Dog colour patterns explained by modular promoters of ancient canid origin

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Distinctive colour patterns in dogs are an integral component of canine diversity. Colour pattern differences are thought to have arisen from mutation and artificial selection during and after domestication from wolves but important gaps remain in understanding how these patterns evolved and are genetically controlled. In other mammals, variation at the ASIP gene controls both the temporal and spatial distribution of yellow and black pigments. Here, we identify independent regulatory modules and their effect on patterning phenotypes in domestic dogs.

The expression of ASIP in cells of the hair follicle regulates a switch from making eumelanin (black or brown pigment) to pheomelanin (yellow to nearly white pigment) 

Results

Expression of ASIP promotes pheomelanin synthesis; therefore, ASIP alleles associated with a yellow colour are dominant to those associated with a black colour. Although dominant yellow (DY) is common in dogs from diverse geographic locations, the most common coat pattern of modern wolves is agouti (AG), in which the dorsum has banded hairs and the ventrum is light. Three additional colour patterns are recognizable but all have been described historically by different, inconsistent and sometimes overlapping names that predate genomic analysis; we refer to these as shaded yellow (SY), black saddle (BS) and black back (BB) (Fig. 1 and Supplementary Table 1).

We analysed skin RNA-sequencing (RNA-seq) data available from dogs of dominant yellow and black back patterns and identified three alternative untranslated first exons for dog ASIP (Fig. 2a, Extended Data Fig. 1 and Supplementary Table 2). As described below, two of the three transcripts vary in abundance between dominant yellow and black back dogs and the corresponding 5′-flanking promoters have sequence variation associated with dog pattern phenotypes. The 5′-flanking promoter regions for these two transcripts are orthologous to the ventral promoter (VP) and hair cycle promoter (HCP) in the laboratory mouse; however, our genetic analysis (Fig. 2) reveals that the dog VP and HCP give rise to more complex patterns than their mouse counterparts. Transcripts associated with the third promoter, which lies ~16 kilobases (kb) upstream of the VP (Fig. 2b), did not vary in abundance in our dataset.

To better understand the relationship between promoter usage and pattern phenotypes, we inspected whole genome sequence data from 77 dog and wolf samples with known colour patterns (Supplementary Table 3). We used dogs that were homozygous at
the ASIP locus to infer two VP haplotypes and five HCP haplotypes, consisting of multiple structural variants that lie within 1.5 kb of each transcriptional start site. VP1 contains a SINE element in reverse orientation relative to the transcription of ASIP and an A-rich expansion not found in VP2 (Fig. 2b left and Supplementary Table 1); the five HCP haplotypes differ according to the number and identity of SINE elements, all in the same orientation as ASIP, as well as additional insertions and deletions (Fig. 2b right and Supplementary Table 1). All structural variants were precisely delineated with Sanger sequencing.

These results were extended by developing PCR-based genotyping assays for the VP and HCP structural variants, examining their association with different pattern phenotypes in 352 dogs from 34 breeds and comparing these results to previously published variants (Table 1, Extended Data Fig. 2 and Supplementary Tables 1 and 4–7). As depicted in Fig. 2c and Table 1, dipotype combinations of VP1 or VP2 with HCP1, 2, 3, 4 or 5 are correlated perfectly with variation in ASIP pattern phenotype. For example, homozygotes for VP1-HCP1, VP2-HCP1, VP2-HCP2 are dominant yellow, shaded yellow and agouti, respectively (Supplementary Tables 4–7). Black saddle and black back dogs differ in their VP configuration but all carry HCP3, 4 and/or 5 in homozygous or compound heterozygous configurations. Because the level of ASIP activity is directly related to the amount of yellow pigment production, these genetic association results suggest that VP1 has greater activity than VP2, HCP1 has greater activity than HCP2 and HCP3, 4 and 5 all represent loss-of-function, since the HCP4 haplotype includes a large deletion of the hair cycle first exon (Fig. 2b) and fails to complement HCP3 or HCP5 (Fig. 3 and Supplementary Table 6). Importantly, increased activity from the ventral promoter (VP1 versus VP2) correlates with dorsal expansion of yellow pigment in black saddle compared to black back phenotypes (Figs. 1 and 2c), which indicates that the VP and HCP haplotypes function separately from each other.

The relationship between structural variation that delineates the different VP and HCP haplotypes and ASIP transcriptional activity was explored more directly in RNA-seq data from biopsies of dorsal and ventral dog skin (Supplementary Table 8 and Extended Data Fig. 1). Read counts from the RNA-seq data were consistent with expectations from the genetic association results: VP1 has greater transcriptional activity and is spatially broadened relative to VP2 (which is only expressed ventrally), HCP1 has greater transcriptional activity relative to HCP2 and no reads are detected from HCP3 or 4 (Fig. 2b and Extended Data Fig. 1). Taken together, these results provide a molecular explanation for ASIP pattern variation in dogs in which the VP and HCP function independently and for which structural variants in close proximity to VP and HCP modulate promoter activity.

Genetic relationships between variant ASIP regulatory modules were examined by comparing haplotypes in 18 homozygous dogs (for the structural variants at the VP and HCP and coding sequences) to those from ten contemporary grey wolves (Fig. 4a and Supplementary Table 9). Overall, agouti dog haplotypes were similar to those from grey wolves. However, dominant yellow and, to a lesser extent, shaded yellow dog haplotypes were similar to those from grey wolves, including sequence, the Arctic grey wolf haplotypes are identical except for one loss-of-function polymorphic site (Fig. 4a, chr24: 23,337,523) and are distinguished from dog dominant yellow haplotypes by only six single nucleotide variants (SNVs) (Supplementary Table 10). Taken together, these observations suggest a common origin of dominant yellow in dogs and white coat colour in wolves without recent genetic exchange.

The evolutionary origin of ASIP haplotypes was explored further by constructing maximum likelihood phylogenetic trees for dogs,
wolves and eight additional canid species (Supplementary Table 9). On the basis of differences in SNV frequency, the 48-kb VP segment was considered separately from the 16-kb HCP-exon 2/3/4 segment (Supplementary Information and Fig. 4a). In the VP tree, all dogs and grey wolves form a single clade, consistent with known species relationships9. However, in the HCP tree, the dominant yellow and shaded yellow dogs lie in a separate clade together with Arctic grey wolves; remarkably, this clade is basal to the golden jackal and distinct from other canid species (Fig. 4b and Extended Data Figs. 3 and 4).

| Table 1 | ASIP diplotype association with pattern phenotype |
|---|---|
| Pattern phenotype | Promoter diplotype | Concordant | Discordant |
| Dominant yellow (n=114)* | VP1-HCP1 / VP1,2-HCP1,3,4,5 | 113 | 1* |
| Shaded yellow (n=64)* | VP2-HCP1 / VP2-HCP1,3,5 | 52 | 12* |
| Agouti (n=46) | VP2-HCP2 / VP2-HCP2,3,5 | 46 | 0 |
| Black saddle (n=53) | VP1-HCP4 / VP1,2-HCP3,4 | 53 | 0 |
| Black back (n=89) | VP2-HCP3,4,5 / VP2-HCP3,4,5 | 89 | 0 |

*Previous studies did not differentiate between the dominant yellow and the shaded yellow phenotype. These dogs had a eumelanistic masking pattern, which prevented reliable phenotype distinction between dominant yellow and shaded yellow.

The pattern of derived allele sharing provides additional insight (Fig. 4d and Extended Data Fig. 5). As depicted in Figs. 2c and 4d, HCP2 is characterized by three small repeat elements that are shared by all canids and is therefore the ancestral form. In the branch leading to core wolf-like canids (golden jackal, coyote, Ethiopian wolf and grey wolf), there are nine derived SNV alleles within the HCP2-exon 2/3/4 segment (Extended Data Fig. 5 and Supplementary Table 11), four of which flank the repeat elements close to HCP1 (Fig. 4d and Extended Data Fig. 5). None of the nine derived alleles is present in the dominant yellow HCP1-exon 2/3/4 segment haplotype which also carries an additional SINE close to HCP1; therefore, this haplotype must have originated before the last common ancestor of golden jackals and other wolf-like canids.
>2 million years ago (Ma)\(^1\). Although the 16-kb HCP1-exon 2/3/4 segment haplotype could have originated on a branch leading to the core wolf-like canids, it would have had to persist via incomplete lineage sorting and absence of recombination for >2 million years and through three speciation events (Supplementary Information). A more likely scenario is that HCP1 represents a ghost lineage from an extinct canid (Figs. 4d and 5b) that was introduced by hybridization with grey wolves during the Pleistocene (below), as has
been suggested for an ancestor of the grey wolf and coyote and in high-altitude Tibetan and Himalayan wolves.

We expanded our analysis of VP and HCP haplotypes to a total of 45 North American and 23 Eurasian wolves. The VP1-HCP1 haplotype combination is found mostly in the North American Arctic in a distribution parallel to that of white coat colour (Extended Data Fig. 6a) and is not observed in Eurasia. We also identified an ancestral HCP1 haplotype variant, referred to hereafter as HCP1A, that does not extend to exons 2/3/4 and lacks the 24-bp insertion found in Arctic grey wolves and dominant yellow dogs (Fig. 4d and Extended Data Fig. 7). The distribution of derivative haplotypes in various combinations (Fig. 5a and Extended Data Fig. 7). Ancient dogs from the Lake Taimyr and Yana River areas of Arctic Siberia had at least one HCP1 haplotype, while ancient dogs from central Europe, Ireland and Siberia carried HCP1A, HCP1 and HCP4, respectively (Supplementary Table 12). Thus, diversity in ASIP regulatory sequences responsible for colour variation today was apparent by 35 ka in ancient wolves and by 9.5 ka in ancient dogs.

Together with our phylogenetic results, comparative analysis of wolf and dog ASIP haplotypes suggests an evolutionary history in which multiple derivative haplotypes and associated colour patterns arose by recombination and mutation from two ancestral configurations corresponding to a white wolf (VP1-HCP1) and a grey wolf (VP2-HCP2), both present in the late Pleistocene (Fig. 5a and Extended Data Fig. 7). The distribution of derivative haplotypes explains colour pattern diversity not only in dogs but also in modern wolf populations across the Holarctic, including white wolves in the North American Arctic (VP1-HCP1) and yellow wolves in the Tibetan highlands (VP2-HCP1A) and is consistent with natural selection for light coat colour. A likely timeline for the origin of modules driving high levels of ASIP expression is depicted in Fig. 5b and indicates a dual origin. The HCP1 haplotype represents introgression into Pleistocene grey wolves from an extinct canid lineage that diverged from grey wolves >2 Ma. This introgression as well as the mutation from VP2 to VP1 occurred before 33.5 ka, on the basis of comparative analysis of wolf and dog ASIP haplotypes suggesting an evolutionary history in which multiple derivative haplotypes and associated colour patterns arose by recombination and mutation from two ancestral configurations corresponding to a white wolf (VP1-HCP1) and a grey wolf (VP2-HCP2), both present in the late Pleistocene.
in Supplementary Table 5. PCR products amplified using LA Taq polymerase (Takara) and Sanger sequencing of PCR amplicons was carried out to validate and characterize structural variants in the promoter regions. 

Methods

Ethics statement. All animal experiments were done in accordance with the local regulations. Experiments were approved by the "Cantonal Committee For Animal Experiments" (Canton of Bern; permits 48/13, 75/16 and 71/19).

Skin biopsies and total RNA extraction. Skin biopsies (6-mm punch) were recovered from three dogs (black back miniature Pinscher and dominant yellow Border Terrier and Irish Terrier) at necropsy and/or surgery for reasons unrelated to this study. Biopsies were recovered from the ventral abdomen and dorsal thorax and are not matched for age or hair growth cycle. The biopsies were immediately put in RNAlater (Qiagen) for at least 24h and then frozen at −20°C. Before RNA extraction, the skin biopsies were homogenized mechanically with the TissueLyser II device from Qiagen. Total RNA was extracted from the homogenized tissue using the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. RNA quality was assessed with a Fragment Analyzer (Agilent) and the concentration was measured using a Qubit Fluorometer (ThermoFisher Scientific).

Whole transcriptome sequencing (RNA-seq). From each sample, 1 μg of high-quality total RNA (RNA integrity number >9) was used for library preparation with the Illumina TruSeq Stranded mRNA kit. The libraries were individually barcoded and pooled and sequenced on an S1 flow cell with 2 × 50bp paired-end sequencing using an Illumina NovaSeq 6000 instrument. On average, 31.5 million paired-end reads per sample were collected. One publicly available Beagle sample was used (SRX1884098). All accession numbers and descriptive read statistics are given in Supplementary Table 8. All reads that passed quality control were mapped to the CanFam3.1 reference genome using STAR aligner (v.2.6.0c).

Transcript coordinates. The STAR-aligned bam files were visualized in the integrated genomics viewer (IGV) browser26. Three different alternate untranslated first exons with splice junctions to the coding exons of ASIP were defined on the basis of the visualizations of the read alignments in IGV on the basis of the RNA-seq data just described. These exact transcripts have not been documented in the National Center for Biotechnology Information (NCBI), however, the three transcripts of NCBI annotation release 105 are virtually identical except for minor differences (VP1 > VP2 > HCP1 > HCP2) are caused by the SINE element, the small insertion or both. We note, however, that modularity of ASIP regulatory variation is a general theme in vertebrates, with non-coding changes driving adaptation in natural populations of deer mice1, mountain hares2, snowshoe hares and several species of parulid warblers3,4-6. Likewise, artificial selection in goats3,7 and laboratory mice2 is associated with structural variation in ASIP regulatory regions that may lead to acquisition of promoters that mediate region-specific expression of ASIP.

ASIP colour pattern diversification was probably an early event during dog domestication, since our analysis of ancient DNA data reveals several different VP and HCP haplotypes in Eurasia by 4.8ka. This is consistent with the wide distribution of dominant yellow across modern dog breeds from diverse locations, as well as the dingo (Supplementary Table 9), a feral domesticate, frequently dominant yellow, introduced to Australia at least 3.5ka (ref. 27). Of particular interest is the Zhokov island dog from Siberia4-6,9,29. On the basis of a haplotype combination of VP2-HCP4, this sled dog that lived 9.5ka exhibited a black back colour pattern, allowing it to be easily distinguished from white-coloured wolves in an Arctic environment.

In wolves, natural selection for VP1 and HCP1 are a likely consequence of Pleistocene adaptation to Arctic environments and genetic exchange in glacial refugia, driven by canid and megafaunal dispersal during interglacial periods. Modern grey wolves are thought to have arisen from a single source ~25ka close to the last glacial maximum9,21; during the North American glacial retreat that followed, the VP1-HCP1 haplotype combination was selected for in today's white-coloured Arctic wolves. Our results show how introgression, demographic history and the genetic legacy of extinct canids played key roles in shaping diversity in dogs and modern grey wolves.
In a few dogs that were heterozygous at both VP and HCP, the phasing of the VP and HCP haplotype combinations was performed on the basis of haplotype frequency within the same breed as noted. A family of Chinooks was used to determine the segregation of extended haplotypes and the phenotype equivalency of HCP3 and 5 (Fig. 3). Summaries of genotyping results and exclusion of previously associated variants are shown in Table 1 and Supplementary Table 7. Supplementary Table 7 lists the genotype–phenotype association in aggregated form; it also contains the genotypes for variants that were previously reported to be associated with the pattern phenotypes.

### Comparison of promoter haplotype effects on transcripts

Transcript data were generated from a second set of samples. Sample descriptions and colours are shown in Supplementary Table 8 for all RNA experiments. Skin samples were collected from a male Swedish Elkhound (agouti), female German Pinscher (dominant yellow), and male Rottweiler (black back) that was conditionally used due to behavioural or health problems not related to skin. Samples were collected in RNAlater Stabilization Solution and stored at −80 °C. RNA was extracted using the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. Integrity of RNA was evaluated with Agilent 2100 Bioanalyzer or TapeStation system (Agilent), and concentration measured with a Qubit 2.1 Spectrophotometer (Invitrogen Corporation). The libraries for STRT (single cell reverse tagged) RNA-seq were prepared using the STRT method with unique molecular identifiers and modifications including longer unique molecular identifiers of the Globin lock method with LNA-primers for the canine alpha- and beta-globin genes. The libraries were sequenced with an Illumina NextSeq 500. Reads were mapped to the CanFam3.1 genome build using HISAT2 mapper v.2.1.0 (ref. 39). The alignment-free quantification method Kallisto was used to estimate the abundance and quantified as transcripts per million mapped reads (TPM) on the basis of an index built from CanFam3.1 Ensembl transcriptome (release 88). The curated VP and coding exons were used to visualize haplotypes (Supplementary Table 3). SNVs that had 100% call rate in these samples were colour coded and displayed relative to the genome assembly and previously associated variants.

### Haplotype construction

Haplotypes were constructed from two publicly available VCF files from the NCBI short read archive as aligned (bam format) or unaligned (fastq format) reads. Fastq data were aligned to the dog genome (CanFam3.1) using BW A-MEM (v.0.7.17) after trimming (Supplementary Table 9). The alignment-free quantification method Kallisto (v.0.46.0) was used to estimate the abundance and quantified as transcripts per million mapped reads (TPM) on the basis of an index built from CanFam3.1 Ensembl transcriptome (release 88). The curated VP and coding exons were used to visualize haplotypes (Supplementary Table 3). SNVs that had 100% call rate in these samples were colour coded and displayed relative to the genome assembly and previously associated variants.

### ASIP phylogenetic analysis in canids

Illumina whole genome sequence for 36 canids, including seven extant species and the dog, were downloaded from the NCBI short read archive as aligned (bam format) or unaligned (fastq format) reads (Supplementary Table 9). Fastq data were aligned to the dog genome (CanFam3.1) using BW A (v.0.7.17) after trimming with Trim Galore (v.0.6.4). SNVs within a 110-kb interval (chr24:23,300,000–23,410,000), which includes the ASIP transcriptional start and regulatory sequences, were identified with Platypus (v.0.8.1) and filtered with VCFtools (v.0.1.15) to include 2,008 biallelic SNVs. Phasing was inferred with BEAGLE (v.4.1) for the 34 of 36 individuals.

### Data availability

All data generated or analysed during this study are included in this published article and its Supplementary Information. GenBank accession numbers for promoter sequence variants are MT319114.1, MT319115.1, MT319116.1 and MT319117.1.

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**Author contributions**

D.B. was involved in conceptualization, investigation, writing, visualization and formal analysis. C.B.K. was involved in investigation, visualization, formal analysis and writing. A.L., P.H. and R.L. were involved in validation and resources. V.I. provided software. P.R. and J.H. undertook validation. K.M.M. and J.R.M. obtained resources. M.K.H., H.L., M.A. and the Dog Genome Annotation (DoGa) consortium obtained resources and STRT analyses. C. Drogemuller was involved in supervision and resources. G.S.B. was involved in supervision, writing and reviewing and editing. T.L. was involved in conceptualization, funding acquisition, investigation, supervision, resources, writing and reviewing and editing.

**Competing interests**

R.L. is associated with a commercial laboratory that offers canine genetic testing. All other authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | Relative transcription of promoter variants. RNA-seq counts (transcripts per million reads, TPM) from dorsal (D) and ventral (V) regions of two dominant yellow, three black back and four agouti dogs obtained from skin biopsies as described in Methods and in Supplementary Table 8. HCP samples also included a black saddle dog. Black dots are from RNA-seq data and grey dots are from STRT RNA-seq data.
Extended Data Fig. 2 | Dog haplotypes across the ASIP locus with comparison to previously associated variants for colour patterns. Dog coat pattern phenotypes are listed on the left. The genomic organization of the ASIP gene with its alternative promoters is illustrated at the top. Yellow indicates a homozygous match to the reference genome, grey deleted, white heterozygous and blue homozygous alternate allele. The black rectangles highlight the promoter regions. Green triangles represent the location of variants that were previously identified to distinguish different alleles for coat colour patterns: (i) The previously identified intronic duplication, “RALY dup”, associated with BS vs. BB haplotypes in some breeds, lies 86 kb to the left of the VP but recombinants (Supplementary Table 7) exclude a causal role for ASIP pattern variation. Similarly, (ii), a SINE insertion associated with BB and BS haplotypes in some breeds and, (iii) missense variants in exon 4 associated with DY haplotypes, are also excluded from a causal role in ASIP pattern variation by rare recombinants (Supplementary Table 7). In the samples presented here, the dominant yellow haplotype extends through the coding sequence where the missense variants associated with this haplotype were previously identified. The results shown here will allow more accurate genetic testing in the future. Samples used are listed in Supplementary Table 3. Raw genotyping results are in Supplementary Table 4 and summary results comparing previously identified variants are in Table 1 and Supplementary Table 7.
Extended Data Fig. 3 | Expanded canid phylogenetic tree inferred from 48-kb region including the ventral promoter. An expanded version of the maximum likelihood tree shown in Fig. 4b, with 34 canids, representing 7 of 9 extant species.
Extended Data Fig. 4 | Expanded canid phylogenetic tree inferred from 16-kb region within and downstream of the hair cycle promoter. An expanded version of the maximum likelihood tree shown in Fig. 4b, with 34 canids, representing 7 of 9 extant species.
Extended Data Fig. 5 | Genomic distribution of derived substitutions across the ASIP locus. a, Canid phylogenies for the ventral (48 kb) and hair cycle (16 kb) promoter regions, with relevant internal branches marked by the occurrence of derived variants plotted in (b). b, Derived substitutions shared by grey wolf and dogs (cyan). Ancestral alleles on DY/Arctic wolf haplotypes (red) or BB and DY/Arctic wolf haplotypes (orange) that correspond to derived substitutions among the core group of wolf-like canids (Supplementary Table 12). The broken lines demarcate the HCP region (chr24:23,375,800–23,380,000). The solid line signifies the downstream boundary for phylogenetic analysis. The solid green and orange lines indicate the positions of the SINE and 24 bp insertion, respectively, associated with the DY/Arctic wolf haplotype.
Extended Data Fig. 6 | The distribution of ASIP haplotypes in modern grey wolves. Modern grey wolves (squares) from (a) North America (n = 45) or (b) Eurasia (n = 23) were genotyped for 5 structural variants and 6 SNVs using whole genome sequencing data. Wolves are coloured by inferred VP and HCP haplotypes, as indicated in the figure legend and in Supplementary Table 11. The asterisk indicates an SY-like haplotype without the HCP1 insertion.
**Inferred ancestral ASIP haplotypes**

- **extinct Pleistocene canid**
  - HCP1\(^A\) exons 2-4

- **Pleistocene grey wolf**
  - VP2
  - HCP2 exons 2-4

**Observed ASIP haplotypes**

- **grey wolf**
  - VP2
  - HCP2
  - & agouti dog

- **black back dog**
  - VP2
  - HCP5

- **ancient grey wolf (i.e. Yana site)**
  - VP1
  - HCP1

- **arctic grey wolf**
  - & dominant yellow dog
  - VP1
  - HCP1

- **shaded yellow dog**
  - VP2
  - HCP1

- **grey wolf (i.e. Tibetan wolf)**
  - VP2
  - HCP1\(^A\)

- **black saddle dog**
  - VP1
  - HCP4

- **black back dog**
  - VP2
  - HCP3

- **black back dog**
  - VP2
  - HCP4

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Extended Data Fig. 7 | Evolutionary diversification of ASIP haplotypes observed in grey wolves and dogs. The colour (red or blue) of ASIP haplotype segments indicates ancestral species of origin, inferred from phylogenetic analysis (Fig. 4b, Extended Data Figs. 3, 4). Relevant structural variants near the ventral (VP) and hair cycle (HCP) promoters are depicted as yellow triangles (polynucleotide expansions), green bars (SINE insertions) and white bars (deletions). Modified promoter activity is indicated by an X mark (no activity) or an additional arrow (elevated expression), based on RNA-seq (Extended Data Fig. 1) and/or inference from coat colour (Figs. 1, 2c, 4c).
Reporting Summary

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

No software was used for data collection.

Data analysis

All software used was referenced in the methods section and the version used was provided.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files). GenBank accession numbers for promoter sequence variants are MT319114.1, MT319115.1, MT319116.1, MT319117.1.
Field-specific reporting

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Life sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

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All studies must disclose on these points even when the disclosure is negative.

Sample size

Dogs and breeds were selected for genotyping based on segregation of coat colors within and between breeds. Some breeds were included if they had been reported to have coat colors inconsistent with previously identified variants. Publicly available whole genome sequenced samples of dogs, wild canids and ancient samples were used. For phylogenetic analysis, whole genome sequence from at least one representative for each extant canid species was included, based on data availability.

Data exclusions

All data exclusion was noted. Rational for excluding individuals samples in phylogenetic analysis are addressed in the methods section and based on evidence in prior studies.

Replication

Phylogenies were inferred using Maximum Likelihood (Tamura-Nei model) with 250 bootstrap replications.

Randomization

Dogs are grouped by color pattern as outlined in Figure 1 and described in the main text.

Blinding

Classification of dogs by color pattern was done prior to genetic and genomic analyses, based on standard practices for genetic association studies.

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Materials & experimental systems

Involved in the study

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Palaeontology and archaeology

Animals and other organisms

Human research participants

Clinical data

Dual use research of concern

Methods

Involved in the study

n/a

ChIP-seq

Flow cytometry

MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The study does not involve the use of laboratory animals.

Wild animals

Genomic data from natural populations of wild animals was obtained from prior studies.

Field-collected samples

DNA samples from owned pet dogs.

Ethics oversight

All animal experiments were done in accordance with the local regulations. Experiments were approved by the “Cantonal Committee For Animal Experiments” (Canton of Bern; permits 48/13, 75/16 and 71/19).

Note that full information on the approval of the study protocol must also be provided in the manuscript.