Materials and Methods

Sample collection and DNA Isolation

DNA was isolated from blood samples collected from registered dogs of established breeds with at least three generations of available pedigree data. DNA samples were collected from dog shows, competitions, and specialty events, by our group and the Waltham Center for Pet Nutrition (Supplemental Table 2). Pedigree analyses were used to ensure that only dogs with no common grandparents were included. Eight to 12 dogs from each breed from each of 76 breeds were selected for the initial data set. Additional dogs included in the mutation screen were selected without regard to pedigree relationships. Samples were collected by licensed veterinarians or trained veterinary technicians through venipuncture of the cephalic vein using standard protocols approved by the NHGRI Animal Care and Use Committee. In all cases blood samples were collected into ACD or EDTA anticoagulant and shipped at room temperature to the Ostrander laboratory. Samples were stored at 4^0C prior to DNA extraction. DNA isolation was performed off-site using standard proteinase K/phenol-chloroform isolation methods by HealthGene, Inc (Ontario, Canada). Final samples were suspended in 10 mM Tris base, 0.1 mM EDTA, aliquoted and stored at -80^0C.

Phenotype determination:

Phenotype was determined based on the written ideal of each breed called the "breed standard". Breed standards are developed by the breed club and published by the kennel club and include descriptions of traits, both physical and behavioral, that are considered

important or necessary to the individual breed. Dogs were considered chondrodysplastic and cases in the GWAS if their descriptions met the following three criteria:

1) Height ≤ 15 inches and height to length ratio < 1. Not symmetrical.
2) Skeletal structure of limbs described as heavy, ample, or well-boned especially in relation to overall size.
3) Forelimbs bowed, curved, and/or feet turned out.

All three criteria had to be met before a breed was considered a case in the GWAS. Mutation screening revealed that two of three criteria are sufficient to identify a chondrodysplastic breed. Breeds with descriptions that suggested variation in these traits were removed from the analysis.

SNP genotyping

Samples were genotyped using the Affymetrix Version 2, Canine SNP chip which lists approximately 127,000 features. The standard Affymetrix "GeneChip® Mapping 500K Assay" protocol was followed using 250 ng of phenol/chloroform extracted-DNA for each dog (http://www.affymetrix.com/support/downloads/manuals/500k_assay_manual.pdf). Before the hybridization step, the volume of the reaction was reduced to 35 µl by heated evaporation to allow the entire reaction to be hybridized to the SNP chip. Genotypes were called using the BRLMM-p algorithm on batched sets of CEL files (http://www.affymetrix.com/support/technical/whitepapers/brlmmp_whitepaper.pdf) (*1*). All calls were combined into one data set. SNPs with a call rate of <90%, were heterozygous in >60% of individuals, or had discordant genotype calls in >1/3 of 158

duplicate pairs were discarded. Of these SNPs, 43 had minor allele frequencies of <1%. The resulting set of 41,635 informative SNPs was used in the association analysis described in this study. Complete list of SNPs is available at http://research.nhgri.nih.gov/dog_genome/.

Association Analysis

The data set was divided into cases and controls based on breed assignment with Pembroke and Cardigan Welsh Corgi, Dachshund, Basset Hound, Petit Basset Griffon Vendéen, Pekingese, Glen of Imaal Terrier, and Scottish Terrier considered "cases" and dogs from the remaining 64 breeds assigned as "controls". Four breeds, (Jack Russell Terrier, West Highland White Terrier, Havanese and Sussex Spaniel) were assigned a "missing" phenotype because the standard description, history, or our own measurement data suggested that the leg length or shape was variable (*2, 3*). Single marker chi-squared association, model-based Fisher association, and haplotype association was performed using PLINK (*4*). Analysis was repeated with five overlapping subsets of 2-3 case breeds each to determine if all breeds were contributing to the association at the same locus.

Because dog breeds are highly structured populations, there is an inflation of *p*-values in association tests that is caused by the relatedness between samples within populations. One strategy for removing the cryptic relatedness is to use the allele frequencies within the population instead of the individual genotypes. For each breed, we first calculated the relative frequency of the minor allele at each marker and then conducted a Mann-Whitney U test comparing the frequencies in chondrodysplastic breeds with the frequencies in all other dog breeds. The test rejects the null hypothesis of

no association if there is a large difference in the median allele frequency between the two groups.

Fine Mapping

For fine mapping additional SNPs were chosen from public databases utilizing the canine genome sequence (*5*) (http://www.broad.mit.edu/node/459, http://www.ncbi.nlm.nih.gov/projects/SNP/). Assays were designed for 95 SNPs from the 600 kb region around the peak of strongest association with an average spacing of 6.5 kb. SNPs were genotyped using the SNPlex genotyping system and an ABI 3730XL genome analyzer with standard protocols (Applied Biosystems, Santa Clara, CA). Thirty-one SNPs either failed the genotyping assay or were not polymorphic and were therefore removed from further analysis.

Chi-squared analysis revealed 10 SNPs in the region with *p*-value of association $<10^{-50}$. The single strongest association was found at position 23,445,875, with a single marker uncorrected *p*-value of $4x10^{-109}$.

T-test of significance in difference between heterozygosity measures in cases vs. controls was performed using the statistical package R.

Primers to surround all conserved segments within the haplotype and to tile across region of particular interest were designed using PrimerTile software (Peter Chines, personal communication) and the Primer3 program (*6*). Segments were amplified using standard PCR protocols and a 40 cycle, touch-down thermocycler program with the annealing temperature reduced by ½ a degree for the first 20 cycles, then held steady at the lowest temperature for the last 20 cycles. Primer sequences and annealing

temperatures can be found in Supplemental Table 4. Each segment was sequenced using BigDye terminator sequencing kits and standard protocols (Applied Biosystems, Santa Clara, CA) in 26 chondrodysplastic dogs from nine breeds and 18 non-chondrodysplastic dogs from 11 breeds.

Sequencing the Insert

Primers surrounding the localized insertion were designed from the CanFam2 reference genome and used to generate a PCR product of ~5 kb using KOD Xtreme Hot Start DNA polymerase with an extension time of 9 min (Novagen, San Diego CA). The product was extracted from an agarose gel using QIAquick Gel Extraction Kit (Qiagen Corp., Valencia, CA) and then sequenced on an Illumina Genome Analyzer following the manufacturer's protocol. A total of 536,492 high-quality, 48-base reads were generated and assembled into contigs using the short-read assembly program Velvet (*7*). A variety of parameters were tested, and we determined that the optimal settings for this data set were a k-mer size of 29 and coverage-cutoff of 27. This produced five distinct contigs, one of which was 1,570bp in length and matched the *fgf4* cDNA using BLAT (http://genome.ucsc.edu/).

Additional primers were developed from the exon sequences and 3'UTR of the annotated *FGF4* gene and matched with sequence surrounding the insert in order to obtain the complete sequence of the insert and look for polymorphisms. Sequencing was performed as described in the fine mapping section. A complete list of primers can be found in Table S6.

Tissue collection

Fetal Tissue:

Tissues were collected in accordance with the animal care and use protocols approved by Cornell University. Fetal tissues were obtained from pregnant dogs presenting at a non-profit spay and neuter clinic for reproductive surgery. Upon collection, fetuses were placed in RNAlater and stored for 1-3 days at 4^{o}C. Excess RNAlater was poured off and the fetus was frozen at -80^{o}C. Frozen limb samples were shipped on dry ice. Femoral and tibial heads from knee joints were removed using sterile procedures with the aid of a dissecting microscope and used for RNA extraction.

Neo-natal tissue:

Peripheral blood and articular cartilage were collected from a four week old F2 dachshund – beagle cross that was chondrodysplastic and homozygous for the insert. Blood was collected into ACD via cardiac puncture. Cartilage was dissected using sterile procedure from scapular wing, humeral joints, femoral joints, xiphisternum, and rib and diced into fragments approximately 1 mm thick. Fragments were placed in a 5x volume of RNA later (Ambion) in 1.7 mL eppendorf tubes and frozen by immersion in LN2. Frozen samples were shipped on dry ice. All procedures were performed in accordance with the animal care and use guidelines established and approved by the Baker Institute and Cornell University.

Adult tissue:

Sections of liver and full-thickness articular cartilage specimens from the humeral head were obtained from a 10 year-old male castrated Shih Tzu and a 10 year-old male castrated Siberian Husky immediately after euthanasia performed for reasons unrelated to this study. Collected specimens were placed in RNAlater (Ambion) immediately after

harvest, stored at 4°C overnight and then transferred to –80°C until shipped on dry-ice. Collection was performed in accordance with the animal care and use protocols of the University of Missouri.

RNA Analysis

RNA was isolated from testes, cartilage and liver tissues stored in RNA-later® (Ambion, Austin TX). Adult cartilage was ground over dry ice using a mortar and pestle. Cartilage fragments were then forced through a BioMasher™ (Investigen, Hercules CA) in TRI reagent (Ambion) and extraction was completed using RiboPure® RNA extraction kit (Ambion, Austin, TX). Neonatal cartilage samples were ground in liquid nitrogen over dry ice using a mortar and pestle then extracted using the RiboPure® RNA extraction kit (Ambion, Austin, TX). Testes and liver were homogenized in TRI reagent using a disposable plastic pestle in 1.7 mL eppendorf tubes. DNA was extracted from the same tissues using manufacturer's protocols (http://www.ambion.com/techlib/append/supp/tri.pdf). Fetal cartilage samples were homogenized using a motorized mixer and disposable plastic pestle in a 1.7 mL eppendorf tube in lysis solution from the RNAqueous®-4PCR Kit (Ambion) and extraction was completed following manufacturers protocols. DNA extraction on fetal tissue was performed using a DNEasy Blood & Tissue Kit (Qiagen Corp., Valencia CA). The mass of tissue used for DNA extraction varied, but did not exceed 25mg, as is suggested by the manufacturer. The initial tissue lysis and homogenization step was performed using the Kontes 2mL dounce and 180uL of lysis buffer. After tissue homogenization, the extraction procedure follows the routine protocol provided.

Residual DNA was removed from the RNA samples using the Turbo DNA free kit (Ambion, Austin, TX) and samples were stored at -20^{o}C. First-strand cDNA was prepared using SMART MMLV reverse transcriptase and SMARTScript RT (Clontech, Mountain View, CA) using an oligo dT primer with 100 ng to 1 μg of RNA per reaction and standard protocols. PCR across the identifying nucleotide was performed using F-CTGAGTTTTCTAGTAAGACCGGTGAAG and R-ACAGAGAAACATGGGCTGTGG primers. PCR to detect expression of surrounding genes was performed using the following primer pairs: CD36F- CGGAGACATGCTGATTGAGAAG, CD36R-AGCCAGATTGAGAACGGTCATAG; Sema3cF- agtgaatgctgctgacggtaga, Sema3cR-gatgcagagacacttgggaaac; ActinF- GATCTGGCACCACACCTTCTAC, ActinR-CCCAGAGTCCATGACAATACCA; negative control for DNA contamination SQ7415F- GGCTGGTCACCTTGGTCTTCTC, SQ7415R-TATCACTCGGGCTTTGGTTGTG. The products were sequenced using Sanger sequencing methods and BigDye Terminator Sequencing on the ABI3730xl (Applied Biosystems. Santa Clara, CA).

Figure S1. A 5 Kb insert is found only in chondrodysplastic breeds. PCR amplification of a region on chromosome 18 using primers F- cacacagatggaccatgaaataagt and R- ccaatttgttccctccatttc produced a 132 bp fragment in non-chondrodysplastic dogs (controls) and a band of ~5000 bp in chondrodysplastic dogs (cases). 1Kb ladder and Low mass ladder are both produced by Invitrogen Corp. (Invitrogen, Carlsbad, CA)

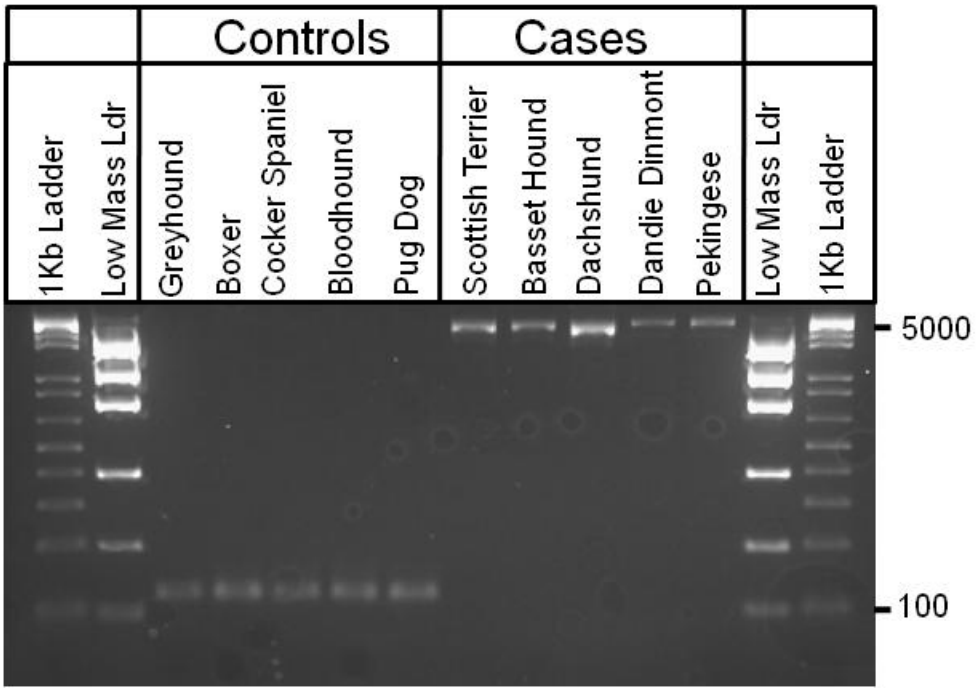


Figure S2. Expression of FGF4 and the genes on either side of the fgf4-retrogene in cartilage from a) neonatal chondrodysplastic dog and b) fetal non-chondrodysplastic dogs. The negative control amplifies an intronic region of FGF4 (1147 bp) only in genomic DNA. The molecular weight markers used are 100 bp ladder and 1 Kb plus ladder (Invitrogen). Actin is amplified as a positive control for expression in all cell types.

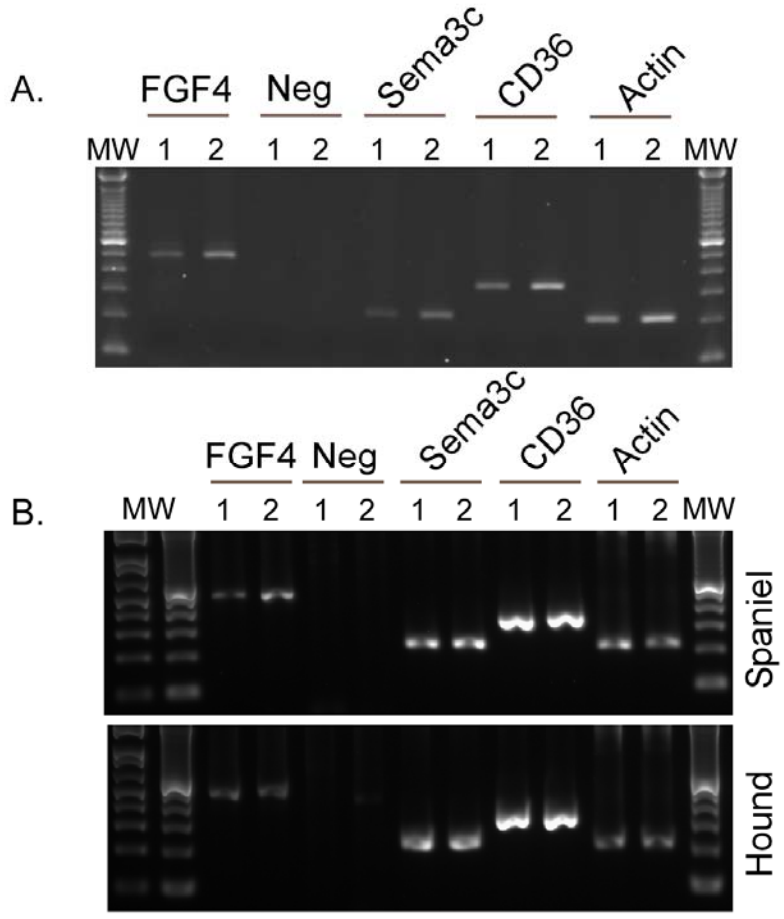


Figure S3. Distrubution of haplotypes in the FGF4 gene and the retrogene insertion site in dog and wolf populations. The combination of haplotypes necessary to produce the first chondrodysplastic dog is shown at the top of the figure. The correct combination was identified only in wolves from Europe and the Middle East. No dogs carried both the insertion site and the *FGF4* haplotype of the ancestor. Breeds or wolf populations are listed if they carry one of the two required haplotypes or if they carry a dog or wolf specific haplotype. See Table S6 for frequency distribution.

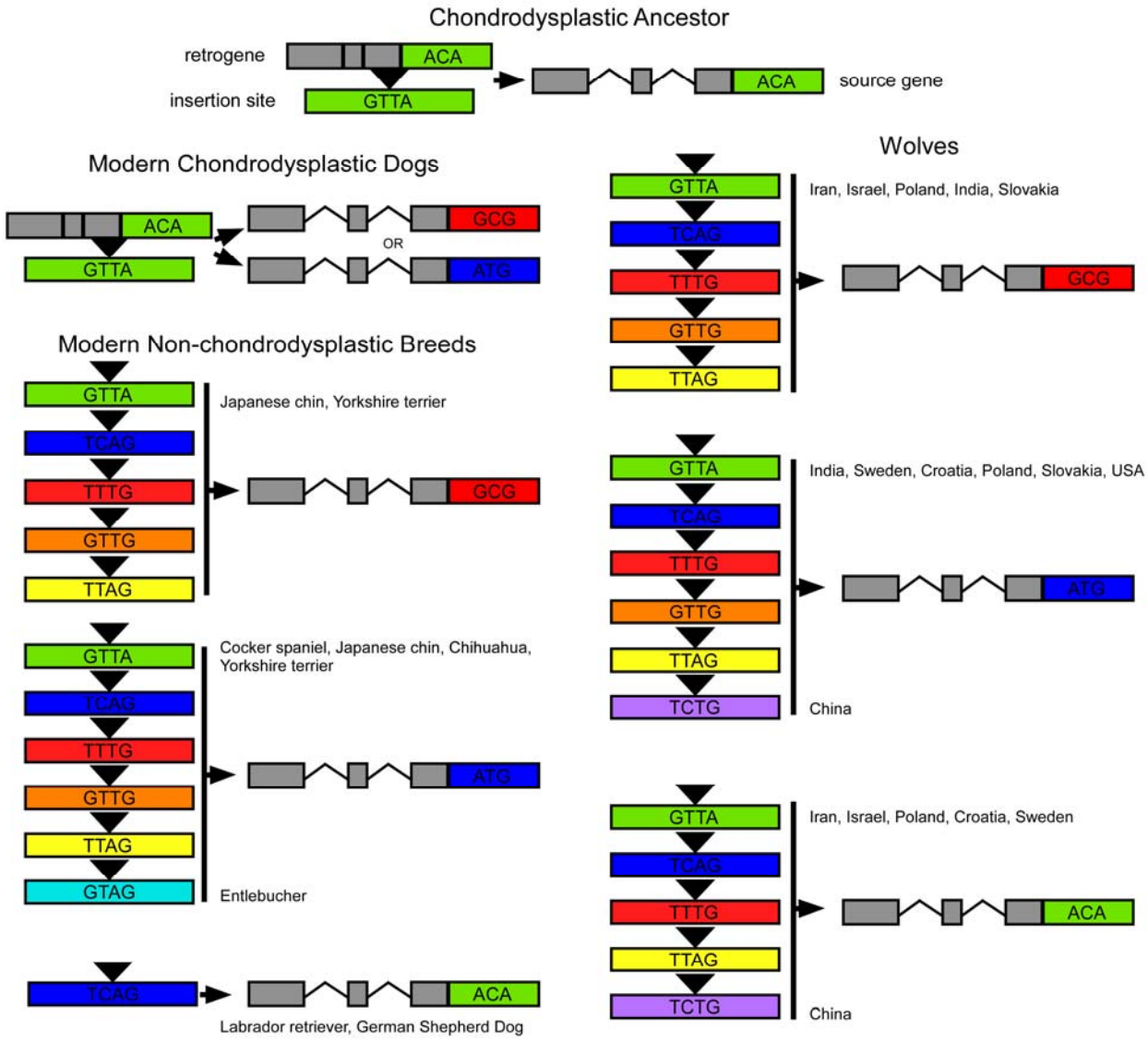


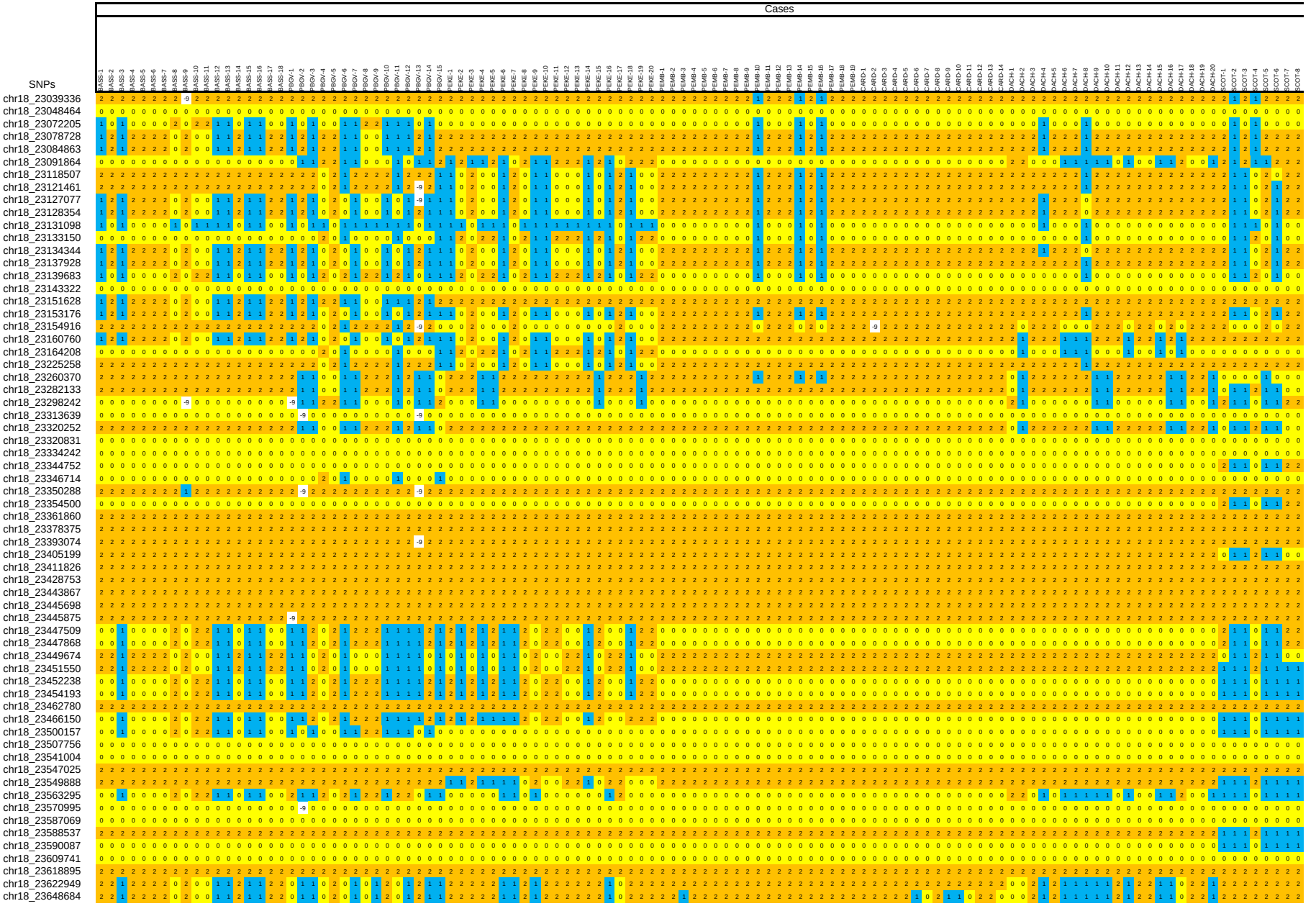
Table S1. List of breeds and number of individuals genotyped for whole genome association analysis.

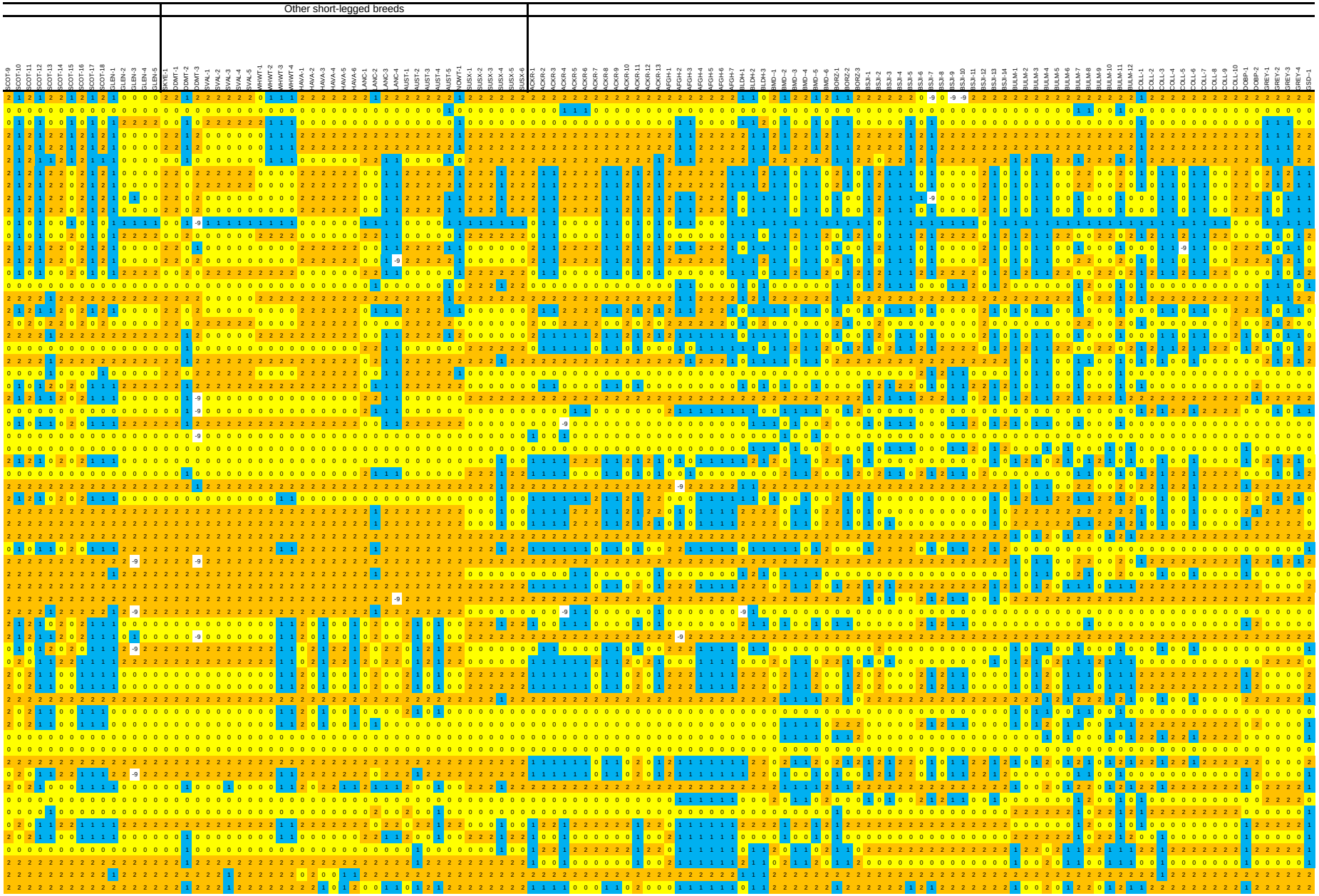
Breed	Breed_ID	Number	Females	Males	Unspecified Sex	Phenotype[a]
American Cocker Spaniel	ACKR	12	5	7	0	Control
American Eskimo Dog	AESK	1	1	0	0	Control
Afghan Hound	AFGH	12	5	5	2	Control
Akita	AKIT	12	4	7	1	Control
Alaskan Malamute	AMAL	6	1	3	2	Control
Australian Shepherd	AUSS	12	7	5	0	Control
Australian Terrier	AUST	12	5	7	0	Control
Basset Hound	BASS	12	9	3	0	Case
Beagle	BEAG	12	9	3	0	Control
Bloodhound	BLDH	9	4	5	0	Control
Bernese Mountain Dog	BMD-	12	7	4	1	Control
Border Collie	BORD	12	1	1	10	Control
Borzoi	BORZ	11	4	4	3	Control
Boston Terrier	BOST	6	4	1	1	Control
Boxer	BOX-	12	9	2	1	Control
Briard	BRIA	12	10	2	0	Control
Brittany	BRIT	12	0	0	12	Control
Brussels Griffon	BRUS	5	3	2	0	Control
Basenji	BSJI	12	5	7	0	Control
Bulldog	BULD	11	6	5	0	Control
Bullmastiff	BULM	12	9	3	0	Control
Bull Terrier	BULT	3	3	0	0	Control
Cairn Terrier	CAIR	7	5	2	0	Control
Cardigan Welsh Corgi	CARD	12	6	6	0	Case
Chihuahua	CHIH	7	5	2	0	Control
Chow Chow	CHOW	12	0	0	12	Control
Cavalier King Charles Spaniel	CKCS	9	5	3	1	Control
Collie	COLL	12	5	7	0	Control
Dachshund	DACH	12	6	6	0	Case
Great Dane	DANE	8	6	2	0	Control
Scottish Deerhound	DEER	12	2	3	7	Control
Doberman Pinscher	DOBP	12	6	6	0	Control
English Springer Spaniel	ESSP	12	1	8	3	Control
French Bulldog	FBUL	10	7	2	1	Control
Glen of Imaal Terrier	GLEN	12	5	6	1	Case
Golden Retriever	GOLD	12	1	1	10	Control
Greyhound	GREY	9	3	6	0	Control
German Shepherd Dog	GSD-	12	6	4	2	Control
German Shorthaired	GSHP	10	4	5	1	Control

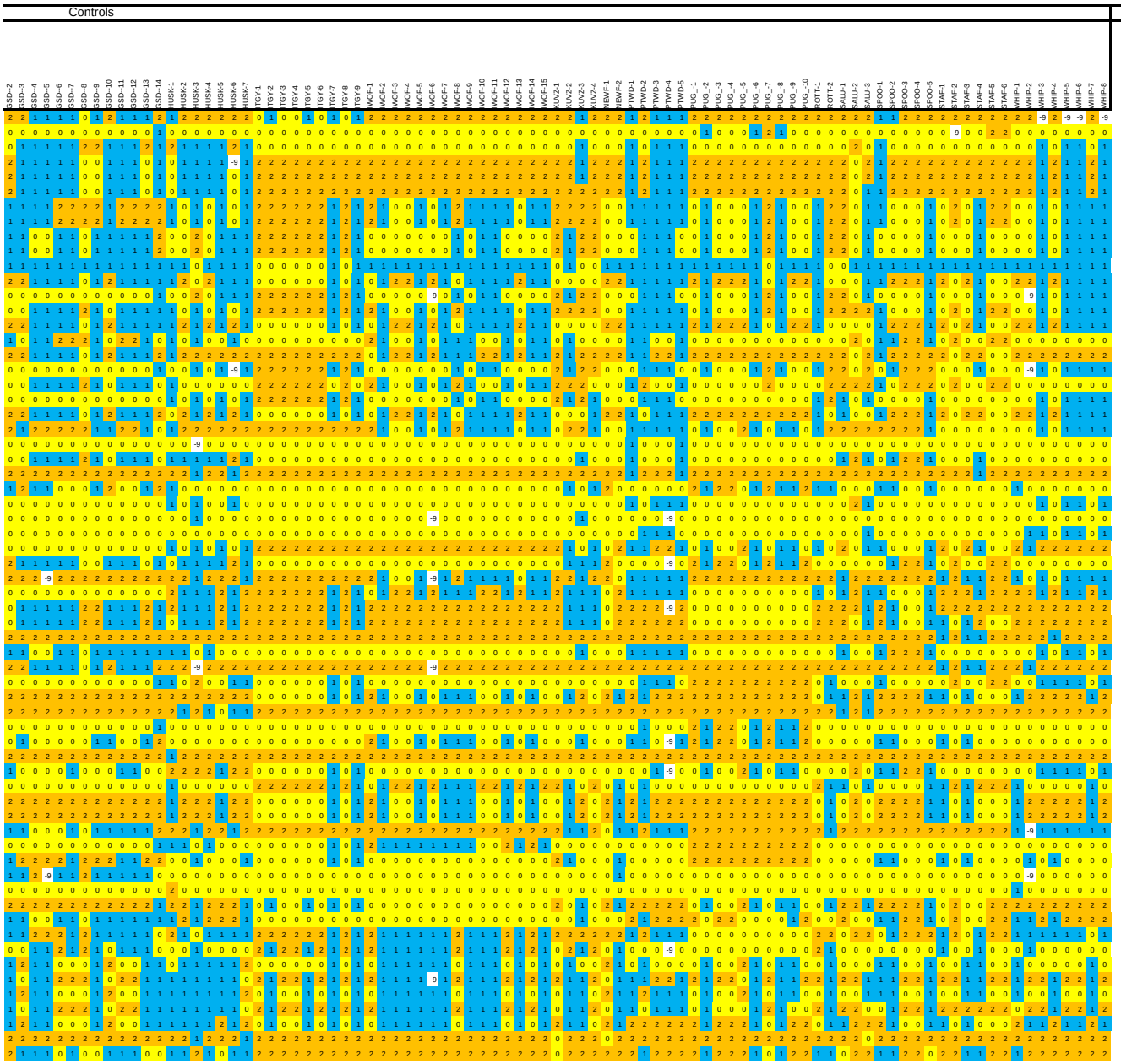
Pointer						
Giant Schnauzer	GSNZ	12	7	5	0	Control
Havanese	HAVA	9	6	3	0	n.d.
Siberian Husky	HUSK	12	8	4	0	Control
Ibizan Hound	IBIZ	12	8	4	0	Control
Italian Greyhound	ITGY	12	9	3	0	Control
Irish Wolfhound	IWOF	13	4	7	2	Control
Irish Water Spaniel	IWSP	12	4	8	0	Control
Jack Russell Terrier	JACK	12	7	5	0	n.d.
Kuvasz	KUVZ	12	5	7	0	Control
Labrador Retriever	LAB-	12	3	2	7	Control
Mastiff	MAST	12	8	4	0	Control
Miniature Bull Terrier	MBLT	12	8	3	1	Control
Miniature Pinscher	MPIN	12	7	5	0	Control
Newfoundland	NEWF	12	3	7	2	Control
Norwich Terrier	NOWT	10	5	5	0	Control
Old English Sheepdog	OES-	12	8	4	0	Control
Papillon	PAPI	8	3	3	2	Control
Petit Basset Griffon Vendeen	PBGV	11	8	3	0	Case
Pekingese	PEKE	12	5	7	0	Case
Pembroke Welsh Corgi	PEMB	12	6	6	0	Case
Pomeranian	POM-	12	10	2	0	Control
Portuguese Water Dog	PTWD	12	6	6	0	Control
Pug Dog	PUG	12	6	6	0	Control
Rottweiler	ROTT	12	4	7	1	Control
Saluki	SALU	12	4	8	0	Control
Samoyed	SAMO	6	1	4	1	Control
Scottish Terrier	SCOT	12	4	8	0	Case
Chinese Shar-Pei	SHAR	12	6	6	0	Control
Shih Tzu	SHIH	12	7	5	0	Control
Standard Poodle	SPOO	13	6	7	0	Control
Shetland Sheepdog	SSHP	12	6	4	2	Control
Standard Schnauzer	SSNZ	12	5	6	1	Control
Staffordshire Bull Terrier	STAF	12	5	7	0	Control
Saint Bernard	STBD	12	5	2	5	Control
Sussex Spaniel	SUSX	5	2	3	0	n.d.
Toy Poodle	TPOO	12	5	7	0	Control
Whippet	WHIP	12	6	6	0	Control
West Highland White Terrier	WHWT	12	3	9	0	n.d.

a. Case and control status refer to strict chondrodysplastic phenotype assigned in initial analysis and required that the dogs meet all three criteria put forth in materials and methods.

Table S2. Genotypes of 64 SNPs across the associated locus. Dog breeds are listed at the top of the chart, grouped by leg length phenotype. SNP positions are listed on the left in order from the centromeric to the telomeric end of the locus. Homozygous genotypes are shown as 0 (yellow) or 2 (gold), heterozygous genotypes are 1 (blue) and missing data is labeled -9 (white).


Cases
SNPs
BASS-1
BASS-2
BASS-3
BASS-4
BASS-5
BASS-6
BASS-7
BASS-8
BASS-9
BASS-10
BASS-11
BASS-12
BASS-13
BASS-14
BASS-15
BASS-16
BASS-17
BASS-18
PBOV-1
PBOV-2
PBOV-3
PBOV-4
PBOV-5
PBOV-6
PBOV-7
PBOV-8
PBOV-9
PBOV-10
PBOV-11
PBOV-12
PBOV-13
PBOV-14
PBOV-15
PEKE-1
PEKE-2
PEKE-3
PEKE-4
PEKE-5
PEKE-6
PEKE-7
PEKE-8
PEKE-9
PEKE-10
PEKE-11
PEKE-12
PEKE-13
PEKE-14
PEKE-15
PEKE-16
PEKE-17
PEKE-18
PEKE-19
PEKE-20
PEMB-1
PEMB-2
PEMB-3
PEMB-4
PEMB-5
PEMB-6
PEMB-7
PEMB-8
PEMB-9
PEMB-10
PEMB-11
PEMB-12
PEMB-13
PEMB-14
PEMB-15
PEMB-16
PEMB-17
PEMB-18
PEMB-19
CARO-1
CARO-2
CARO-3
CARO-4
CARO-5
CARO-6
CARO-7
CARO-8
CARO-9
CARO-10
CARO-11
CARO-12
CARO-13
CARO-14
DACH-1
DACH-2
DACH-3
DACH-4
DACH-5
DACH-6
DACH-7
DACH-8
DACH-9
DACH-10
DACH-11
DACH-12
DACH-13
DACH-14
DACH-15
DACH-16
DACH-17
DACH-18
DACH-19
DACH-20
SCOT-1
SCOT-2
SCOT-3
SCOT-4
SCOT-5
SCOT-6
SCOT-7
SCOT-8
chr18_23039336
chr18_23048464
chr18_23072205
chr18_23078728
chr18_23084863
chr18_23091864
chr18_23118507
chr18_23121461
chr18_23127077
chr18_23128354
chr18_23131098
chr18_23133150
chr18_23134344
chr18_23137928
chr18_23139683
chr18_23143222
chr18_23151628
chr18_23153176
chr18_23154916
chr18_23160760
chr18_23164208
chr18_23225258
chr18_23260370
chr18_23282133
chr18_23298242
chr18_23313639
chr18_23320252
chr18_23320831
chr18_23334242
chr18_23344752
chr18_23346714
chr18_23350288
chr18_23354500
chr18_23361860
chr18_23378375
chr18_23393074
chr18_23405199
chr18_23411826
chr18_23428753
chr18_23443867
chr18_23445698
chr18_23445875
chr18_23447509
chr18_23447868
chr18_23449674
chr18_23451550
chr18_23452238
chr18_23454193
chr18_23462780
chr18_23466150
chr18_23500157
chr18_23507756
chr18_23541004
chr18_23547025
chr18_23549888
chr18_23563295
chr18_23570995
chr18_23587069
chr18_23588537
chr18_23590087
chr18_23609741
chr18_23618895
chr18_23622949
chr18_23648684


Other short-legged breeds



Summary Statistics

het cases	het controls	AA cases	AA controls	BB cases	BB controls	He cases	He controls	Ho cases	Ho controls
10	27	4	9	114	121	0.131	0.246	0.078	0.172
0	10	129	150	0	3	0.000	0.093	0.000	0.061
26	38	94	119	9	7	0.283	0.267	0.202	0.232
26	38	9	5	94	120	0.283	0.251	0.202	0.233
26	38	9	6	94	120	0.283	0.258	0.202	0.232
34	42	70	7	25	115	0.439	0.283	0.264	0.256
20	69	18	44	91	51	0.340	0.499	0.155	0.421
21	69	17	44	90	51	0.337	0.499	0.164	0.421
32	68	24	64	72	31	0.430	0.480	0.250	0.417
31	68	25	65	73	31	0.431	0.479	0.240	0.415
56	134	73	30	0	0	0.340	0.483	0.434	0.817
21	72	91	47	17	45	0.335	0.500	0.163	0.439
31	59	25	72	73	30	0.431	0.466	0.240	0.366
31	71	24	47	74	46	0.425	0.500	0.240	0.433
31	71	74	46	24	47	0.425	0.500	0.240	0.433
0	43	129	105	0	16	0.000	0.353	0.000	0.262
15	34	5	7	109	123	0.175	0.250	0.116	0.207
32	58	24	72	73	32	0.428	0.470	0.248	0.358
2	23	42	93	83	48	0.448	0.462	0.016	0.140
28	63	18	83	83	18	0.373	0.421	0.217	0.384
17	72	101	38	11	54	0.257	0.495	0.132	0.439
12	50	11	40	106	74	0.229	0.479	0.093	0.305
23	10	24	151	82	2	0.399	0.082	0.178	0.061
26	43	11	109	92	12	0.303	0.325	0.202	0.262
27	18	89	2	11	144	0.311	0.125	0.213	0.110
0	39	127	107	0	18	0.000	0.353	0.000	0.238
23	28	11	131	95	4	0.288	0.196	0.178	0.172
0	6	129	156	0	0	0.000	0.036	0.000	0.037
0	25	129	136	0	3	0.000	0.171	0.000	0.152
9	50	113	61	7	53	0.162	0.499	0.070	0.305
3	48	125	87	1	28	0.038	0.434	0.023	0.294
1	35	0	12	126	114	0.008	0.299	0.008	0.217
9	62	113	60	7	42	0.162	0.494	0.070	0.378
0	42	0	39	129	82	0.000	0.465	0.000	0.258
0	49	0	43	129	72	0.000	0.484	0.000	0.299
0	9	0	3	128	152	0.000	0.087	0.000	0.055
10	52	7	99	112	13	0.169	0.363	0.078	0.317
0	19	0	7	128	136	0.000	0.183	0.000	0.117
1	32	0	115	128	17	0.008	0.321	0.008	0.195
0	47	0	33	129	84	0.000	0.452	0.000	0.287
0	12	0	7	129	145	0.000	0.146	0.000	0.073
2	10	0	147	125	5	0.016	0.116	0.016	0.062
28	40	76	112	25	11	0.422	0.308	0.217	0.245
30	1	74	0	25	162	0.428	0.006	0.233	0.006
28	39	25	110	75	14	0.424	0.327	0.219	0.239
32	48	20	82	77	34	0.402	0.457	0.248	0.293
32	48	77	44	20	72	0.402	0.485	0.248	0.293
32	48	77	44	20	72	0.402	0.485	0.248	0.293
0	37	0	14	129	112	0.000	0.319	0.000	0.227
31	22	78	129	20	13	0.399	0.250	0.240	0.134
25	31	97	96	7	37	0.257	0.435	0.194	0.189
0	23	129	128	0	11	0.000	0.239	0.000	0.142
0	1	129	162	0	1	0.000	0.018	0.000	0.006
0	49	0	45	129	70	0.000	0.488	0.000	0.299
19	52	9	78	100	34	0.247	0.464	0.148	0.317
37	53	76	21	16	90	0.392	0.411	0.287	0.323
0	46	128	94	0	23	0.000	0.405	0.000	0.282
1	44	128	97	0	23	0.008	0.398	0.008	0.268
13	63	2	24	114	76	0.123	0.449	0.101	0.387
13	65	114	83	2	16	0.123	0.417	0.101	0.396
0	66	129	21	0	77	0.000	0.442	0.000	0.402
0	67	0	68	129	29	0.000	0.472	0.000	0.409
27	6	12	3	90	155	0.317	0.070	0.209	0.037
30	46	15	22	84	96	0.357	0.398	0.233	0.280

Table S3. Positions of SNPs discovered in this study with their genotypes and allele frequencies in 40 dogs. Primers used to obtain the sequences from which the SNPs were discovered are listed in the last two columns of the table.

						Cases							Controls								
Name	Position	Allele A	Allele B	count A	count B	Ho	He	MAF	AA	AB	BB	count	Ho	He	MAF	AA	AB	BB	count	Forward Primer	Reverse Primer
SQ6686_snp1	23281790	C	T	43	45	0.12	0.23	0.13	2	3	21	26	0	0	1	18	0	0	18	TTGACTTCGGGATTTCCTACTT	TTGAAGCTATTCCAAAAATTACACC
SQ6686_snp2	23281792	A	G	42	46	0.15	0.26	0.15	2	4	20	26	0.11	0.1	0.94	16	2	0	18	TTGACTTCGGGATTTCCTACTT	TTGAAGCTATTCCAAAAATTACACC
SQ6686_snp3	23281800	C	T	48	40	0.15	0.2	0.12	21	4	1	26	0.11	0.1	0.94	0	2	16	18	TTGACTTCGGGATTTCCTACTT	TTGAAGCTATTCCAAAAATTACACC
SQ6686_snp4	23281891	C	T	1	87	0	0	0	0	0	26	26	0.06	0.05	0.03	0	1	17	18	TTGACTTCGGGATTTCCTACTT	TTGAAGCTATTCCAAAAATTACACC
SQ6686_snp5	23281991	A	G	2	86	0	0	0	0	0	26	26	0.11	0.1	0.06	0	2	16	18	TTGACTTCGGGATTTCCTACTT	TTGAAGCTATTCCAAAAATTACACC
SQ6686_snp6	23281994	A	G	48	40	0.15	0.2	0.12	21	4	1	26	0.11	0.1	0.94	0	2	16	18	TTGACTTCGGGATTTCCTACTT	TTGAAGCTATTCCAAAAATTACACC
SQ6686_snp7	23282133	C	G	34	52	0.15	0.2	0.12	1	4	21	26	0.35	0.29	0.82	11	6	0	17	TTGACTTCGGGATTTCCTACTT	TTGAAGCTATTCCAAAAATTACACC
SQ6687_snp1	23287316	G	T	83	3	0.04	0.04	0.02	25	1	0	26	0.12	0.11	0.06	15	2	0	17	CAAATTTCATGTTTCATAAGCACA	CTCAGACTAGATTGGGACAAGTCA
SQ6687_snp2	23287414	A	G	45	41	0.12	0.23	0.13	21	3	2	26	0	0	1	0	0	17	17	CAAATTTCATGTTTCATAAGCACA	CTCAGACTAGATTGGGACAAGTCA
SQ6687_snp3	23287678	C	T	83	1	0	0	0	26	0	0	26	0.06	0.06	0.03	15	1	0	16	CAAATTTCATGTTTCATAAGCACA	CTCAGACTAGATTGGGACAAGTCA
SQ6687_snp4	23287719	C	T	83	1	0	0	0	26	0	0	26	0.06	0.06	0.03	15	1	0	16	CAAATTTCATGTTTCATAAGCACA	CTCAGACTAGATTGGGACAAGTCA
SQ6760_snp1	23298053	C	T	42	46	0.15	0.2	0.12	1	4	21	26	0	0	1	18	0	0	18	TGAAGGCAACACAAAGTAAACAG	ACTTGAATAAGCCCACATCACC
SQ6760_snp2	23298242	G	T	47	41	0.15	0.2	0.12	21	4	1	26	0.06	0.05	0.97	0	1	17	18	TGAAGGCAACACAAAGTAAACAG	ACTTGAATAAGCCCACATCACC
SQ6688_snp1	23320252	A	G	38	50	0.15	0.2	0.12	1	4	21	26	0.22	0.2	0.89	14	4	0	18	TCAAGTAAAGATTAGGTCTGTCAACATT	AACCATAACAAAGAATGAACAGTGA
SQ6688_snp2	23320369	A	G	46	42	0.15	0.2	0.12	21	4	1	26	0	0	1	0	0	18	18	TCAAGTAAAGATTAGGTCTGTCAACATT	AACCATAACAAAGAATGAACAGTGA
SQ6688_snp3	23320471	C	T	47	41	0.15	0.2	0.12	21	4	1	26	0.06	0.05	0.97	0	1	17	18	TCAAGTAAAGATTAGGTCTGTCAACATT	AACCATAACAAAGAATGAACAGTGA
SQ6688_snp4	23320472	A	G	46	42	0.15	0.2	0.12	21	4	1	26	0	0	1	0	0	18	18	TCAAGTAAAGATTAGGTCTGTCAACATT	AACCATAACAAAGAATGAACAGTGA
SQ6688_snp5	23320831	C	T	87	1	0	0	0	26	0	0	26	0.06	0.05	0.03	17	1	0	18	TCAAGTAAAGATTAGGTCTGTCAACATT	AACCATAACAAAGAATGAACAGTGA
SQ6690_snp1	23329007	G	T	51	37	0.12	0.17	0.1	22	3	1	26	0.22	0.2	0.89	0	4	14	18	TCCCTGTAATCAAGTACTCCAAAA	GATGCAGAATCAAATCCAAGTT
SQ6690_snp2	23329014	C	G	11	77	0.08	0.14	0.08	1	2	23	26	0.17	0.31	0.19	2	3	13	18	TCCCTGTAATCAAGTACTCCAAAA	GATGCAGAATCAAATCCAAGTT
SQ6693_snp1	23334242	C	G	85	3	0	0	0	26	0	0	26	0.17	0.15	0.08	15	3	0	18	AACCTGGTGAGTCCATCTCATT	TTTCTCTGTTAACCAAAGCACAAC
SQ6693_snp2	23334363	A	G	81	7	0.04	0.04	0.02	25	1	0	26	0.11	0.28	0.17	14	2	2	18	AACCTGGTGAGTCCATCTCATT	TTTCTCTGTTAACCAAAGCACAAC
SQ6762_snp1	23335392	A	T	80	8	0.04	0.04	0.02	25	1	0	26	0.17	0.31	0.19	13	3	2	18	ACCTTGAGAGTGTGTGTCCTG	TGAATGGATGAGAGAAGTAAAGG
SQ6696_snp1	23347988	A	G	7	81	0.04	0.04	0.02	0	1	25	26	0.22	0.28	0.17	1	4	13	18	GTACAGTTCATTTTCCCCCATT	TTGCATTAAAATAGGACGTTGC
SQ6696_snp2	23348271	A	T	1	87	0	0	0	0	0	26	26	0.06	0.05	0.03	0	1	17	18	GTACAGTTCATTTTCCCCCATT	TTGCATTAAAATAGGACGTTGC
SQ6696_snp4	23348398	A	C	38	48	0.08	0.14	0.08	1	2	23	26	0	0	1	17	0	0	17	GTACAGTTCATTTTCCCCCATT	TTGCATTAAAATAGGACGTTGC
SQ6697_snp1	23349270	A	G	74	6	0	0	0	24	0	0	24	0.25	0.3	0.19	11	4	1	16	TCTAAGATAATTACAACGAATAACCAG	AGAAATTTTTGAAGGGAAAAGC
SQ6697_snp2	23349311	A	G	4	84	0	0	0	0	0	26	26	0.22	0.2	0.11	0	4	14	18	TCTAAGATAATTACAACGAATAACCAG	AGAAATTTTTGAAGGGAAAAGC
SQ6697_snp3	23349475	A	G	54	32	0	0.08	0.04	24	0	1	25	0.22	0.28	0.83	1	4	13	18	TCTAAGATAATTACAACGAATAACCAG	AGAAATTTTTGAAGGGAAAAGC
SQ6697_snp4	23349659	A	C	81	7	0	0	0	26	0	0	26	0.28	0.31	0.19	12	5	1	18	TCTAAGATAATTACAACGAATAACCAG	AGAAATTTTTGAAGGGAAAAGC
SQ6698_snp1	23351610	A	G	13	69	0.04	0.04	0.02	0	1	23	24	0.35	0.46	0.35	3	6	8	17	TGGCCAAGTTATTGTGACAT	TTCTTCAACCAATAGGTCTATGTGTACT
SQ6700_snp1	23355666	C	T	23	63	0.04	0.11	0.06	1	1	23	25	0.33	0.49	0.56	7	6	5	18	GTAAAGTGGGCACAAACAGG	ACACCACACAACCCATTTTT
SQ6700_snp3	23356140	A	T	81	5	0	0	0	25	0	0	25	0.17	0.24	0.14	14	3	1	18	GTAAAGTGGGCACAAACAGG	ACACCACACAACCCATTTTT
SQ6702_snp1	23357519	G	T	34	50	0	0.08	0.04	1	0	24	25	0	0.11	0.94	16	0	1	17	TCATACAACTTCTTCCCCCATC	ATAGATCTTGCCCCGAATTCAC
SQ6702_snp2	23357594	G	T	38	50	0	0.14	0.08	2	0	24	26	0	0.1	0.94	17	0	1	18	TCATACAACTTCTTCCCCCATC	ATAGATCTTGCCCCGAATTCAC
SQ6702_snp3	23357621	C	T	38	50	0	0.14	0.08	2	0	24	26	0	0.1	0.94	17	0	1	18	TCATACAACTTCTTCCCCCATC	ATAGATCTTGCCCCGAATTCAC
SQ6703_snp1	23358668	A	G	36	50	0	0.08	0.04	1	0	24	25	0	0.1	0.94	17	0	1	18	AAAACCATTTTTGGTAAATACCTC	TCCTGCAACTTGGTTAAGGTC
SQ6704_snp1	23358950	A	G	75	9	0	0	0	25	0	0	25	0.29	0.39	0.26	10	5	2	17	GACCTTAACCAAGTTGCAGGAG	CATACTAGGCTTCATTGCTTCAGA
SQ6706_snp1	23362488	A	C	49	39	0.04	0.11	0.06	24	1	1	26	0	0	1	0	0	18	18	TCCATAACATCCATTCGAGAAC	TTTGCATAATCAGGGACAAAAT
SQ6707_snp2	23363105	A	G	2	82	0	0	0	0	0	26	26	0	0.12	0.06	1	0	15	16	CATGAGCACTGACTGTTTTGA	CATGAGGAGCTTTTTAGGAGGA
SQ6707_snp3	23363128	A	G	2	82	0	0	0	0	0	26	26	0	0.12	0.06	1	0	15	16	CATGAGCACTGACTGTTTTGA	CATGAGGAGCTTTTTAGGAGGA
SQ6707_snp4	23363177	A	T	77	7	0	0	0	26	0	0	26	0.19	0.34	0.22	11	3	2	16	CATGAGCACTGACTGTTTTGA	CATGAGGAGCTTTTTAGGAGGA
SQ6707_snp5	23363487	G	T	3	81	0.12	0.11	0.06	0	3	23	26	0	0	0	0	0	16	16	CATGAGCACTGACTGTTTTGA	CATGAGGAGCTTTTTAGGAGGA
SQ6861_snp1	23388940	A	C	37	49	0.04	0.11	0.06	1	1	24	26	0	0	1	17	0	0	17	TGTGATACTGCCAACCTCTTCA	CATGGAACACTACTAACCCAAGAA
SQ6978_snp1	23422125	C	G	50	38	0.04	0.23	0.13	22	1	3	26	0.17	0.24	0.86	1	3	14	18	AGGATTGGCTCACCTTACTCTG	AATCAGCAGCATGGCACTTAC
SQ6978_snp2	23422296	C	T	87	1	0.04	0.04	0.02	25	1	0	26	0	0	0	18	0	0	18	AGGATTGGCTCACCTTACTCTG	AATCAGCAGCATGGCACTTAC
SQ6863_snp1	23422559	A	G	1	87	0	0	0	0	0	26	26	0.06	0.05	0.03	0	1	17	18	AGTAAGTGCCATGCTGCTGAT	AGCTGTTGTTTGCTAAATTGTCAT
SQ6864_snp1	23423632	A	T	87	1	0	0	0	26	0	0	26	0.06	0.05	0.03	17	1	0	18	TGTGTCAGTTGAATCCCAGAGT	CAACCAGGCTCATTACAGCTAAA
SQ7122_snp2	23424673	G	T	53	35	0	0	0	26	0	0	26	0.06	0.05	0.97	0	1	17	18	gggcaaggtagttcaagatgac	CCTAACAGTTTAGTGGGTCGTTG
SQ6865_snp1	23427327	C	T	16	70	0	0	0	0	0	26	26	0.24	0.5	0.47	6	4	7	17	AACATTCTTCGGGATAGATCA	TTGGAATGGTATGGCCTGGT
SQ6866_snp1	23428753	A	T	29	59	0	0	0	0	0	26	26	0.17	0.31	0.81	13	3	2	18	AAATTTTGGCCATTCATTTC	CACACTGATTGGTTTGTGGA
SQ6866_snp2	23429085	A	G	81	7	0	0	0	26	0	0	26	0.17	0.31	0.19	13	3	2	18	AAATTTTGGCCATTCATTTC	CACACTGATTGGTTTGTGGA
SQ6953_snp3	23429150	A	T	85	1	0	0	0	26	0	0	26	0.06	0.06	0.03	16	1	0	17	ATGCTACTGAAAGCCAATGGA	CCTCCAGCTTTGTGCCTGTAT
SQ6953_snp5	23429633	A	G	52	36	0	0	0	26	0	0	26	0	0	1	0	0	18	18	ATGCTACTGAAAGCCAATGGA	CCTCCAGCTTTGTGCCTGTAT
SQ6953_snp6	23429758	A	G	76	10	0	0	0	25	0	0	25	0.22	0.4	0.28	11	4	3	18	ATGCTACTGAAAGCCAATGGA	CCTCCAGCTTTGTGCCTGTAT
SQ6953_snp7	23429809	A	C	85	1	0	0	0	25	0	0	25	0.06	0.05	0.03	17	1	0	18	ATGCTACTGAAAGCCAATGGA	CCTCCAGCTTTGTGCCTGTAT
SQ6954_snp2	23430032	C	T	80	6	0	0	0	26	0	0	26	0.12	0.29	0.18	13	2	2	17	AAAGGAGACTGAAATGAATAGAAAGA	TTGGCTACTAATCCCTTATTGGAC
SQ6954_snp3	23430303	A	G	60	28	0	0	0	26	0	0	26	0	0.35	0.78	4	0	14	18	AAAGGAGACTGAAATGAATAGAAAGA	TTGGCTACTAATCCCTTATTGGAC

insert	23431136	N	Y	36	52	0	0	0	0	0	26	26	0	0	1	18	0	0	18	tggaccatgaaataagtcagacag	ccaatttgttccctccatttc
SQ6867_snp1	23432408	A	G	52	34	0	0	0	26	0	0	26	0	0	1	0	0	17	17	TTACCCACAAGGAAGATACAGC	CTTTGCCATTAGAGCCACAA
SQ6867_snp2	23432759	C	T	76	10	0	0	0	26	0	0	26	0.35	0.42	0.29	9	6	2	17	TTACCCACAAGGAAGATACAGC	CTTTGCCATTAGAGCCACAA
SQ6867_snp4	23432963	C	T	10	74	0	0	0	0	0	26	26	0.38	0.43	0.31	2	6	8	16	TTACCCACAAGGAAGATACAGC	CTTTGCCATTAGAGCCACAA
SQ6867_snp5	23432974	C	T	10	72	0	0	0	0	0	26	26	0.4	0.44	0.33	2	6	7	15	TTACCCACAAGGAAGATACAGC	CTTTGCCATTAGAGCCACAA
SQ6958_snp1	23433369	A	G	8	70	0	0	0	0	0	26	26	0	0.43	0.31	4	0	9	13	GAACATAGCCTTGGCCTTAACA	GAGAAATGAGTATGCCTTTATTTGC
SQ6958_snp2	23433377	C	T	70	8	0	0	0	26	0	0	26	0	0.43	0.31	9	0	4	13	GAACATAGCCTTGGCCTTAACA	GAGAAATGAGTATGCCTTTATTTGC
SQ6958_snp3	23433435	C	G	8	70	0	0	0	0	0	26	26	0	0.43	0.31	4	0	9	13	GAACATAGCCTTGGCCTTAACA	GAGAAATGAGTATGCCTTTATTTGC
SQ6958_snp4	23433720	A	C	8	68	0	0	0	0	0	25	25	0	0.43	0.31	4	0	9	13	GAACATAGCCTTGGCCTTAACA	GAGAAATGAGTATGCCTTTATTTGC
SQ7002_snp1	23434231	C	T	75	11	0	0	0	26	0	0	26	0.06	0.44	0.32	11	1	5	17	ACTCTGCTGCTCACCTTTCTTC	CCTCAGGGTCACAATGACAAC
SQ7002_snp2	23434855	C	G	60	8	0	0	0	20	0	0	20	0.14	0.41	0.29	9	2	3	14	ACTCTGCTGCTCACCTTTCTTC	CCTCAGGGTCACAATGACAAC
SQ6960_snp1	23434956	C	T	75	7	0	0	0	26	0	0	26	0.33	0.36	0.23	9	5	1	15	ATCCCCAGTACCTGTGGTTG	AAAGTGATTTTTAATGTTGGTGAT
SQ6960_snp2	23435142	A	C	10	78	0	0	0	0	0	26	26	0.11	0.4	0.28	4	2	12	18	ATCCCCAGTACCTGTGGTTG	AAAGTGATTTTTAATGTTGGTGAT
SQ6960_snp3	23435322	C	T	8	78	0	0	0	0	0	26	26	0.12	0.36	0.24	3	2	12	17	ATCCCCAGTACCTGTGGTTG	AAAGTGATTTTTAATGTTGGTGAT
SQ6960_snp4	23435327	C	T	8	76	0	0	0	0	0	25	25	0.12	0.36	0.24	3	2	12	17	ATCCCCAGTACCTGTGGTTG	AAAGTGATTTTTAATGTTGGTGAT
SQ6961_snp1	23435847	A	G	87	1	0	0	0	26	0	0	26	0.06	0.05	0.03	17	1	0	18	ACCTGTCCTCACACTTTTCACC	GAGAAGATACCATTAGAAGTCAATAAGG
SQ6961_snp2	23436094	A	C	10	76	0	0	0	0	0	25	25	0.11	0.4	0.28	4	2	12	18	ACCTGTCCTCACACTTTTCACC	GAGAAGATACCATTAGAAGTCAATAAGG
SQ6962_snp9	23436299	T	C	78	4	0	0	0	24	0	0	24	0.24	0.21	0.12	13	4	0	17	TTTACTGAAACTGAATGCACAAAG	ATCGCAATTCTCCAAAAGAAG
SQ6962_snp8	23436310	G	C	2	78	0	0	0	0	0	24	24	0.13	0.12	0.06	0	2	14	16	TTTACTGAAACTGAATGCACAAAG	ATCGCAATTCTCCAAAAGAAG
SQ6962_snp7	23436404	G	A	77	3	0	0	0	24	0	0	24	0.19	0.17	0.09	13	3	0	16	TTTACTGAAACTGAATGCACAAAG	ATCGCAATTCTCCAAAAGAAG
SQ6962_snp6	23436476	T	C	4	78	0	0	0	0	0	24	24	0.12	0.21	0.12	1	2	14	17	TTTACTGAAACTGAATGCACAAAG	ATCGCAATTCTCCAAAAGAAG
SQ6962_snp5	23436611	T	A	3	79	0	0	0	0	0	24	24	0.18	0.16	0.09	0	3	14	17	TTTACTGAAACTGAATGCACAAAG	ATCGCAATTCTCCAAAAGAAG
SQ6962_snp4	23436662	G	T	80	2	0	0	0	24	0	0	24	0.12	0.11	0.06	15	2	0	17	TTTACTGAAACTGAATGCACAAAG	ATCGCAATTCTCCAAAAGAAG
SQ6963_snp1	23436774	A	G	15	69	0	0	0	0	0	26	26	0.19	0.5	0.47	6	3	7	16	TCACATATTTCATTATTTACTCCATTCA	TTTATCCTTGCTGTTTCCACAA
SQ6962_snp2	23436864	T	C	2	78	0	0	0	0	0	24	24	0.13	0.12	0.06	0	2	14	16	TTTACTGAAACTGAATGCACAAAG	ATCGCAATTCTCCAAAAGAAG
SQ6963_snp2	23436962	G	A	34	50	0	0	0	0	0	25	25	0	0	1	17	0	0	17	TCACATATTTCATTATTTACTCCATTCA	TTTATCCTTGCTGTTTCCACAA
SQ6964_snp1	23437569	C	T	2	78	0	0	0	0	0	24	24	0.13	0.12	0.06	0	2	14	16	AAGGAAAAACCTATGCACTTTCA	ACTGAAGATACAATGCAACATGATTA
SQ6964_snp2	23437685	A	T	70	6	0	0	0	23	0	0	23	0.13	0.32	0.2	11	2	2	15	AAGGAAAAACCTATGCACTTTCA	ACTGAAGATACAATGCAACATGATTA
SQ6964_snp3	23438050	C	T	11	69	0	0	0	0	0	25	25	0.2	0.46	0.37	4	3	8	15	AAGGAAAAACCTATGCACTTTCA	ACTGAAGATACAATGCAACATGATTA
SQ6964_snp4	23438173	C	T	80	2	0	0	0	25	0	0	25	0	0.12	0.06	15	0	1	16	AAGGAAAAACCTATGCACTTTCA	ACTGAAGATACAATGCAACATGATTA
SQ6964_snp5	23438199	A	G	83	1	0	0	0	25	0	0	25	0.06	0.06	0.03	16	1	0	17	AAGGAAAAACCTATGCACTTTCA	ACTGAAGATACAATGCAACATGATTA
SQ6965_snp3	23438534	A	G	22	60	0	0	0	0	0	25	25	0.13	0.43	0.69	10	2	4	16	TCAAAGCAGTTGGTTAGAGACC	AAAAAGAGGAAAATCTAAGTTGAAGA
SQ6965_snp4	23438581	C	T	1	81	0	0	0	0	0	25	25	0.06	0.06	0.03	0	1	15	16	TCAAAGCAGTTGGTTAGAGACC	AAAAAGAGGAAAATCTAAGTTGAAGA
SQ6965_snp5	23438978	C	T	81	1	0	0	0	25	0	0	25	0.06	0.06	0.03	15	1	0	16	TCAAAGCAGTTGGTTAGAGACC	AAAAAGAGGAAAATCTAAGTTGAAGA
SQ6966_snp1	23439394	C	T	76	2	0	0	0	24	0	0	24	0	0.12	0.07	14	0	1	15	GGCAGAGGGAGAAGGATTGA	TCAACAGTTACAGCACCATCAG
SQ6967_snp1	23440116	C	T	2	74	0	0	0	0	0	23	23	0	0.12	0.07	1	0	14	15	CAAATCTAGGCATTCTGACTCCA	TCCATATACCTCTTCTTGCTCTACAA
SQ6660_snp1	23445698	A	C	1	81	0	0	0	0	0	26	26	0.07	0.06	0.03	0	1	14	15	GGCAGTCATGTTGAAGAAGAA	GAACCTTTTATTCCAAATAGCAA
SQ6660_snp2	23445875	C	T	25	55	0	0	0	0	0	26	26	0.07	0.19	0.89	12	1	1	14	GGCAGTCATGTTGAAGAAGAA	GAACCTTTTATTCCAAATAGCAA
SQ6662_snp1	23447509	C	G	58	24	0.12	0.41	0.29	17	3	6	26	0.2	0.42	0.3	9	3	3	15	GATGCCCTCTTTCTTGACTTGT	CTGAAATGGTGGCTGTTGTG
SQ6663_snp1	23447868	G	T	40	44	0.12	0.38	0.75	18	3	5	26	0.06	0.06	0.03	0	1	15	16	TAAGCCCCAAGGAAGAGAACAT	TCCGAATCCAACCAAAACTC
SQ6664_snp1	23450779	A	T	65	19	0.12	0.34	0.22	18	3	4	25	0.24	0.36	0.24	11	4	2	17	AGCTTGGGATTGTATCCATTG	ATATGAACATCTTTTTATGTCTTTGG
SQ6664_snp2	23450797	C	T	40	44	0.12	0.34	0.78	18	3	4	25	0.06	0.06	0.03	0	1	16	17	AGCTTGGGATTGTATCCATTG	ATATGAACATCTTTTTATGTCTTTGG
SQ6664_snp3	23450879	C	T	62	24	0	0	0	26	0	0	26	0.12	0.42	0.71	4	2	11	17	AGCTTGGGATTGTATCCATTG	ATATGAACATCTTTTTATGTCTTTGG
SQ6664_snp4	23451308	A	G	43	41	0.15	0.36	0.77	4	4	18	26	0.06	0.06	0.03	15	1	0	16	AGCTTGGGATTGTATCCATTG	ATATGAACATCTTTTTATGTCTTTGG
SQ6664_snp5	23451342	A	T	41	41	0.15	0.36	0.23	18	4	4	26	0.07	0.06	0.97	0	1	14	15	AGCTTGGGATTGTATCCATTG	ATATGAACATCTTTTTATGTCTTTGG
SQ6665_snp6	23451742	A	C	13	71	0	0	0	0	0	25	25	0.29	0.47	0.38	4	5	8	17	TTTTTATGTTTGATCTGAACCTCAC	AATTATTTAAGGTTACACAGCCAGT
SQ6665_snp5	23451807	C	G	39	45	0.16	0.36	0.76	17	4	4	25	0.06	0.06	0.03	0	1	16	17	TTTTTATGTTTGATCTGAACCTCAC	AATTATTTAAGGTTACACAGCCAGT
SQ6665_snp4	23451827	A	C	32	52	0.16	0.36	0.24	4	4	17	25	0.35	0.48	0.59	7	6	4	17	TTTTTATGTTTGATCTGAACCTCAC	AATTATTTAAGGTTACACAGCCAGT
SQ6665_snp3	23451841	C	T	52	32	0.16	0.36	0.24	17	4	4	25	0.35	0.48	0.59	4	6	7	17	TTTTTATGTTTGATCTGAACCTCAC	AATTATTTAAGGTTACACAGCCAGT
SQ6665_snp2	23452108	A	C	43	39	0.16	0.36	0.76	4	4	17	25	0.06	0.06	0.03	15	1	0	16	TTTTTATGTTTGATCTGAACCTCAC	AATTATTTAAGGTTACACAGCCAGT
SQ6665_snp1	23452115	A	G	41	39	0.16	0.36	0.76	4	4	17	25	0.07	0.06	0.03	14	1	0	15	TTTTTATGTTTGATCTGAACCTCAC	AATTATTTAAGGTTACACAGCCAGT
SQ6869_snp1	23468179	C	T	28	58	0.16	0.36	0.24	4	4	17	25	0.44	0.49	0.44	4	8	6	18	GGGACCTAAGCAAAGCAACTTA	CTTGGGCTGCATCTTCTTCA
SQ6869_snp2	23468257	A	C	20	66	0.16	0.36	0.24	4	4	17	25	0.22	0.35	0.22	2	4	12	18	GGGACCTAAGCAAAGCAACTTA	CTTGGGCTGCATCTTCTTCA
SQ6869_snp3	23468387	C	T	66	20	0.16	0.36	0.24	17	4	4	25	0.22	0.35	0.22	12	4	2	18	GGGACCTAAGCAAAGCAACTTA	CTTGGGCTGCATCTTCTTCA
SQ6870_snp1	23468632	A	T	68	20	0.15	0.36	0.23	18	4	4	26	0.22	0.35	0.22	12	4	2	18	GGGCCTAGTATTTGCCACAG	TTTTCTTGTTTTATTGCTTTCAACTC
SQ6870_snp2	23468637	C	T	34	54	0.15	0.36	0.23	4	4	18	26	0.33	0.48	0.61	8	6	4	18	GGGCCTAGTATTTGCCACAG	TTTTCTTGTTTTATTGCTTTCAACTC
SQ6870_snp3	23469105	A	T	57	25	0.12	0.34	0.22	18	3	4	25	0.38	0.49	0.44	6	6	4	16	GGGCCTAGTATTTGCCACAG	TTTTCTTGTTTTATTGCTTTCAACTC
SQ6867_snp3	23482890	A	G	9	75	0	0	0	0	0	26	26	0.44	0.4	0.28	1	7	8	16	TTACCCACAAGGAAGATACAGC	CTTTGCCATTAGAGCCACAA
SQ6684_snp1	23539415	A	G	76	12	0.19	0.17	0.1	21	5	0	26	0.17	0.31	0.19	13	3	2	18	TGAAGCTAGACAGTGAGAATTGAAC	TTGATTACAAACATAAAACATTGAGAGA
SQ6684_snp2	23539437	A	G	86	2	0	0	0	26	0	0	26	0	0.1	0.06	17	0	1	18	TGAAGCTAGACAGTGAGAATTGAAC	TTGATTACAAACATAAAACATTGAGAGA
SQ6764_snp1	23587069	C	G	70	18	0	0	0	26	0	0	26	0.22	0.5	0.5	7	4	7	18	GGAATGGATGAAAAGAAAGAGG	TAATTTGGGAAAGACGTTGTTG
SQ6764_snp2	23587152	C	T	87	1	0	0	0	26	0	0	26	0.06	0.05	0.03	17	1	0	18	GGAATGGATGAAAAGAAAGAGG	TAATTTGGGAAAGACGTTGTTG
SQ6764_snp3	23587227	C	T	11	77	0.19	0.33	0.21	3	5	18	26	0	0	0	0	0	18	18	GGAATGGATGAAAAGAAAGAGG	TAATTTGGGAAAGACGTTGTTG
SQ6764_snp4	23587239	A	T	80	8	0	0	0	26	0	0	26	0.11	0.35	0.22	13	2	3	18	GGAATGGATGAAAAGAAAGAGG	TAATTTGGGAAAGACGTTGTTG
SQ6685_snp1	23622780	C	G	12	68	0.26	0.29	0.17	1	6	16	23	0.24	0.21	0.12	0	4	13	17	GGAACTCCTTTTGGACATGG	AAGACTTTCAGACACAAATGAATC

Table S4. Insert frequency and genotypes in multiple breeds of dog and the wolf.

Breed	Number of Dogs	Insert Frequency	Genotypes[a] NN	IN	II
Afghan Hound	4	0.00	4	0	0
Akita	3	0.00	3	0	0
Anatolian Shepherd	4	0.00	4	0	0
Australian Cattle Dog	1	0.00	1	0	0
Basenji	9	0.00	9	0	0
Basset Hound	15	1.00	0	0	15
Beagle	8	0.00	8	0	0
Bloodhound	4	0.00	4	0	0
Border Collie	4	0.00	4	0	0
Boxer	1	0.00	1	0	0
Bulldog	3	0.00	3	0	0
Bullmastiff	8	0.00	8	0	0
Cairn Terrier	7	1.00	0	0	7
Cardigan Welsh Corgi	13	1.00	0	0	13
Cavalier King Charles Spaniel	4	0.00	4	0	0
Chihuahua[b]	3	0.33	0	2	1
Clumber Spaniel	4	0.00	4	0	0
Cocker Spaniel	8	0.00	8	0	0
Collie	4	0.00	4	0	0
Dachshund	14	1.00	0	0	14
Dandie Dinmont Terrier	3	1.00	0	0	3
Doberman Pinscher	7	0.00	7	0	0
English Foxhound	1	0.00	1	0	0
English Setter	2	0.00	2	0	0
Entlebucher	6	0.00	6	0	0
French Bulldog	4	0.00	4	0	0
Geman Shepherd Dog	10	0.00	10	0	0
Giant Schnauzer	4	0.00	4	0	0
Glen of Imaal Terrier[c]	12	0.92	0	2	10
Golden Retriever	4	0.00	4	0	0
Grand Basset Griffon Vendeen[d]	4	0.63	0	3	1
Greyhound	4	0.00	4	0	0
Havanese[c]	7	0.93	0	1	6
Ibizan Hound	8	0.00	8	0	0
Irish Setter	2	0.00	2	0	0
Irish Wofhound	8	0.00	8	0	0
Italian Greyhound	4	0.00	4	0	0
Jack Russell Terrier	7	0.00	7	0	0
Japanese Chin[b]	4	0.13	3	1	0

Labrador Retriever	8	0.00	8	0	0
Lancashire Heeler	6	1.00	0	0	6
Mastiff	4	0.00	4	0	0
Miniature Pinscher	5	0.00	5	0	0
Miniature Poodle	4	0.00	4	0	0
Norwich Terrier	8	1.00	0	0	8
Old English Sheepdog	11	0.00	11	0	0
Pekingese	13	1.00	0	0	13
Pembroke Welsh Corgi	15	1.00	0	0	15
Petit Basset Griffon Vendeen	14	1.00	0	0	14
Pointer	4	0.00	4	0	0
Pomeranian	4	0.00	4	0	0
Pug Dog	8	0.00	8	0	0
Rhodesian Ridgeback	3	0.00	3	0	0
Schipperke	3	0.00	3	0	0
Scottish Terrier[c]	14	0.96	0	1	13
Shetland Sheepdog	6	0.00	6	0	0
Shiba Inu	3	0.00	3	0	0
Shih Tzu	10	1.00	0	0	10
Siberian Husky	4	0.00	4	0	0
Skye Terrier	3	1.00	0	0	3
Soft Coated Wheaten Terrier	8	0.00	8	0	0
Sussex Spaniel	9	0.00	9	0	0
Swedish Valhund	5	1.00	0	0	5
Tibetan Spaniel	4	1.00	0	0	4
Tibetan Terrier	7	0.00	7	0	0
Vizsla	3	0.00	3	0	0
West Highland White Terrier	7	1.00	0	0	7
Yorkshire Terrier[b]	4	0.50	1	2	1
Wolf	26	0.00	26	0	0
Mixed Breeds[e]	12	0.63	2	5	5

a) N = no insert, I = insert
b) Three of the smallest breeds (adult body weight < 7lbs), the Chihuahua, Japanese chin, and Yorkshire terrier, carry the insert at a much higher frequency than expected since they do not show the phenotype as it is described in this study. We suggest that the extremely low growth rate in these breeds due to unrelated mutations in additional growth factors (*9, 10*) disguise the effect of the retrogene allowing it to segregate without selective pressure.
c) Four dogs from three chondrodysplastic breeds were found to be heterozygous for the mutation when it was assumed to be fixed within the breed. The first breed, the Glen of Imaal terrier is new to the AKC, having been accepted only 5 years ago. Judging records show that leg length was not completely fixed within the breed as recently as 2002 and was the primary

critique for young dogs (http://www.irishglenofimaalterrier.nl). Current records of the same show very little variation and suggest that the trait is now approaching fixation. Sampling of today's puppies would likely reveal little or no heterozygosity at this locus. The Havanese are also a newly recognized breed though not as new as the Glen. This breed struggles with a second form of dwarfism, osteochondrodysplasia, that correlates with a number of health problems (*11*). The presence of the second mutation may disguise the absence of the retrogene allowing a low level of segregation within the breed. The final breed in which we found one heterozygous individual is the Scottish terrier. This breed is neither new nor variable and the lack of fixation may indicate an unplanned outcrossing, faulty breeding records, or that absolute fixation on a dominant trait is not easy to maintain.

d) The grande basset griffon Vendéen (GBGV) presents a special case. This breed is the taller sister to the petit basset griffon Vendéen (PBGV). They originally came from the same litters with the smaller offspring called Petit and the larger called grande though both are disproportionately short legged compared to their cousins the griffon Vendéen and the grande griffon Vendéen. The two are now separate breeds however the GBGV is not yet recognized by the AKC. The genotypes suggest that the breed may be under balancing selection to maintain the slightly more moderate phenotype of the heterozygote in order to distinguish it from the PBGV. Additional sampling five and ten generations from now would help to address this issue.

e) The mixed breed dogs were identified as possibly chondrodysplastic based on photographic observations. Breed testing was done using the Wisdom Panel™ (Mars, Inc. McLean VA) and suggested that 7 of 12 mixes had a medium or high probability of ancestry with breeds carrying the retrogene. Two of the dogs showed traces of shared ancestry with chondrodysplastic breeds and three (including the two without the retrogene) showed no indication of having ancestors from breeds that we have identified as carrying the *fgf4* retrogene.

Table S5. List of primers use to sequence the *fgf4* retrogene containing insert.

Primer Name	Primer Sequence	Tm	Region covered
SQ7130	F-CACACAGATGGACCATGAAATAAGT	61	
	R-ccaatttgttccctccatttc	60	Amplifies entire insert
SQ7342	F-TCGAAACCCTTAACCCACTCATC		
	R-cctttccctctggcaaccac	64	from 3'UTR of the retrogene to region on chr18 telomeric to the insert
SQ7343	F-AAGGCTGATTGGAGTTTGTGTC	61	
	R-agctggaagtttgtacctcttg	58	from 3'UTR of the retrogene to region on chr18 telomeric to the insert
SQ7344	F-TCACGTTTGAGCTATCTTTACCC	60	
	R-ctttccctctggcaaccac	61	from 3'UTR of the retrogene to region on chr18 telomeric to the insert
SQ7345	F-GCGTCCCATTGAAACCTTG	61	
	R-cctttccctctggcaaccac	64	from 3'UTR of the retrogene to region on chr18 telomeric to the insert
SQ7371C	F-gcaaagatatgcaaacaacccaagtatc	66	
	R-ACATCACCGACCCTGCCTCTTC	67	from region on chr18 centromeric to the insert to the 3'UTR
SQ7409	F-GAGCAAGAACGGGAAGACCAAG	64	
	R-TTTCAGCGGGAGATGGGTTC	64	from exon 3 into 3'UTR, amplifies both source gene and retrogene
SQ7410	F-GAGCAAGAACGGGAAGACCAAG	63	
	R-TTTATCACTCGGGCTTTGGTTG	63	from exon 3 into 3'UTR, amplifies both source gene and retrogene
SQ7411_seq	R-GGGAGATGGAGGAAGATTGACTAC	62	used for sequencing within 3'UTR
SQ7414	F-GGTCTTGGCACCTCAACTTCTC	62	
	R-ACAGAGAAACATGGGCTGTGG	62	PCR across SNPs in source FGF4 gene from intron3 to 3'UTR
SQ7413	F-CTGAGTTTTCTAGTAAGACCGGTGAAG	62	
	R-ACAGAGAAACATGGGCTGTGG	62	sequencing primers for 3'UTR SNPs from SQ7414 or SQ7130 for retrogene

Table S6. The frequency of the haplotypes in the *FGF4* gene and the insertion site required to create the *fgf4* retrogene in the original chondrodysplastic dog.

Insert haplotype	Total	Dogs carrying the insert[a]	Dogs without the insert	Wolves
GTTA[b]	0.33	0.98	0.01	0.29
GTAG	0.00	0.00	0.01	0.00
TCAG	0.25	0.00	0.41	0.05
GTTG	0.02	0.00	0.01	0.11
TTTG	0.14	0.00	0.20	0.18
TTAG	0.25	0.02	0.36	0.29
TCTG	0.01	0.00	0.00	0.08
FGF4 haplotype				
ACA[c]	0.04	0.00	0.03	0.20
GCG	0.29	0.38	0.24	0.36
ATG	0.67	0.63	0.73	0.44

a. Includes some dogs that are heterozygous for the insert. b. GTTA is the four marker haplotype that represents the region on CFA18 at positions 23,424,674; 23,427,327; 23,428,753; and 23,432,408, into which the retrogene was transposed. The insert is found between the 3rd and 4th markers. c. ACA is the three marker haplotype from the 3'UTR of *FGF4* that is identical to that found in the *fgf4* retrogene. It is common in the wolf but rare in the domestic dog.

Supplemental References:

1. N. Rabbee, T. P. Speed, *Bioinformatics* **22**, 7 (2006).
2. N. B. Sutter, D. S. Mosher, M. M. Gray, E. A. Ostrander, *Mamm Genome* **19**, 713 (2008).
3. American Kennel Club, *The Complete Dog Book*. Official Publication of the American Kennel Club (Howell Book House, New York, NY, ed. 19th Edition Revised, 1998).
4. S. Purcell *et al.*, *Am. J. Hum. Genet.* **81**, 559 (2007).
5. K. Lindblad-Toh *et al.*, *Nature* **438**, 803 (2005).
6. S. Rozen, H. Skaletsky, *Methods Mol Biol* **132**, 365 (2000).
7. D. R. Zerbino, E. Birney, *Genome Res* **18**, 821 (2008).
8. C. Guo *et al.*, *Cell Signal* **20**, 1471 (Aug, 2008).
9. N. B. Sutter *et al.*, *Science* **316**, 112 (2007).
10. P. Jones *et al.*, *Genetics* **179**, 1033 (2008).
11. A. N. Starr *et al.*, *J Hered* **98**, 510 (2007).