Collins-Franschetti Syndrome Mandibulofacial dysostosis | 5q32; *TFOF1*, | Macrostomia (wide mouth), hypoplastic zygomatic arches, micrognathia, dysmorphic ears, maxillofacial dysmorphogenesis |
| Treacher-Collins Type II (#613717) | -- | 13q12.2; *POLR1D* | Hypoplasia of the facial bones, maxillofacial dysmorphogenesis |
| Treacher-Collins Type III (TCS3, #248390) | Mandibulofacial Dysostosis Treacher Collins Type, Autosomal Recessive | 6p21.1; *POLR1C* | Hypoplasia of the facial bones, maxillofacial dysmorphogenesis |
| Williams-Beuren (WBS, #194050) | Chromosome 7q11.23 Deletion Syndrome, 1.5-1.8 MB Williams syndrome | Haploinsufficiency related to large deletion on chromosome 7 | Malar (frontal bone) flattening, anteverted nostrils. |